United Nations Scientific Committee on the Effects of Atomic Radiation

SOURCES, EFFECTS AND RISKS OF IONIZING RADIATION

UNSCEAR 2020/2021 Report

Volume III

SCIENTIFIC ANNEX C:

Biological mechanisms relevant for the inference of cancer risks from low-dose and low-dose-rate radiation

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United Nations Scientific Committee on the Effects of Atomic Radiation

UNSCEAR 2020/2021
Report to the General Assembly, with Scientific Annexes

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Scientific Annex C

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NOTE

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ANNEX C

BIOLOGICAL MECHANISMS RELEVANT FOR THE INFERENCE OF CANCER RISKS FROM LOW-DOSE AND LOW-DOSE-RATE RADIATION

CONTENTS

LIST OF ABBREVIATIONS

BrdU	Bromodeoxyuridine
CpG	CpG dinucleotide, cytosine-phosphate-guanine
CT	Computed tomography
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
DoReMi	Low Dose Research towards Multidisciplinary Integration [European Commission funded project]
DSB	Double-strand break
dsDNA	Double-stranded DNA
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial-to-mesenchymal transition
G_0	Quiescent phase during cell-division cycle
G_1/S	Stage in the cell cycle at transition point between G_1 and S stage
G_2/M	Transition between G_2 and M phases of the cell cycle
GSEA	Gene-set-enrichment-analysis
hCG	Human celera genome
HBRA	High-level natural background radiation area
HRR	Homologous recombination repair
HZE	High atomic-number element
kb	Kilo-base pair
ICR	Institute of Cancer Research
LET	Linear energy transfer
LINAC	Linear accelerator
lncRNA	Long non-coding RNA
LOH	Loss of heterozygosity
miRNA	MicroRNA
mRNA	Messenger RNA
NBS	Nijmegen Breakage Syndrome caused by NBS1 (nibrin) gene mutation
NHEJ	Non-homologous end joining
NHF	Normal human fibroblast
NK cell	Natural killer cell
NKT cell	Natural killer T-cell
PCR	Polymerase chain reaction

phospho	Phosphorylated
Ku (e.g. Ku70, Ku80)	ATP-dependent DNA helicase II subunit 1
PMA	Phorbol-12-myristate-13-acetate
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTC	Papillary thyroid cancer
RBE	Relative biological effectiveness
RIDDLE syndrome	Radiosensitivity, ImmunoDeficiency Dysmorphic features and LEarning difficulties syndrome
RIFs	Radiation-induced foci
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RT-PCR	Reverse transcription-polymerase chain reaction
SCID	Severe combined immunodeficiency
siRNA	Small interfering RNA
ssDNA	Single-stranded DNA
sSPE	Simulated solar particles event
Tconv	Conventional T-cells
TCR	T-cell receptor
TF	Transcription factor
Th (e.g. Th1, Th2, etc.)	T-helper cells (1, 2, etc.)
TRECs	T-cell receptor excision circles
Treg	Regulatory T-cells
TSCE	Two-stage clonal expansion
TUNEL	Terminal deoxynucleotidyl transferase assay
WLM	Working level month

I. INTRODUCTION

1. In 1955, the General Assembly of the United Nations established the Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) to assess and report levels and effects of exposure to ionizing radiation. The Scientific Committee issues detailed reports and reviews which are widely regarded as authoritative. Governments and organizations throughout the world rely on the Committee's estimates as the scientific basis for evaluating radiation risk and for establishing protective measures.

2. Since the establishment of the Scientific Committee, its mandate has been to undertake broad estimates of the sources of ionizing radiation and its effects on human health and the environment. In 1973, the mandate expanded to include scientific estimates of radiation risk. These assessments of the Scientific Committee provide the scientific foundation used, inter alia, by the relevant agencies of the United Nations system in formulating international standards for the protection of the general public and workers against ionizing radiation; those standards, in turn, are linked to important legal and regulatory instruments. In its UNSCEAR 2012 Report, the Committee considered the attribution of health effects and the inference of risks from radiation exposure, as well as on the uncertainties in risk estimates [U10]. The understanding of the biological mechanisms by which radiation-induced effects such as cancer may occur is a relevant element for the inference of radiation risk. This annex is intended to synthesize the current knowledge on biological mechanisms of radiation actions at doses mostly in the low to moderate range relevant for cancer risk inference. It is emphasized that this is not a report on radiation effects; in particular, it is not a report on cancers that can be attributed to radiation exposure situations.

3. The Committee's strategic objectives adopted in 2019 included giving priority to "Health and environmental effects, and inferred risks". This included a commitment that "The Committee will collate and evaluate information such as epidemiological data, in particular for the low dose range and for long-term low-dose-rate exposure and analyse data from research on mechanisms of biological reactions at such exposures. The Committee will pursue innovative approaches to integrate radiobiological and epidemiological research, to enhance the understanding of *(a)* radiation-induced health effects and associated inferred risks related to the induction of cancer, non-cancer effects, and hereditary disease; and *(b)* environmental effects. Attention will be given to individual-related factors affecting the incidence of such effects, including age, sex, lifestyle, genetic or familial predisposition and health status."

4. This evaluation has been undertaken to fulfil a commitment made by the Committee in the UNSCEAR 2012 White Paper, "Biological mechanisms of radiation actions at low doses" to further review the biological mechanisms that influence the health risks from low-dose radiation exposure [U9].

5. A number of relevant evaluations have been carried out by the Committee over many years. These include annex B of the UNSCEAR 1986 Report [U2], annexes E and F of the UNSCEAR 1993 Report [U3], annex B of the UNSCEAR 1994 Report [U4], annexes F, G and H of the UNSCEAR 2000 Report [U5], annex of the UNSCEAR 2001 Report [U6], and annexes C and D of the UNSCEAR 2006 Report [U7]. Additionally, the Committee has provided a summary of low-dose radiation effects on health in the UNSCEAR 2010 Report [U8]. The rationale for the judgements on the mechanisms relevant to the health effects of low-dose and dose-rate radiation exposures were summarized in the UNSCEAR 2012 White Paper [U9] that also presents a brief review of the post-2006 literature on this topic.

6. This evaluation is intended to synthesize the current knowledge on biological mechanisms of radiation actions at low doses (i.e. those less than or equal to 100 milli-Gray (mGy) low-linear energy transfer (LET) exposure, or less than or equal to one track traversal per cell of high-LET exposure) and

low dose rates (i.e. those of 0.1 mGy/min or less low-LET exposure, or no more than one high-LET track traversal per cell per hour), assess their implications for understanding the processes of cancer development after exposure to ionizing radiation, and to explore the implications for dose–response relationships of radiation-induced cancers. By comparison, the Committee takes moderate doses to be those of 0.1 to about 1 Gy for low-LET radiations [U10]. This annex addresses the following questions:

(a) For which biological mechanisms and pathways is there evidence that indicates that they can affect the frequency of cancers following exposure to ionizing radiation, including at low doses and low dose rates? What are the differences in utilization and/or activation of these pathways and mechanisms at low doses compared with moderate doses? What evidence is available on the form of the dose–response relationships for these mechanisms?

(b) Considering the mechanisms identified above, can any conclusions be drawn as to their overall influence on the dose–response relationships for cancers associated with radiation exposure at low doses compared with moderate doses?

(c) Are there ways to link information on the biological processes and mechanisms found to be relevant to human cancer and existing epidemiological data on incidence of disease in exposed populations?

(d) Is there evidence for tissue-specific variation in the mechanisms of response to ionizing radiation that relate to the differing sensitivity of tissues to radiation-induced cancers?

(e) Are the mechanisms that can be associated with cancer development similar following low- and high-LET exposures?

7. To address these questions, the evaluation focuses on particular topic areas that include the established mechanisms of targeted DNA damage/mutation and additionally a range of mechanisms that may contribute to radiation carcinogenesis at low doses and low dose rates. The areas are as follows:

(a) DNA damage;

(b) DNA damage signalling, chromatin remodelling and epigenetics;

(c) Effects on other signal transduction pathways;

(d) Gene and protein expression;

(e) DNA repair and effects on somatic cells;

(f) Genomic instability, bystander effects, damage/effects on non-nuclear cellular components, adaptive response and hyper-radiosensitivity;

(g) Stem cells and target cell populations for radiation carcinogenesis;

(h) Effects at the whole organism level.

8. Relevant literature was identified using searches of PubMed, current review articles, from the knowledge of members of the Expert Group, and suggestions from delegations to the Committee. The evaluation considers that peer-reviewed primary scientific papers provide the best evidence on which to base conclusions. A limited number of review articles and reports were also taken into account. Given that the most recent published substantial work of the Committee considered the literature up to 2006, this evaluation draws mainly on publications from 2006 to 2020*;* some earlier publications were included which had not previously been considered by the Committee or are of particular relevance to the mechanistic arguments presented. Papers were evaluated against the following criteria:

(a) Is this an original publication (and not a review, editorial, commentary or correspondence)?

(b) Is there experimental evidence indicating that the endpoints described can be linked directly or indirectly to radiation carcinogenesis?

(c) Is the experimental design adequate and free of substantial flaws, including in dosimetry?

(d) Are the results statistically sound?

(e) Have the results been replicated or otherwise substantiated?

9. In gathering and reviewing the literature, the focus has been on reports that consider low-dose and low-dose-rate effects. However, for context, and particularly for consideration of implications for approaches to risk extrapolation, it is necessary to consider studies that use higher doses. Where apparent, qualitative and/or qualitative differences in response to low and moderate doses are taken into account.

II. PREVIOUS FINDINGS OF THE COMMITTEE UP TO 2012 AND THE BROADER CONTEXT

A. Previous findings of the Committee

10. In its summary of low-dose radiation effects on health in the UNSCEAR 2010 Report [U8], the Committee noted that the health concerns at low doses and low dose rates were *(a)* the risk of cancer in several tissues (for which there was substantial evidence of an increased incidence of cancer at moderate and high levels of exposure) and *(b)* the risk of heritable effects (which are assumed to occur in human populations, although they have not been observed directly). In terms of the possible mechanisms underlying these health effects, the Committee noted the importance placed upon mutational mechanisms. The paradigm of radiation-induced damage to DNA, which may be converted to a DNA sequence mutation, provided important underpinning of the use of a linear no-threshold model for estimating risk of disease at low doses and low dose rates. The rationale for the judgements on the mechanisms relevant to the health effects of low-dose and low-dose-rate radiation exposures made in previous evaluations of the Committee were summarized in the UNSCEAR 2012 White Paper [U9] to guide the Committee's future programme of work.

11. In addition to direct mutational mechanisms, the Committee has considered "non-targeted" effects of radiation, that is, cellular action of radiation exposure that occur in cells neighbouring the cell that was actually "hit", or in the descendents of "hit" cells (UNSCEAR 2006 Report, annex C [U7]). The judgement of the Committee in its 2006 report was that non-targeted and delayed effects of radiation may be associated with radiation-induced disease but no evidence for disease causation had been found. It was noted that any contribution of non-targeted and delayed effects would be implicitly incorporated in the estimates of risk of radiation-induced health effects derived epidemiologically.

12. The Committee also provided an assessment of the effects of ionizing radiation on the immune system (UNSCEAR 2006 Report, annex D [U7]). The immune system could act to modify the cancer risk if radiation exposure served to enhance or diminish the capacity of the body to mount an immune response against developing cancers, whatever their cause. While many studies were examined, it remained impossible to judge clearly whether the effects of radiation at low doses on the immune system served to stimulate or suppress or have no effect on immune responses.

13. In developing the UNSCEAR 2012 White Paper [U9], the Committee additionally undertook a limited literature review to identify any major advances in understanding the biological mechanisms acting at low doses. The Committee concluded that:

(a) Since 2006, significantly more data had become available on the biological consequences of low-dose radiation exposure and non-targeted effects. The White Paper [U9] considered advances in the areas of genomic instability, bystander and abscopal effects, adaptive response, reactive oxygen metabolism and mitochondrial function, DNA sequence analysis and genetic polymorphisms, gene and protein expression, cellular interactions and tissue-level phenomena and systems biology approaches;

(b) While mechanistic understanding of non-targeted effects was improving, many studies remained observational;

(c) There were reports of differential gene and protein expression responses at high and low radiation doses and dose rates, but these reports were mixed in outcome, and there was little of the coherence required of robust data that could be used confidently for risk assessment;

(d) There was at the time no indication of a causal association of non-targeted phenomena with radiation-related disease and indeed, some may not operate at low doses in vivo;

(e) A systems-level framework should provide a useful guide for future integration of mechanistic data into risk estimation methods;

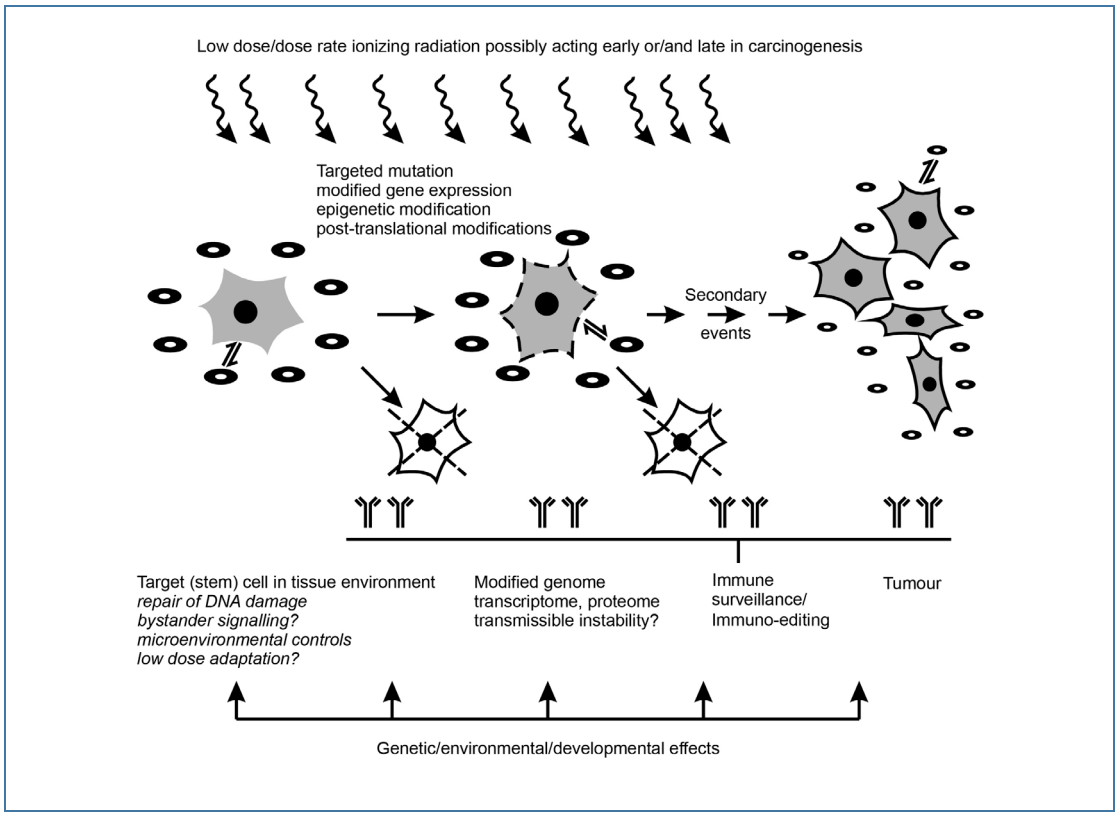
(f) In the case of radiation-induced perturbation of immune function or inflammatory reactions, there was a clearer association with disease, but the impact of radiation was less well understood.

14. The Committee agreed to: *(a)* continue to encourage research into the mechanistic understanding of low-dose radiation action that may contribute to improved understanding of disease risk in humans and into the factors that can modulate risk, and *(b)* consider developing further biologically-based risk models and the systems-level framework to integrate mechanistic data into a risk-assessment framework. The overall schema for radiation carcinogenesis proposed in the UNSCEAR 2012 White Paper [U9] is reproduced in figure I. In this schema, carcinogenesis progresses through a series of steps, or key events that persist in the target cell population (caused by DNA mutation, epigenetic alteration or other process), that convert a normal cell into a fully malignant cancer cell; this central path can be modified by a range of influencing factors including signalling from the surrounding micro-environmental cells, rates of cell death, immune surveillance and immunoediting of cancer cells. This schema and the general approach is similar to the use of adverse outcome pathways in toxicology (see [L17, O7]). The use of adverse outcome pathways is increasingly advocated for improving the assessment of the risks of radiation health effects [C12, P23], and recently the National Council on Radiation Protection and Measurements has published a report on the topic and more generally the integration of radiobiological and epidemiological data for radiation risk assessment [N11]. Thus, there is growing consensus internationally that such approaches are promising.

15. Additionally, to the above considerations, the Committee notes that there are reports of effects of ionizing radiation on other cellular processes and systems. For example, effects of radiation on cell membrane systems have been reported. High dose exposure effects have been summarized by Huber et al. [H40], notably effects on ion channels, with effects on membrane permeability associated with thymocyte apoptosis also reported [P2], along with similar permeability and integrity effects in yeast [C5]. Damage to membranes as assessed by levels of lipid peroxidation has also been suggested to be caused by low dose occupational exposures [D21]. Further, the Committee is aware of effects on other bio-molecules, e.g. RNA and proteins that are not considered in detail here as most information relates to high dose exposures.

Figure I. The systems view of radiation carcinogenesis modified from [U9]

The systems view takes account of the tissue context in which target stem and early progenitor cells reside, and of intercellular signalling and bystander signalling of responses to radiation exposure between cells. DNA damage may be repaired or converted to mutations; additionally, radiation may affect gene expression, protein modification and epigenetic status. Cells may die through apoptosis or other pathways, enter terminal differentiation or senescence (or otherwise be selected against – unshaded, crossed cells) thereby removing them from cancer-development pathways. Radiation may affect these processes at any stage of cancer development. The induced alterations to genome, epigenome, transcriptome and proteome together affect cellular differentiation (phenotype) – some of these altered states of differentiation may lead to cells having growth or survival advantage and thus are linked to cancer development. Genomic instability may be another outcome. Cells with altered phenotype may be subject to detection and killing by the immune system (immunoediting). Effects of radiation on all the processes noted above have been observed; however, it is only mutations in DNA sequences sustaining radiation damage that have an established role in radiation carcinogenesis. The availability of robust quantitative data on radiation-induced changes associated with disease development will provide the way to integrate mechanistic findings into risk estimation. Superimposed onto the somatic pathway(s) of cancer development are influences from the individual's inherited genome (e.g. disease susceptibilities, variations in target/stem cell numbers), history of exposure to other environmental agents (e.g. the known interaction between tobacco smoke and radon in lung carcinogenesis) and developmental/age-related factors (e.g. stem-cell development and numbers, life expectancy). Large grey cells – target cell and its descendents, large white cells – cells removed due to apoptosis, immuno-editing or other process, smaller oval cells – surrounding tissue cells, two-way arrow – bystander signalling and inter-cellular communication, "Y" shaped symbols – representative of immune responses, including both humoral and cellular immunity.



B. Broader context on current understanding of mechanisms of carcinogenesis

16. Cancer is prevalent in the population. Current estimates indicate that in the United Kingdom, which is representative of Western European and North American populations, some 50 per cent of the population will develop cancer during life and around 30 per cent will die from cancer [C2]. About half of cancers are diagnosed in those over 70 years of age, and cancer incidence is highest in those over 85 years of age [C2]. There is considerable global variation in cancer incidence and mortality and this information is collated by the International Agency for Research on Cancer [I1], global cancer incidence rates are highest in the 75+ year age group. Therefore, in general, cancer is a disease of older ages, though there are cases of cancer and leukaemia among children and young adults. The observation that human cancers are more common later in life is interpreted as suggesting that multiple events have to accumulate in cells over a long latency period before they become fully cancerous. Cancers are also multifactorial in origin with contributions from "spontaneous" events in DNA replication, exposures to occupational and environmental carcinogens, and lifestyle factors such as diet, exercise levels, alcohol consumption and tobacco consumption having an impact.

17. Cancer, which we use here to include both solid cancers and leukaemias, can be defined as the uncontrolled proliferation of abnormal cells in a body tissue; these abnormal cells may spread to other parts of the body to form metastases. Research efforts over decades have yielded a good understanding of the characteristics of cancers and the differences between normal cells and cancer cells. These are best summarized in the hallmarks of cancer as defined by Hanahan and Weinberg [H9, H10]. The hallmarks (figure II) are defined as the biological capabilities acquired through the multistep development of cancers in humans. The original hallmark capabilities [H9] that are distinctive for cancers are:

(a) Sustained proliferative signalling;

(b) Evading growth suppressors;

(c) Resisting cell death;

(d) Enabling replicative immortality;

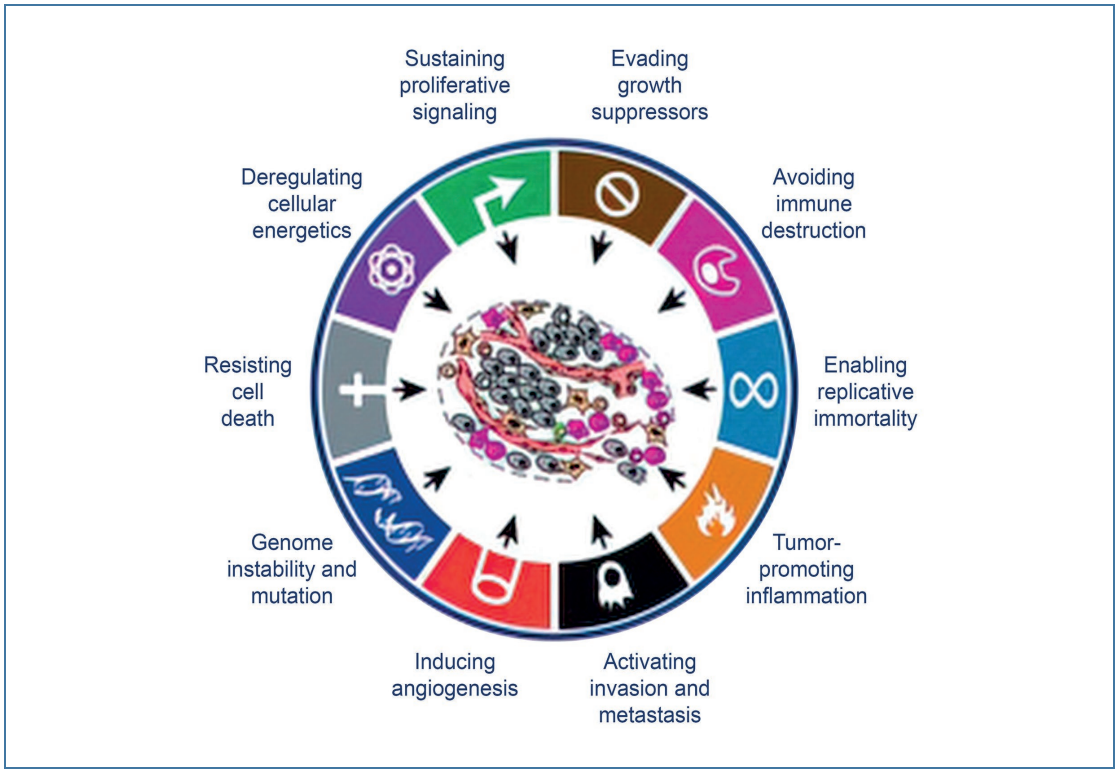
(e) Inducing angiogenesis;

(f) Activating invasion and metastasis.

18. These hallmarks were extended in the 2011 publication [H10] to include two "enabling" characteristics: *(a)* genome instability and mutation, and *(b)* tumour-promoting inflammation; and two "emerging" hallmarks: *(i)* deregulating cellular energetics and *(ii)* avoiding immune destruction.

19. Not all malignancies necessarily have all hallmarks; for example, angiogenesis is not a characteristic of leukaemia. It is generally the case that fewer alterations are required for leukaemogenesis than for the development of solid cancers. This is reflected in the relatively short latent periods between exposure to radiation and excess leukaemia presentation in the survivors of the Japanese atomic bombings, for example.

Figure II. The hallmarks of cancer: a framework summarizing the capabilities of cancer cells and the mechanisms contributing to carcinogenesis (adapted from [H10])



20. Understanding the underlying drivers of the acquisition of hallmarks has changed over the years as a widening range of contributory processes have been discovered [H11]. Clearly though, cancer cells acquire specific phenotypic traits, as defined by the hallmarks; as such, cancer can be viewed as a disease where the normal patterns of differentiation in specific tissues are altered (or reprogrammed) allowing cancer cells to escape the normal tight regulation of proliferation in tissues.

21. The acquisition of multiple biological capabilities as set out in the hallmarks depends on the accumulation of changes in several pathways. There is a long-held understanding that these changes can be caused by somatic mutation of the genomes of cancer cells. Recent large DNA sequencing studies bear this out. For example, Martincorena et al. [M19] have analysed exome sequences for over 7,500 cancer cases representing 29 different tumour types. This analysis concluded that on average there are four (range 1–10) coding substitution mutations driving the cancer process that are recurrent in specific tumour types, and these are under positive selection. Whole genome sequencing has additionally revealed that many genotoxic agents produce distinct damage-related mutational signatures in DNA, and in some cases similar damage-related mutational signatures are observed in cancers – for example a simulated solar radiation signature in UV-associated skin cancers, and a polyaromatic hydrocarbon signature in smoking-related lung cancers [K46]. It has also become increasingly clear that there are additional non-mutational routes to modify cellular phenotypes; collectively these are known as epigenetic pathways. The epigenetic pathways lead to long-lasting alterations in gene expression, and these include alterations to DNA methylation of cytosine residues in gene upstream regulatory elements, modification of histones and chromatin status through acetylation, phosphorylation, ubiquitination plus a range of other chromatin modifications, and expression of non-coding RNAs that modify transcript stability and abundance [D5]. Given that targeting of epigenetic regulators in cancer therapy holds promise, it would appear that epigenetic processes play an important role in carcinogenesis [D5]. Epigenetic alterations and non-coding RNA (largely microRNA) alterations have also been observed as recurrent changes in cancers [V9].

Inflammation can also contribute to the acquisition of hallmark capabilities through modification of the tumour microenvironment, and in some cases, can lead to the release of reactive oxygen species (ROS) that can actively contribute to mutagenesis during tumour pathogenesis [H10].

22. It is also important to consider which of the cells within the body represent the target cells for carcinogenesis. Most cells in the body are terminally differentiated, many with rather short existence before loss, skin and intestinal epithelia being good examples. Given the observation that cancers are present predominantly in older age groups, the cells of origin must necessarily be long lived. Thus, attention tends to focus on the stem and early progenitor cells that serve to maintain tissues throughout life. It is also possible that the elderly are more vulnerable to cancer due to impaired repair/defence mechanisms; however for induced cancers from instantaneous exposures, such as experienced by the survivors of the atomic bombings in Japan, effects take from a few to many years to manifest.

23. The hallmarks provide a current overview of the pathways that need to be considered in order to understand the mechanisms involved in the development of cancer. Consideration of the enabling characteristics (i.e. somatic mutation, epigenetic modification and inflammation) is clearly important in order to understand how radiation contributes to carcinogenesis. Thus, the hallmarks framework and the broader understanding of cancer mechanisms in general are of considerable value in directing judgements on the mechanisms associated with carcinogenesis following low-dose and low-dose-rate radiation exposure. In this annex, the Committee considers the recognized mechanisms of carcinogenesis and the observed impacts of low-dose and/or low-dose-rate radiation on these mechanisms, including some consideration of moderate/high-dose effects for context and potential implications for risk extrapolation. This evaluation aims to inform judgements on the pathways relevant to low-dose and low-dose-rate radiation carcinogenesis and further inform judgements on the best justified models for extrapolation of radiation cancer risk for exposures in the 10–100 mGy range.

III. BIOLOGICAL MECHANISMS RELEVANT TO DOSE–RESPONSE RELATIONSHIPS

24. In order to identify papers relevant to the evaluation of biological mechanisms relating to the inference of cancer risks from low-dose and low-dose-rate radiation exposure, literature databases were searched with defined search terms as set out below. Most papers were identified through searching of PubMed, and the papers identified were supplemented by papers identified by the expert group as of relevance during review and discussion of the evaluation. All identified papers, irrespective of source, underwent a standard evaluation as described in appendix A. The search terms employed were not always sufficiently specific to exclude papers using for example UV radiation, such papers were deemed out of scope by the expert group and thus not included among the papers reviewed. Table 1 provides a summary of the information on search terms used for each section of this chapter, the number of papers reviewed, and the number positively evaluated.

25. A wide range of potentially relevant literature is available that utilizes a wide range of experimental approaches, including cells, tissue and animal models. All experimental systems have limitations in their applicability to cancer risk inference in humans. Cell models are unable to fully recapitulate the process of carcinogenesis, but human material can be utilized. While able to follow entire disease development, experimental animals may differ in response by comparison with humans but whole organism responses can be examined. The use of genetically or otherwise modified animal models can provide useful insights into the pathways of disease pathogenesis, but the nature of genetic alterations can differ from those found

in human populations naturally. In terms of cell models, greater weight is placed on non-transformed human cells studies, and particularly those cells that are targets for radiation carcinogenesis (stem and early progenitor cells). As transformed cells carry cancer-related alterations they are not considered to be the best models. Most experimental animal work relates to mice, but a wide range of organisms have been used. More generally there is the issue of the robustness of findings in terms of reproducibility and from a statistical point of view; all experimental systems and assays have limitations of sensitivity and reproducibility, these factors are considered in greater detail in appendix A, "Principles and criteria for ensuring the quality of the Committee's reviews of experimental studies of radiation exposure". The aim here has been to consider evidence from all systems and search for responses that are consistent among a range of models that credibly relate to human cancer.

26. Most papers identified relate to studies of external low-LET radiation exposures. Where available papers concerning internal exposure and high-LET exposures were also considered (see table 1).

Table 1. Summary of literature search terms utilized, number of papers identified and positively evaluated

DSB: double-strand break, ROS: reactive oxygen species

Section	*Search terms utilized*	*Number of papers reviewed*	*Number of papers positively evaluated*[a]
III.A. DNA damage	Radiation, low-dose radiation, DSB, base damage, ROS, complex DNA lesions	25	19
III.B. DNA damage signalling, chromatin remodelling and epigenetics	High- and/or low-dose radiation, DNA damage response, post-translational modification, chromatin remodellers, histone modifiers, DNA methylation, microRNAs, transcription	132	82
III.C. Effects on other signal transduction pathways	Same as "Immune system and cytokine responses"	19	18
III.D. Gene and protein expression	Radiation, ionizing, low dose, NOT therapy, gene expression, protein expression	164	95
III.E. DNA repair and effects on somatic cells	High- and/or low-dose radiation, repair of DSB, repair of complex DNA lesions, repair of base damage, induction of genetic alterations, repair mutants and radiation-induced cancer	112	74
III.F. Genomic instability, bystander effects, damage/effects on non-nuclear cellular components, adaptive response and hyper-radiosensitivity	Genomic instability/ionizing radiation/low dose	183	11
III.G. Stem cells and target cell populations for radiation carcinogenesis	Stem cell/ionizing radiation/low dose	334	35

Section	*Search terms utilized*	*Number of papers reviewed*	*Number of papers positively evaluated*[a]
III.H. Effects at the whole organism level	Immune system and cytokine responses: – radiation, ionizing, irradiation, low, low dose, cancer, immune*[b], inflamm*, excluding therapy, radiotherapy, review; – low-dose radiotherapy.	117	101
III.H.5. Effects on vascularization, cell migration/invasion and epithelial-mesenchymal transformation	Cell migration/invasion AND ionizing radiation AND low dose	92	5
IV. Integration of data at different levels of organization and modelling of cancer mechanisms	Radiation, carcinogenesis, modelling, NOT therapy Radiation, modelling, ionizing, NOT therapy	67	45

[a] A positive evaluation indicates that a paper is considered suitable for consideration in this annex.

[b] The asterisk indicates that the word stem (e.g. Immun-) can be followed by any string of letters.

A. DNA damage

27. The important role of radiation damage to DNA in radiation carcinogenesis was made clear in the UNSCEAR 1993 Report, annex E, "Mechanisms of radiation oncogenesis" [U3] and the UNSCEAR 2000 Report, annex F, "DNA repair and mutagenesis" and annex G, "Biological effects at low radiation doses" [U5]. These reports indicated that radiation acts mainly through the induction of damage to nuclear DNA in somatic cells, and that DNA double-strand breaks (DSBs) and complex lesions are most likely of greatest importance in causing long-lived mutations. Carcinogenesis was described as a multistage process originating from single cells that have sustained mutations to critical growth-regulating genes.

28. The DNA damaging effects of radiation on somatic cells have been investigated over many decades and there is a rich literature available. Many studies, both historically and currently, utilize in vitro cell culture systems where it is common that cells are proliferating, often exponentially. This is unlike the situation for a substantial proportion of somatic cells in the body, where a majority reside in a G_0 state. As discussed in section III.G, it is generally considered that stem and early progenitor cells are the main target cell populations for radiation carcinogenesis. These cell populations can reside in a quiescent G_0 or be actively cycling. The responses of G_0-phase cells to radiation exposures are known to differ in substantial respects from the responses of actively cycling cells, and this has to be borne in mind when translating findings from cell culture systems to the in vivo situation.

29. As noted previously biophysical arguments support the observations that damage to DNA is induced as a simple linear function of dose. The Committee has placed emphasis on the importance of mis-repaired and un-repaired DNA double-strand breaks and complex damage sites in determining the cellular effects of radiation exposure. Double-strand breaks are relatively rare (around 40 DSBs per cell per Gy low-LET), at doses of a few mGy and below and so DSBs will be present in only a fraction of irradiated cells. The use of immunostaining and fluorescence tagging of DNA damage response-related chromosomal proteins (see section III.B), γH2AX and 53BP1 [R18] in particular, have brought some

new information on the quantitative aspects of DSB formation and their repair following radiation exposure. These experimental approaches use knowledge of DNA damage signalling, i.e. the early and rapid assembly of protein foci at sites of DNA double-strand breaks, rather than direct physical assessment of strand breakage to estimate frequencies of DSBs at low dose.

30. Neumaier et al. [N12] assessed 53BP1 focus formation (termed radiation-induced foci (RIF)) using live cell imaging of immortalized mammary epithelial cells transiently transfected with fluorescently tagged *53BP1* (*53BP1-GFP*). They observed a nonlinear dose response in human cells (0.1–2 Gy X-rays). A greater number of smaller RIFs than expected (35 RIF/Gy) were formed per unit dose following low-dose X-ray exposure (100 mGy) by comparison with high-dose exposure, with time the small foci were observed to aggregate into larger foci. This phenomenon appears to be cell type dependent, i.e. normal human diploid fibroblasts displayed the expected RIF frequency (30 RIF/Gy). Similar observations of supra-linear induction of foci have been made using immune-fluorescence methods [B26]. These observations may reflect the aggregation of DNA breaks into "repair complexes" following higher dose exposures. Besides cell type specificity, there remain some technical issues that might be relevant to these findings and their interpretation [B21]; therefore, the findings of higher focus numbers at low by comparison with high doses require further substantiation.

31. With conventional staining for γH2AX in confluent human fibroblasts exposed to X-rays, a linear relationship has been reported between the number of foci per cell and the ionizing radiation dose ranging from 1.2 mGy to 2 Gy [R17]. Also in vivo (human lymphocytes, mouse retinae) linear relationships for γH2AX foci formation within the dose range 3–30 mGy have been observed [L39, M34, R20]. In contrast, evidence for low-dose supra-linear induction of foci (<10 mGy) after in vivo (paediatric patients with congenital heart disease) and in vitro X-ray radiation of human lymphocytes has been reported [B26]. The earlier step of ataxia-telangiectasia-mutated (ATM) protein activation (phospho-ATM) precedes 53BP1 focus formation. Induction of phospho-ATM foci has a linear dose–response relationship with doses ranging for 10 mGy to 1 Gy, with an average 50 foci per unit dose (Gy); furthermore, the size of foci was independent of dose [S70]. These findings are further considered in Mullenders et al. [M45].

32. In order to study the DNA damage induction from diagnostic procedures such as computed tomography (CT), angiography etc., blood samples from 45 patients who have been subjected to CT or angiography and 15 controls were stained for γH2AX [K23]. The exposed cohort was further subgrouped into patients undergoing one CT scan, multiple scans and angiography groups. For the exposed group, blood was collected 1 hour after the diagnostic exposure. γH2AX staining was carried out and 10,000 cells per sample were analysed for γH2AX positivity using a flow-cytometer. Increased numbers of γH2AX positive cells were identified in exposed samples compared to control samples. Multiple CT exposures produced the highest increase in γH2AX positive cells. It was concluded that multiple CT scans produce more DSBs compared to the single scans as observed in the form of γH2AX positive cells in blood cells. Yoon et al. [Y11] measured two established radiation biomarkers, γH2AX and pCHK2 (activated checkpoint kinase 2), in individuals exposed to X-rays during dental examinations. Exfoliated oral epithelial cells were collected from 100 healthy individuals undergoing routine dental radiographic examination (23.4 mGy) before and after the radiographic examinations. Both biomarkers displayed statistically significant up-regulated expression after exposure.

33. The spectrum of damage to DNA caused by ionizing radiation exposure, particularly high-LET radiation, also includes complex lesions/clustered damage sites (two or more oxidized bases, abasic sites or strand breaks on opposing DNA strands within a few helical turns). These complex lesions/clustered damages are more difficult to repair than isolated lesions and are thought to trigger carcinogenic events via formation of complex and persistent genomic alterations. The formation and repair of clustered damage and the impact of type of radiation have been assessed in modelling studies, in vitro studies employing plasmid templates with engineered damage clusters, and rarely in in vivo studies with

mammalian cells. A study with tumour and immortalized epithelial human cells [A22] compared the formation of clustered damage at single cell level by low- and high-LET irradiations (X-rays, Si ions and Fe ions) and H_2O_2 (control). Complex lesions/clustered damage sites were identified in single-cells by 3D-analysis of three types of repair foci: DSBs, single-strand breaks and base damage using specific molecular probes for DSBs (53BP1), ssDNA (XRCC1) and 8-oxoGuanine (OGG1), respectively. The spatial distribution of these repair foci tagged by different colours, was monitored in the same cell and colocalization of three colours was scored as clustered damage. The result indicated colocalization 30 minutes after irradiation (1 Gy) by Si ions (40%), Fe ions (75%), X-rays (4%) but not after H_2O_2 (0%). These results are supported by unified stochastic modelling based on radiation tracks, that predicts both the induction, spatial distribution and complexity of DNA damage. This modelling approach is coupled to experimental data (γH2AX foci) from 2D fluorescence microscopy of human cells exposed to X-rays and neutron beams (broad-energy neutron beam with an average energy of 2.4 MeV) [B17]. In the dose range of 1–5 Gy, DSB multiplicity (average number of DSBs/focus) is found to be 1.20 and 11.09 for X-rays and neutrons, respectively.

34. Ionizing radiation induces ROS, i.e. highly reactive metabolites of oxygen, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), Their lifetime in biological systems ranges from nanoseconds to seconds depending on their reactivity [D17]. ROS detection therefore requires probes that very rapidly react with ROS and produce stable quantifiable products such as chemiluminescent and fluorescent probes.

35. The ROS production as function of the X-ray dose was examined in human endothelial cells. Fluorescent measurements of ROS induced by doses of 0.03, 0.1 and 0.5 Gy and profiled three hours after irradiation, showed a linear dose response [K37]. Analysis of ROS (using 20,70-dichlorofluorescin diacetate (DCFDA)) in chronically low dose X-rays (0.010 or 0.05 Gy/d for 31 days) treated immortalized human fibroblasts revealed persistent ROS production when measured 24 hours after the last radiation. Surprisingly, radiosensitive *ATM* and *NBS* (Nijmegen breakage syndrome gene) deficient cells lacked induction of ROS [S36]. In view of the short lifespan of ROS, these data reflect radiation induced perturbations in cellular redox homeostasis rather than induced ROS per se.

36. The induction of various types of base damage (DNA single-strand breaks, apurinic/apyrimidinic sites, alkali labile sites) was assessed by the alkaline comet assay in human peripheral blood mononuclear cells obtained from 20 individuals exposed to gamma radiation and processed immediately. These data reveal a linear dose response (100 mGy–2 Gy) [T20]. Alkaline comet assay measurements on whole blood exposed to X-rays (29 kV, 220 kV) and gamma irradiation (^{137}Cs, ^{60}Co) (0.5–3.0 Gy) also reveal for all types of radiation a linear dose–response relationship with the same slope when analysed for "% DNA in the tail" or "tail moment" [R15]. Of note, the induction frequency of DNA base modifications per unit dose (Gy) of X-rays is close to the steady-state level of oxidized bases in fibroblasts and lymphocytes cultured under common (high) oxygen conditions, i.e. approximately one modification per 10^7 normal bases measured in various studies using different methodologies, including HPLC-EC, comet assay, enzymatic assays and DNA repair labelling [C29, P16]. On a population level, basal amounts of DNA damage (DNA strand breaks, alkali-labile sites and oxidative DNA damage (Endo III and hOGG1 sensitive sites)) were determined using comet assay in peripheral blood mononuclear cells of 67 healthy adult male individuals from normal and high-level natural background radiation areas (HBRAs) of the Kerala coast [K47]. The study included 45 individuals from HBRA (>1.50 mGy/y) and 22 individuals from a normal level natural background radiation areas (≤1.50 mGy/y). Overall frequencies of DNA strand breaks and oxidative damage observed in HBRA individuals were not significantly different from normal level natural background radiation area individuals. In the same cohort, a lack of increase of DNA double-strand break in the HBRA individuals when compared to normal level natural background radiation area individuals was observed as well [J9]. In contrast, after in vitro irradiation (2 or 4 Gy) of peripheral blood mononuclear cells initial levels of DNA strand breaks were lower in subjects from HBRAs compared to subjects of normal level natural background radiation areas; an opposite effect was

observed when DSBs were measured by premature chromosome condensation [K48, K49]. A preliminary study of γH2AX foci in blood of residents living in the HBRA of Mamuju, West Sulawesi, showed a trend towards higher (albeit not significant) average values relative to the control area [B22].

37. In summary, ionizing radiation induced DNA damage has been quantified in mammalian cells exposed to low and moderate doses, exploiting various methodologies, i.e. direct measurement of DNA breaks by single cell electrophoresis and by indirect measurements using immunostaining and fluorescence tagging of DNA damage response-related proteins. In almost all studies the induction of different types of DNA lesions are in accordance with linear dose response. Experimental data on dose dependence of clustered damage induction are sparse; the data available from modelling and experimental studies suggest a low induction of clustered damage by photon radiation (X-rays) compared to high-LET alpha particles.

B. DNA damage signalling, chromatin remodelling and epigenetics

38. The UNSCEAR 2000 Report, annex F, "DNA repair and mutagenesis" [U5] recognized that DNA damage, particularly DSBs, was responsible for initiating a signalling process that resulted in repair or other responses to the damage. Ionizing radiation activates base excision repair, single-strand break repair and DSB repair as major responses to the types of DNA damage that it induces. Only DSB repair has a separation of damage recognition, signalling and repair. It was recognized that these signalling pathways could impact on individual sensitivity and response to radiation exposure. The potential importance of epigenetic effects in carcinogenesis was acknowledged in the UNSCEAR 2000 Report, annex G, "Biological effects at low radiation doses" [U5] but the rudimentary understanding of this topic precluded any substantive conclusions being drawn.

1. DNA damage signalling

39. Cellular surveillance mechanisms are essential to maintain genome integrity as DNA in cells is continually being damaged in many ways. To counteract threats posed by DNA damage, the cell has evolved a capability to detect DNA damage as well as aberrant DNA structures generated by DNA damage (chromatin perturbations, stalled transcription and replication) and to elicit cellular responses (i.e. the DNA damage response. The DNA damage response is initiated by a cellular signalling cascade to promote DNA damage removal through activation of DNA repair, alteration of chromatin, pausing of cell-cycle progression (checkpoint activation), modulation of transcription and replication, and induction of apoptosis. Cells defective in these protective mechanisms often manifest enhanced sensitivity towards DNA-damaging agents including ionizing radiation, and many such defects cause human disease [J1].

40. Experiments dealing with the biological effects of deficiencies in DNA damage response factors on signalling, repair, cell cycle, genetic alterations etc. in low-dose irradiated cells are limited. On the contrary, mechanistic studies often exploit cell-based assays that utilize single-copy fluorescent reporters to measure repair of an enzymatically-induced single DSB. These assays have been applied to assess the impact of DNA damage response deficiencies on repair of a single DSB by non-homologous end joining (NHEJ) and homologous recombination repair (HRR) [P18]. These assays measure a cellular response to low-level DNA damage. While enzymatically-generated breaks are not structurally identical to radiation-induced DSBs, they represent a somewhat simplified model; nonetheless the simpler enzymatic

induced breaks, even when only one is present, do trigger a standard DNA damage response as demonstrated by foci formation [D19].

41. The main molecular mechanism governing DNA damage response activation consists of post-translational modification of specific proteins including phosphorylation, ubiquitylation and acetylation. One of the most frequent modifications is the reversible and dynamic phosphorylation of proteins at specific serine, threonine and tyrosine residues [O16] by protein kinases catalysing the transfer of phosphate to their substrates. The key kinases that are considered to orchestrate DNA double-strand break damage signalling, are ATM kinase, ATR (ataxia telangiectasia and Rad3-related) kinase and DNA-PKcs [B36]; each kinase is activated by a distinct DNA structure. Recruitment to DNA damage requires a specific co-factor for each kinase: NBS1 for ATM, ATRIP (ATR-interacting protein) for ATR and Ku80 for DNA-PKcs. The DSB is considered to be the main DNA lesion that activates ATM. ATR is activated by single-stranded DNA at ssDNA/dsDNA junctions and cells defective in ATR signalling are sensitive to ultraviolet radiation and mitomycin C, but not to low-LET ionizing radiation [O2]. However, sustained resection at sites of DNA breaks due to complex types of damage by high-LET radiation might lead to a switch from ATM to ATR signalling [S2]. DNA-PKcs is recruited to DSBs by interaction with Ku80 and activated by autophosphorylation thereby promoting repair by NHEJ. DNA-PKcs kinase activity is important for repair of DSBs by NHEJ; moreover, DNA-PKcs mutants are sensitive to low-dose ionizing radiation [L31]. It is important to note that processes other than DNA damage response can be regulated by post-translational modification following low-dose radiation. For instance, radiation differentially regulates protein acetylation in human coronary artery endothelial cells. Notably, ionizing radiation might alter the acetylation status of proteins belonging to several pathways including protein synthesis, cytoskeleton-related processes, protein folding and calcium signalling, by differentially regulating histone acetylase/deacetylase activities in 0.5 Gy irradiated human endothelial cells [B20].

42. Mutations identified in ataxia teleangiectasia patients occur throughout the *ATM* gene with no "hot spots" and generally lead to protein instability. Autophosphorylation of ATM at multiple sites (the main site S1981, and additional sites such as pS367 and pS1893) following ionizing radiation exposure [K42] activates ATM and is functionally important because mutant forms of ATM refractory to phosphorylation are defective in correcting radiosensitivity of ataxia-telangiectasia cells [K41, K42]. Activation of ATM after DSB induction orchestrates the DNA damage response by initiating phosphorylation of multiple downstream targets involving DNA repair factors (such as H2AX, 53BP1), checkpoint kinases, chromatin remodellers and transcription factors [S32]. Phosphorylation of ATM is detectable as discrete foci over a 10 mGy to 1 Gy dose range and appears to be linear with dose. Moreover, ATM foci displayed a similar average size at 20 mGy and 1 Gy [S70] indicating a sensitive and effective induction of DNA damage response at doses as low as 20 mGy of ionizing radiation. Also, phosphorylation of ATM's direct downstream target, H2AX, is linear with dose [L39].

43. Ubiquitin modifications are another type of signalling post-translational modifications within the DNA damage response that regulate the repair of DSBs (and other types of DNA damage) within chromatin [S18, S49]. Formation of γH2AX by ATM initiates binding of the MDC1 protein that thereafter recruits a number of factors to DSBs including ubiquitin ligases (E3 ubiquitin ligases RNF8, RNF168 and others). These ligases generate specific ubiquitin marks on histones near the breaks, providing a platform for recruitment of DSB repair factors. The signalling cascade thus switches from phosphorylation to ubiquitylation events. Defects in ubiquitylation may underlie some human diseases associated with radiosensitivity. For example, mutation in ubiquitin ligase *RNF168* leads to RIDDLE syndrome, a disorder associated with radiosensitivity (1 Gy) and immunodeficiency [P19, S64]; the underlying cause for the disease phenotype might be a deficiency of DSB repair by homologous recombination [L50].

44. The ATM and ATR kinases play key roles in cell-cycle regulation after DNA damage. Cell-cycle checkpoints allow the integrity of genetic material to be monitored before progression through key stages such as replication and mitosis. Checkpoints at G_1/S and G_2/M as well as intra-S-phase stage have been described. Checkpoints are initially activated by phosphorylation of ATM and its downstream checkpoint kinase Chk2 (phosphorylation of Chk2 is linear in the dose range 0.2–2 Gy) and ATR and its downstream checkpoint kinase Chk1 (phosphorylation of Chk1 is linear in the dose range 0.2–2 Gy) in mammalian cells following ionizing radiation exposure [B36, S41]. In addition, experiments with X-irradiated *Drosophila* flies revealed a radiation sensitive spindle checkpoint [R19]. This checkpoint only affects entry into anaphase at high doses (3–6 Gy X-rays). In contrast, low doses of X-irradiation only activate the DNA damage Chk1 checkpoint at metaphase. In spite of sensitive Chk1/Chk2 activation, radiation doses less than 200 mGy fail to activate the G_2/M checkpoint, due to a G_2/M checkpoint activation threshold of 10–20 DSBs per cell [D12, K43].

45. In clonal cell survival assays, mammalian cells often exhibit hyper-radiosensitivity (relatively high cell killing) to low-dose (<100–200 mGy low-LET radiation) radiation followed by increased radioresistance (induced radioresistance, relatively low cell killing) at higher doses (see also section III.F.6). The low dose hypersensitivity described in several cell types following exposure to low doses [K44, M16] is likely attributable at least in part to the lack of G_2/M checkpoint induction. ATM (and ATM activation) correlates with the development of increased radioresistance (the level of cell killing at higher dose), but not to the higher levels of cell killing at low dose, i.e. low dose hyper-radiosensitivity [E16]. ATR function is required for the IR-induced G_2/M checkpoint activation and cooperates with ATM in the mechanism of low dose hyper-radiosensitivity induced by C ion beam [X2]. On the basis of information gathered from studies using short-term endpoints, doses of less than 200 mGy may be predicted to be more effective in terms of carcinogenic potential than higher doses where G_2/M checkpoints are effectively induced [E16]. The low dose hyper-radiosensitivity is associated with induction of apoptosis when cells in G_2 are irradiated [M17]. Thus, low-dose radiation damaged cells may survive and progress through aG_2/M-phase transition with a high frequency of chromosome misalignments and anaphase bridges, subsequently enter apoptosis and so are eliminated from the pool of damaged and therefore potentially pre-cancerous cells. Low-dose irradiation of non-transformed cells (as low as 2 mGy gamma rays and 0.29 mGy alpha particles) can increase the apoptosis of transformed pre-cancer cells through an intercellular process; however, this process also saturates at very low doses (50 mGy for gamma rays and 25 mGy for alpha particles) [B23, P22]. The proposed mechanism is, that non-transformed cells selectively remove transformed cells from co-culture via cytokine and reactive oxygen/nitrogen species (ROS/RNS) signalling. However, it is obvious, that low doses of ionizing radiation (10–100 mGy) can induce apoptosis in normal (stem) cells as shown for mouse spermatogonia [G11]. The relevance of apoptosis induced in either damaged normal or pre-cancer cells for cancer is not entirely clear. The killing of pre-cancer cells would be expected to reduce cancer risk. Likewise, the killing of cells with directly induced damage and/or mutations would be expected to reduce risk. In both cases, cell killing would lead to stimulation of proliferation with risk of induced genomic instability. In addition, low dose-damaged cells might undergo chromothripsis-like rearrangements in the subsequent cell cycle by incorrect reassembly of DNA fragments through erroneous DNA repair (see section III.E) thereby increasing the low-dose cancer risk.

46. Mouse experiments revealed the importance of ATM and DNAPKcs for X-ray induced DNA damage signalling in whole organisms. Homozygous $SCID^{-/-}$, $ATM^{-/-}$ knockout and $ATM^{+/-}$ heterozygotes were compared to C57BL/6 wild-type mice in a publication from Flockerzi et al. [F15]. Animals were given 100 mGy X-rays daily for 2–10 weeks locally to the lungs at dose rate of 2 Gy/min once or five days a week for 2, 4, 6, 8 or 10 weeks. At the conclusion of irradiation, animals were sacrificed at 0.5, 24, or 48 hours post-irradiation. Bronchiolar and alveolar cells were screened for γH2AX foci as a sign of DNA damage. In repair-proficient C57BL/6 mice that received a single dose of

100 mGy or doses in the range of 1–4 Gy (over 2–8 weeks) the numbers of foci decreased with time up to 72 hours after termination of irradiation. In mice that received a dose of 5 Gy (10 weeks exposure) differences in numbers of foci at 24 and 72 hours after termination of exposure were not significant. In *ATM* knockout mice, numbers of foci increased with dose and passage of time (up to 72 hours after termination of irradiation) did not modulate that. In SCID mice, some decrease in the number of foci was seen when 0.5 hours post-irradiation was compared to longer timepoints. Finally, in *ATM* heterozygotes, differences in the number of foci were notable for the post-irradiation time of 0.5 hours compared with longer timepoints, but the differences between post-irradiation times of 24 and 72 hours were negligible. Overall, the numbers of foci in SCID mice were comparable with those in C57BL/6 mice. Hence, the genetic background relating to DNA repair determined the extent of cumulative low-dose radiation damage. Moreover, increased DNA damage during fractionated exposures affected DNA replication and apoptosis in the lung parenchyma, which may influence overall lung function.

47. A similar study from the same group explored persistence of DNA damage foci in cells of irradiated animals [L44]. Mouse strains used in this work were *ATM* wild-type mice C57BL/6, *ATM* heterozygotes, *ATM* null and SCID mice. An X-ray irradiator was used to deliver doses of 100 or 6,000 mGy at dose rate of 2,000 mGy/min. Animals were sacrificed at 0.1, 0.5, 5, 24 or 72 hours after exposure to 6,000 mGy or 0.5, 24 or 72 hours after exposure to a single dose of 100 mGy. In addition, fractionated exposures were carried out, where animals received 100 mGy per day, five days a week for 2, 4 or 8 weeks (10, 20 or 40 fractions accumulating to total doses of 1,000, 2,000 or 4,000 mGy). DNA damage foci stained for 53BP1 were imaged in nuclei of cortical neurons – in all exposure scenarios SCID mice had the most foci, and of all timepoints, 0.5 hours after exposure had the most foci in all strains of mice. In mice exposed to multiple fractions, *ATM* knockouts had the least foci after a single fraction dose of 100 mGy and early post-irradiation timepoints but after multiple fractions cortical neurons of these mice had many more foci than wild-type or *ATM* heterozygotes. Median diameter of foci was not the same in all mice strains – *ATM* knockout mice had the largest foci. Finally, this study also included transmission electron microscopy of cells with developed foci; antibodies against pKu70 repair protein were labelled with gold and scored. Once again, the morphology of DNA damage was different in cells from different genotypes. In their subsequent work, this laboratory perfected these methods and expanded studies to skin and intestine [S12]. In this work C57BL/6 (C57BL/6NCrl) mice received whole-body irradiation with 10 or 100 mGy 6 MV photons (linear accelerator (LINAC) X-rays) at dose rate: 2 Gy/min. Single exposure to 100 mGy dose caused DNA damage that lasted 24 hours in keratinocytes, but only 30 minutes in enterocytes. Moreover, enterocytes showed 72 hours lasting foci only when 50 fractions of 100 mGy were applied, while the same was true for keratinocytes exposed to ten 100 mGy fractions.

48. To summarize, cellular surveillance mechanisms (DNA damage response) are initiated by a cellular signalling cascade to promote DNA damage removal, alteration of chromatin, checkpoint activation, modulation of transcription and replication, and induction of apoptosis. Cells defective in these protective mechanisms are often sensitized towards ionizing radiation, most notably in dose ranges above 0.5 Gy; in humans, these defects may underlie disease including cancer. The main molecular mechanism governing DNA damage response activation consists of post-translational modification of specific proteins including phosphorylation, ubiquitylation and acetylation. One of the most frequent modifications is the reversible and dynamic phosphorylation of proteins at specific serine, threonine and tyrosine residues by protein kinases catalysing the transfer of phosphate to their substrates and by phosphatases removing phosphate. The principal kinases involved in DNA damage response are ATM, ATR and DNA-PKcs. Mutations in enzymes involved in post-translational modification of specific proteins underlie human diseases associated with radiosensitivity (i.e. ataxia telangiectasia ATM, Seckel Syndrome ATR, SCID DNA-PKcs, Riddle syndrome ubiquitin ligase RNF168) and often confer radiosensitivity in mouse models carrying these specific mutations or gene deletions.

2. Chromatin remodelling

49. All DNA metabolizing processes in living cells including repair of DNA damage, are complicated by the packaging of genomic DNA into chromatin. In addition to DNA damage signalling, efficient repair of DSBs in the chromatin context of the cell requires post-translational histone modifications and ATP-dependent chromatin remodelling to allow access to the repair machinery [J14]. Numerous covalent modifications of core histones in chromatin are linked to DNA repair including phosphorylation, ubiquitylation, acetylation and methylation of several different histone tails. The archetypical example of γH2AX is a prerequisite for the binding of MDC1 and the subsequent accumulation of DNA damage response factors at DSBs to promote NHEJ and HRR. Moreover, cells lacking H2AX phosphorylation capacity manifest a G_2/M checkpoint defect after doses of ionizing radiation <1 Gy [F11]. Chromatin flanking DSBs is modified by histone acetyltransferases which acetylate histones H3 and H4. Histone acetyltransferases bind to γH2AX leading to further acetylation, while recruiting acetyl-dependent chromatin remodellers. This creates a chromatin micro-environment that is rich in γH2AX and acetylated H3 and that promotes DSB repair by HRR. Besides HRR, histone acetylation also significantly stimulates NHEJ [L48]. Low/moderate-dose radiation (doses of less than 1 Gy in this context) differentially regulates protein acetylation in human cells as described in section III.B.1. In addition, human primary cells exposed to chronic (7 days) ^{137}Cs gamma radiation delivered at 6–20 mGy/h revealed dose-dependent reduction in cell proliferation and increased cellular senescence and concomitantly up to 40% reduction of core histone protein levels due to reduced histone gene expression. In contrast and consistent with senescence induction, chronic gamma radiation increased the expression of the senescence related histone variant H2AJ [L45].

50. Enzymes involved in the above-mentioned core histone modifications are often involved in radiosensitivity and cancer. For example, it is becoming clear that acetylation, deacetylation, methylation and demethylation of histone tails are all tightly controlled at sites of DSBs by histone acetyltransferases, histone deacetylases, methyltransferases and demethylases [L48]. The spermidine/spermine-N1-acetyltransferase 1 (SAT1) promotes acetylation of histone H3, regulates HRR and is overexpressed in radioresistant glioblastoma tumours. SAT1 depletion leads to reduction in BRCA1 expression, explaining decreased HRR capacity and radiosensitization [B56]. Histone acetylase Tip60 binds to chromatin surrounding DSBs and its depletion impairs both DNA damage-induced histone H4 hyperacetylation and accumulation of repair molecules at sites of DSBs resulting in defective HRR and radiosensitization. Activation of the Tip60 acetyltransferase by DSB, however requires interaction of Tip60 with methylated histone H3 (H3K9me3); the latter modification is generated by the methyltransferase Suv39h1. Cells lacking Suv39h1 display defective activation of Tip60 and ATM, decreased HRR and increased radiosensitivity [A28]. This illustrates the intricate process required to repair DSBs in the context of chromatin. Importantly, the impaired HRR (and NHEJ) of DSBs as a consequence of defects in chromatin modifications are amenable to study at the level of a single DSB in model systems. Tables 2 and 3 summarize the various enzymes involved in chromatin modification and DSB repair.

51. Along with ATM-dependent signalling that promotes post-translational modifications of chromatin in the vicinity of DSBs, ATP-dependent chromatin remodelling is essential to overcome the chromatin barrier and facilitate access of proteins and enzymes to damaged DNA. Emerging evidence shows that ATP-dependent chromatin remodellers are not only involved in DSB repair, but also key in preventing cancer and other human health disorders [O12]. Chromatin remodelling enzymes are large protein complexes that use ATP hydrolysis to alter the nature of the interaction between DNA and histones by virtue of nucleosome sliding, nucleosome disruption, histone eviction or histone exchange. They are classified for ease into 4 families: SWI/SNF (BAF in mammalian cells), ISWI, CHD and INO80 (table 3). All modellers except for INO80, bind to γH2AX and modified histones at the vicinity of DSBs [R16].

Table 2. Histone alterations and enzymatic activities known to be modulated after ionizing radiation exposure (adapted from [L48])

DSB: double-strand break, HRR: homologous recombination repair, NHEJ: non-homologous end joining

Histone modification	*Enzyme*	*DSB repair pathway*	*Reference*
H2AX phosphorylation (γH2AX, S139P)	ATM, ATR, DNA-Pkcs	Checkpoint activation, stimulation of HRR and NHEJ	[B36, F11]
H2AX acetylation (K5ac)	Tip60 P300/CBP	Required for chromatin ubiquitylation and H2AX release Promotion of ionizing radiation survival independently of γH2AX	[I5, J17]
H2A ubiquitylation K13,15ub	RNF8 (mono/di-ub), RNF168 (poly-ub)	Accumulation of BRCA1 and 53BP1 after ionizing radiation, checkpoint activation and repair of DSBs in heterochromatin	[S49]
H2B ubiquitylation K120ub	RNF20 and RNF40	Accumulation of Ku, BRCA1 and RAD51; repair by NHEJ and HRR	[N7]
H3 phosphorylation S10P	CHK1		[S33]
H3 acetylation K56ac	GCN5, SAT1	Recruitment of SWI/SNF complex, promotion of HRR	[D2, M22]
H3 methylation K4me and K9me	Suv39H1/Suv39H2 SET1	Stimulation of Tip60 activation and recruitment of HP1 proteins to DSBs to promote repair	[D18]
H4 acetylation K16ac	MOF1 (reversed by HDAC1/2)	Regulation of HRR and NHEJ by acetylation/deacetylation	[L25]
H4 ubiquitylation K91ub	BBAP	53BP1 recruitment to DSBs	[C10]
H4 methylation K20me2	Suv4-20H1/Suv4-20H2	Recruitment of 53BP1. The 53BP1-H4me-interaction suppresses HRR, but promotes NHEJ	[P10]

52. In mammalian cells, BAF family members are also known as SMARCA. Among these members, SMARCA2 and SMARCA4 have been implicated in DSB repair by facilitating NHEJ and likely HRR by facilitating acetylation of histones and spreading of γH2AX around the DSB. Somatic mutations in SMARCA2 and SMARCA4 predispose to cancer and depletion of BAF subunits results in radiosensitivity in a dose range 0.5–4 Gy [W7]. Also, SMARCA5 affects DSB repair by HRR and NHEJ by a joined action with histone deacetylase SIRT6. Depletion of SIRT prevents the local deacetylation of chromatin and inhibits recruitment of SMARCA5 to DSBs and sensitizes mouse embryonic stem cells to X-rays (1–8 Gy) [T15]. CHD remodellers have common domains that allow the binding to methylated histones and DNA and five members have been classified as being involved in the DSB response [R16]. Among these, CHD1-like or ALC1 (amplified in liver cancer) and CHD2 are recruited to DSBs in a PARP1-dependent fashion, ALC1 interacts with the NHEJ core proteins Ku70 and DNA-PKcs, and cells depleted of ALC1 show distinct sensitivity to ionizing radiation (1–2 Gy) [S22]. In contrast, CHD2 involves a different mechanism namely insertion of histone variant H3.3 in DSB flanking chromatin thereby regulating the assembly of NHEJ core proteins to promote repair. The remodeller CHD3 has been shown to act on DSBs in heterochromatin by binding to SUMOlyated KAP-1 (KRAB (Krüppel associated box)-associated protein-1, which keeps the chromatin compact), thereby facilitating the release of KAP-1. Subsequent release of CHD3 enables chromatin relaxation and allows the recruitment of SMARCA5. These findings showed that a two-step release and recruitment system modulates opposing

chromatin remodelling activities during DSB repair in heterochromatin. Finally, CHD4 has been shown to affect both DNA damage signalling and DSB repair. The signalling of DSBs involves the ubiquitin ligase RNF8-dependent recruitment of CHD4 to DSBs which promotes further decondensation of chromatin to facilitate ubiquitylation of chromatin and subsequent recruitment of BRCA1. The repair of DSBs by HRR is largely dependent on CHD4 and CHD4-depleted cells are sensitive for DSB inducing agents [P1].

53. The exposures of cells depleted of chromatin remodellers or of histone modifiers, have been carried out only at doses of ionizing radiation greater than 0.5 Gy, and hence, no information is available for the effects at low doses. However, in almost all studies survival experiments were complemented with measurement of DSB repair in cellular systems where a single DSB per cell can be induced by enzymatic cutting of the DNA and fluorescent single-cell visualization of repair. The general conclusion is that loss of functional chromatin remodelling and modification generally impairs the repair of DSBs by either HRR or NHEJ or both and confers radiosensitivity of cells to high doses of ionizing radiation. Recent work has explored an alternative NHEJ pathway that involves polymerase theta and requires microhomology repeats as being important when classical NHEJ or HRR are compromised. Tables 2 and 3 provide a summary of information on the functions of chromatin modifiers and chromatin remodellers, respectively. In addition to post-translational modification and chromatin remodelling, chronic low-dose-rate exposures (6–20 mGy/h ^{137}Cs gamma irradiation over 7 days, total doses 1.008–3.36 Gy) of human primary fibroblasts have been observed to deplete histone proteins by up to 40% and this reduction was correlated with histone gene expression changes. Furthermore, cells displayed enlarged nuclear size with increased global transcription including that of pro-inflammatory genes [L45].

Table 3. Chromatin remodellers involved in DSB repair (adapted from [L48])

DSB: double-strand break, HRR: homologous recombination repair, NHEJ: non-homologous end joining

Remodeller	*DSB repair pathway*	*Function*	*Reference*
SWI/SNF (PBAF or BAF)			
SMARCA2 and SMARCA4	HRR and NHEJ	H3 acetylation, Rad51 loading, Ku loading	[L12, P11]
SMARCA5	HRR and NHEJ	RPA and Rad51 loading, BRCA1 loading, Ku loading	[N7]
ISWI			
ACF/CHRAC	HRR, NHEJ	Ku loading	[L5]
RSF1	HRR	RPA and Rad51 loading	[P15]
CHD			
CHD1	HRR	CtIP loading	[K14]
ALC1	NHEJ	Association with Ku and DNA-PKcs	[A7]
CHD2	NHEJ	Chromatin expansion, H3.3 deposition, Ligase 4 loading	[L49]
CHD3	NHEJ	Repair of DSBs in heterochromatin	[K9]
CHD4	HRR, possible NHEJ	RNF8 response (signalling), BRCA1 loading, RPA and Rad51 loading	[L6]
INO80	HRR and NHEJ	Histone H2A.Z, end-resection, Histone H3 removal	[V1]
SMARCAD1	HRR	End-resection	[C27]
p400/Tip60	HRR and NHEJ	Prevents resection by H2A.Z deposition at DSBs	[C28]

54. To summarize, multiple genes have been recently identified that remodel chromatin to facilitate repair of ionizing radiation induced DSB. Chromatin remodelling is achieved by post-translational modification of histones or eviction of histones. ATP-dependent chromatin remodelling is essential to overcome the chromatin barrier and facilitate access of proteins and enzymes to damaged DNA. The exposure of cells depleted of chromatin remodellers (or of histone modifiers) to ionizing radiation confers radiosensitivity, but these experiments have been carried out only at doses of greater than 0.5 Gy. However, DSB repair has been measured in cellular systems in which a single DSB per cell can be induced by enzymatic cutting of the DNA. These experiments reveal that loss of functional chromatin remodelling and modification generally impairs the repair of a single DSB by either HRR or NHEJ or both. Somatic mutations in chromatin remodelling genes (germline mutations and copy number alterations) have been shown to predispose to cancer and radiosensitivity (at moderate/high dose).

3. Epigenetic mechanisms (radiation-induced methylation and others)

55. The concept of epigenetics implies that non-genetic events could generate stable phenotypic differences [S77]. Epigenetic changes include DNA methylation, miRNA expression and chromatin (histone) modifications and are known to play an important role in regulation of cellular processes, most notably gene expression and genomic instability. Epigenetic aberrations can lead to human disease including cancer [J23]. The level of DNA methylation is regulated by DNA methyltransferases (DNMTs) a family of enzymes that catalyse the transfer of a methyl group to DNA. Three active DNA methyltransferases have been identified in mammals named DNMT1, DNMT3A and DNMT3B. DNMT1 is the most abundant DNA methyltransferase in mammalian cells and the key maintenance methyltransferase in mammals. It predominantly methylates hemimethylated CpG di-nucleotides in the mammalian genome.

56. To make firm conclusions on the impact of DNA methylation on phenotype, changes in gene methylation status need to be presented in the context of gene expression patterns. As a paradigm, arsenic, a non-mutagenic human carcinogen, induces tumours through epigenetic changes, particularly in DNA methylation [B45, R4]. Comparison of genome-wide changes in DNA methylation and gene expression revealed several key aspects related to arsenic toxicity and carcinogenicity namely that *(a)* the overall changes in methylation are complex depending on the baseline methylation level and the gene position and *(b)* little overall correlation between arsenic related changes in methylation and gene expression by microarray measurements. Despite the low correlation, a subset of genes that play a role in arsenic-related cancer, showed a relationship between methylation and expression.

57. Since ionizing radiation is known to alter gene expression patterns and to induce several DNA damage response genes, it may alter transcription-associated chromatin marks, including histone modifications, but also DNA methylation status. Indeed, a single DSB can induce DNA methylation in the vicinity of the break. This result was achieved in a model system exploiting breast cancer cells with an exogenous promotor CpG island and expression of a nuclease to induce a defined DSB [O3]. So far, studies on the effects of ionizing radiation on DNA methylation have yielded conflicting results. A recent review summarized the current knowledge of the effects of ionizing radiation on DNA methylation, and is cautious on the implications of the currently available data [M33].

58. Using breast cancer cells irradiated with 2 and 6 Gy of X-rays at 0.86 Gy/min, Antwih et al. [A14] analysed DNA methylation changes over a period of 72 hours after radiation treatment using high density Illumina 450K DNA methylation arrays. Significantly differentially methylated genes related to radiation response pathways, were found without a clear dose or time response. Irradiation of colorectal cancer cells (2 and 5 Gy) and application of the same 450K arrays revealed global DNA methylation changes

and reduced methylation at specific gene loci [B1]. To investigate the relation between radiation-induced methylation, radiosensitivity and genomic instability, mouse embryonic stem cells have been studied containing different levels of methylation due to the presence and absence of DNA methyltransferases [A19]. Functional absence of DNMT1 as well as both DNMT3A and DNMT3B led to a decrease in genomic methylation relative to wild-type mouse embryonic stem cells. None of the mouse embryonic stem cells displayed significant differences in cytosine methylation measured up to 10 days post-irradiation with 3 Gy of X-rays and global methylation levels did not determine radiosensitivity nor radiation-induced (delayed) chromosomal instability. In vivo, phenotypic alterations have been linked to locus specific methylation in the agouti mouse strain. Low doses delivered during gestation by a MicroCT (80 kVp X-rays) caused significantly increased DNA methylation at the viable yellow agouti locus in a sex-specific manner. Average DNA methylation was significantly increased in male offspring exposed to doses between 7 and 76 mGy. Maternal dietary antioxidant supplementation mitigated both the DNA methylation changes and coat colour shift in the irradiated offspring indicating that low-dose exposure during gestation elicits epigenetic alterations particularly by oxidative stress [B31].

59. Examination of normal human fibroblasts and bronchial epithelial cells seven days after gamma irradiation (0.1–1 Gy), did not reveal an appreciable direct effect on DNA cytosine methylation patterns in exposed cells using a methylated CpG island recovery assay combined with microarrays [L3]. Also, at the early phase after irradiation of primary human fibroblasts (6 and 24 hours after 2 or 4 Gy) employing Illumina 450K methylation arrays, there were no detectable methylation differences in genic (promotor and gene body) and intergenic regions between irradiated and control fibroblasts [M6] nor in interspersed *ALU* and *LINE-1* repeats and centromeric alpha satellite DNA. In contrast, peripheral blood cells of occupationally radiation-exposed power plant workers (mean accumulated effective dose 157.74 +/− 85.4 mSv (standard error), approximately 20 years average exposure) displayed increased *LINE-1* methylation after radiation exposure following assessment of global DNA methylation which was lower than in control, unexposed subjects [L14]. Low- and high-dose (0.1, 1.0 and 2.5 Gy) radiation of rats however decreased CpG methylation in the *LINE-1* promoter region in the mammary glands followed by up-regulation of *LINE-1* RNA levels and synthesis of *LINE-1* protein without a specific radiation dose-dependent response [L54].

60. Methylation changes after radiation have been assessed in whole organisms. Examination of DNA methylation in F1 embryos (5.5 hours post-fertilization) of zebrafish with whole genome bisulfite sequencing following parental exposure to 8.7 mGy/h for 27 days revealed 5,658 differentially methylated regions, predominantly gene promoters and enhancers [K11]. The results also indicate that ionizing radiation-related effects in offspring can be linked to DNA methylation changes that partly can persist over generations. BALB/c mice exposed to moderate dose radiation (X-rays, acute exposure, 0.5 Gy, or exposure 0.05 Gy/d for 10 days) revealed global hypomethylation and tissue-specific promoter hypermethylation of particular genes. Notably, promoter hypermethylation, rather than global hypomethylation, was relatively stable [W2]. The key DNA methyltransferase DNMT1 was down-regulated in a tissue-specific manner but down-regulation did not persist in time. Exposure of C3H/HeN mice to doses of high-LET ^{56}Fe ion radiation between 0.1 and 1.0 Gy demonstrated tissue and locus specific as well as dose- and time-dependent DNA methylation changes. However, these changes in DNA methylation did not correlate with significant changes in mRNA level at the same timepoints, nor were they observed for all loci studied [L29].

61. Newman et al. [N13] found that methylation of *L1* repeat elements after 1 Gy X irradiation in spleens of C57BL/6, CBA and BALB/c mice always increased at day 1, at day 6 only in BALB/c and at day 12 decreased in C57BL/6 and CBA mice. However, when detailed sequences of CpG islands were evaluated, changes at day 1 post-irradiation were found to be driven by differences in females in C57BL/6 animals or mostly in males in CBA and BALB/c mice.

62. miRNAs are pleiotropic epigenetic modulators of gene expression. Their modulation in response to radiation is more varied than many other radiation responses. For example, radiation dose responses are rarely observed in in vitro miRNA expression studies, on the contrary, miRNAs are often observed to respond distinctly to different radiation doses [C11]. This is even more noticeable for exposures to low dose fractions of radiation, though total doses may be moderate to high. A recent study by Liang et al. [L28] focused on expression of 11 miRNAs in male 12 weeks old FVB/NJ mice exposed every three days to 28 mGy of gamma rays for four months (for a total dose of 1.12 Gy). miRNAs, including miR-34a, miR-375, miR-185, miR-21, miR-421, miR-193a, miR-199a, miR-146a, miR-155, miR-221, and miR-222, all play significant roles in cancer initiation, promotion or suppression. For example, miR-155 prevents apoptosis and promotes proliferation, invasion and migration in hepatocellular carcinoma [F21]; presence of miR-21 in serum of liver cancer patients is an indicator of poor clinical prognosis [W6], while miR-375 suppresses autophagy in liver cancer [Z16]. Changes in expression for some of these 11 miRNAs were noted either at conclusion of exposure, two months later or, occasionally, at both timepoints. In addition, miRNA expression patterns were different in liver, testis and heart, and few of organ specific miRNA expression changes were statistically significant. These included, for example, increased expression of miR-21 in liver at conclusion of treatment, decreased expression of miR-375 at conclusion of treatment and decreased expression of miR-155 at both timepoints [L28]. miRNA sex differences were studied in 45-day-old C57BL/6 mice exposed to radiation either to whole body or head only (body was shielded) with 1 Gy of X-rays (dose rate 50 mGy/s) [K39]. To simulate scatter dose, some of the "control" animals were exposed to 10 mGy. No changes in miRNA expression were recorded in this group of animals, regardless of sex.

63. Long non-coding RNAs (lncRNAs) are abundant in genomes of higher organisms and regulate transcription fidelity. Exposure to moderate-dose irradiation (0.25 Gy) in human cells causes transiently elevated expression of the lncRNA PARTICLE that regulates the expression of tumour suppressor MAT2A. This altered expression of lncRNA PARTICLE results in repression of MAT2A via methylation and the formation of transcription-repressive complexes [O4]. PARTICLE levels were significantly elevated in exosomes isolated from medium of cells exposed to 0.25 Gy implicating a role of this lncRNA in intercellular communication and transcription-silencing in response to irradiation. A follow-up study demonstrated that elevated expression of PARTICLE operates as a trans-acting mediator of DNA and histone lysine methylation [O5]. Alterations in both miRNA and lncRNA levels following moderate-dose radiation have been also reported in human blood, with dose and time dependence of responses for certain RNAs [A21, K2]. Whole genome expression analysis revealed lncRNA profiles changing in response to radiation (1 to 12 Gy of X-rays) in blood of mice 16–48 hours post-irradiation. Radiation-induction of lncRNAs known for their involvement in DNA damage response has been observed, including lncRNA targets of P53 as well as lncRNAs regulating immune response. The profiles of lncRNAs (and mRNAs) from peripheral blood mononuclear cells of BALB/c mice at 24 hours after gamma radiation (^{60}Co) with repeated 0.05 Gy (for 10 times) and single 0.5 Gy exposures revealed lncRNAs induced by these radiation conditions, that exert a crucial role in the regulation of immune response related gene expression [Q1].

64. To summarize, the data suggest that radiation-induced effects (dose range 0.1–6 Gy) on DNA methylation depend on tissue/cell type, sex, and species. Moreover, radiation type, dose, and time after exposure have to be taken into consideration as well as the relative sensitivity of the technologies used to determine DNA methylation. The data also suggest that dose–response relationships are complex. The evidence from the small number of studies suggests that low-dose exposure is capable of causing changes to DNA methylation and that the methylation changes might differ between low and high dose. The methylation data are inconclusive if related to transcriptional responses and radiation-mediated changes of DNA methyltransferases. Dose–response relationships for miRNA are not yet well characterized.

C. Effects on other signal transduction pathways

65. The recognition of cell signal transduction pathways beyond those involved in DNA damage signalling has been somewhat indirect in prior UNSCEAR reports. Annexes relating to cancer mechanisms acknowledge that signal transduction pathways can be disrupted in cancer development, but no judgements are made in the direct effects of radiation exposures on signal transduction pathways.

66. The physical effects of ionizing radiation (damage to the different cellular and tissue components) are translated into biological effects by the activation or modulation of various intracellular signalling pathways. These events are generally mediated by post-translational modification of proteins and culminate in changes in gene expression to dictate cell fate in response to radiation exposure. Radiation-induced changes in gene expression are discussed in section III.D. In this section, only articles documenting the modulation of these signalling networks at the biochemical level or demonstrating their activity in functional assays will be considered. The UNSCEAR 2006 Report, annex D, "Effects of ionizing radiation on the immune system [U7] did not specifically address the issue of effects on signal transduction pathways and contains few, references to modulation of signal transduction pathways by ionizing radiation.

67. The expression of immune checkpoint proteins such as the Programmed cell Death Ligand (PD-L1) on tumour cells inhibits the activity of tumour-specific cytotoxic T lymphocytes by engaging their inhibitory Programmed Death (PD)-1 receptor. This pathway has received a lot of attention in recent years due to the efficiency of the association of immune checkpoint inhibition with radiotherapy in cancer treatment [B18]. High-dose radiation exposure induces DSBs that can up-regulate the expression of PD-L1 on tumour cells [S11]. The presence of DNA in the cytosol activates the cGAS/STING inflammatory pathway that culminates with the production of interferons (IFNs) [L24]. IFN-γ signalling can also induce up-regulation of PD-L1 expression. However, it seems that radiation exposure does not induce PD-L1 expression on non-transformed cells (primary human fibroblasts) [H2]. Whether cells newly elicited or transformed by radiation exposure use PD-L1 up-regulation to escape immune surveillance and by which pathway and whether this phenomenon occurs after low-dose and low-dose-rate exposure has not been addressed yet.

68. The exposure in vitro of human purified primary monocytes to 0.05, 0.1 and 1 Gy of X-ray radiation dose-dependently modulates the expression of several genes involved in innate immunity signalling. Hence, expression of Toll-like receptor (*TLR*) *4* and *TLR9* genes, and of adaptor signalling proteins MyD88 and IRAK1 is increased after low but not high-dose exposure. The quantification by enzyme-linked immunosorbent assay (ELISA) of the activated (phosphorylated) form of several molecules involved in key signalling pathways showed that low-dose exposure (0.05 and/or 0.1 Gy) specifically resulted in an increase in phospho-IκB, phospho-p38MAPK, phospho-ERK1/2 and phospho-JNK. In contrast, increase in phospho-p53 was found only in 1 Gy irradiated cells [E15]. Thus, low-dose exposure in vitro activates specific signalling pathways involved in signal transduction from innate immune receptors.

69. Musculoskeletal disorders are associated with a destruction of cartilage and bone tissue due to chronic inflammation. Hong et al. [H34] investigated the mechanisms underlying the inhibition by low-dose radiation of chondrocyte de-differentiation in inflammatory situations [H34]. Exposure of human primary chondrocytes to 5–20 mGy (^{137}Cs, 6.7 mGy/min) has no effects on the expression of chondrocyte markers (type II collagen, Sox9) and chondrocyte activation (NF-κB-induced) Cox2 signalling. Treatment with IL-1β induced de-differentiation of chondrocytes over 24 to 48 hours, with loss of specific markers and activation of an NF-κB-mediated inflammation. These events can be inhibited or reversed by exposure of IL-1β treated chondrocytes to 5 or 10 mGy. IL-1β effects are mediated through protein kinase B (Akt) phosphorylation, which results in increase in α, β and γ catenin expression. Low-dose-rate exposure reduces phospho-Akt accumulation and catenin expression. Of note, low-dose-rate

exposure has no effects on catenin expression in un-stimulated chondrocytes. Low dose rate similarly inhibits up-regulation of catenin proteins induced by other stimuli such as Phorbol-12-myristate-13-acetate, EGF or retinoic acid. This showed that moderate dose rate is able to inhibit/attenuate the effects of inflammation in primary chondrocytes, and that the effects of moderate dose rate on Akt-signalling pathways depend on the cellular context.

70. Several immortalized cell lines were used to investigate the mechanisms promoting cell survival or apoptosis following radiation exposure, without and with prior low-dose exposure. The level of phospho-AKT was found to correlate with radiosensitivity of the different cell lines. Transfection of IM-9 (EBV-transformed B-lymphoblastoid cell line) cells with a constitutive active form of AKT provides radioresistance. Prior exposure to 0.01 Gy at a dose rate of 0.01 Gy/h protected cells against apoptosis induced by a subsequent exposure to 2 Gy, with reduced levels of cleaved PARP-1, Casp-3 and Casp-9. Low-dose priming induces in IM-9 cells a transient increase in phospho-ERK1/2, phospho-p38MAPK, phospho-JNK and others over the following 48 hours. Exposure to 2 Gy decreases the level of phospho-ERK, but this effect is abolished by prior low-dose and low-dose-rate exposure. Furthermore, pharmacological or siRNA-mediated inhibition of ERK significantly increase IM-9 death following 2 Gy exposure [P3]. These results showed that AKT and ERK pathways modulate cell fate following low- and high-dose exposures.

71. In a follow-up study, the same laboratory compared the early effects (4 hours) of low dose (0.1 Gy) and high dose (2 Gy) gamma-radiation exposure on protein phosphorylation in IM-9 cells by using phospho-antibody arrays [E17]. As expected, high-dose radiation induced cell cycle arrest, more γH2AX foci, which lasted longer, and some apoptosis as detected by terminal deoxynucleotidyl transferase assay (TUNEL) and the cleavage of Casp-3 and PARP-1. The phosphorylation of ATM and Chk2 was also stronger after exposure to 2 Gy. Low-dose radiation nonetheless induced changes in the phosphorylation of 95 proteins (67 with increased and 28 with decreased phosphorylation). High-dose radiation resulted in the modification of 109 proteins (68 with increased and 41 with decreased phosphorylation). Only 21 proteins with increased and 8 with decreased phosphorylation level were common in the two exposure conditions. While common regulated proteins are mainly involved in cell growth, proliferation, survival and death, low-dose exposure seems to activate cell maintenance and immune cell activation and high-dose exposure activates several cancer-related pathways. Thus, the early responses to low- and high-dose radiation are largely different and affect different signalling pathways.

72. Low to moderate dose radiotherapy is used clinically to control some inflammatory conditions; for example, treatment of benign inflammatory, hyperproliferative and degenerative diseases, including painful elbow syndrome, painful shoulder syndrome, calcaneodynia, achillodynia and osteoarthritis of the joints. The mechanisms underlying the anti-inflammatory effects of low/moderate dose radiotherapy observed around 0.3 to 0.5 Gy were analysed in TNF-α activated EA.hy.926 endothelial cells exposed to doses ranging from 0.1 to 3 Gy. The transcriptional activity of AP-1 was selectively induced at 0.3 Gy [R12]. The induction of X-linked inhibitor of apoptosis protein (XIAP), a protein involved in the modulation of apoptosis and NF-κB activation, was also induced in a bi-phasic pattern, peaking at 0.5 Gy, when apoptosis is minimal. Transfection with a siRNA targeting XIAP expression suppressed the decrease in apoptosis and prevented the increase in radiation-induced NF-κB transcriptional activity observed at 0.5 Gy [R13]. Thus, it appears that the anti-inflammatory effects of low-dose radiotherapy, used to treat chronic inflammatory syndromes, are mediated in part by AP-1 induction of XIAP, which then prevents apoptosis and modulates NF-κB activation in endothelial cells.

73. The effects of radiation exposure on activated immune cells/immune cell activation were also addressed in IgE-mediated allergic responses using either the rat mast cell line, RBL-2H3 [J24], human mast cell lines or an in vivo mouse model [J25]. These responses are induced after the cross-linking of cell surface expressed FcεRI receptor, which initiates a cascade of phosphorylation events that culminate

in the release of mediators (histamine, beta hexosaminidase) and the production of inflammatory cytokine. Exposure (0.01–0.5 Gy, ^{137}Cs source, 0.8 Gy/min) of the different mast cell models before FcεRI cross-linking prevents phosphorylation of proximal (Lyn, Syck, PKC, PLC gamma) and distal (ERK, JNK, p38) kinases and reduces the release of mediators and cytokines. The expression of the FcεRI is also reduced by radiation exposure, in absence of cell death. The same decrease in FcεRI and in cytokine production was observed in vivo in mice exposed to 0.05, 0.1 and 0.5 Gy. Thus, low-dose exposure can inhibit immune cell activation by decreasing the cell surface expression of FcεRI receptors and the phosphorylation of signalling kinases activated by these receptors in mast cells.

74. In a further study, the mechanisms of attenuation of mast cell activation by low-dose gamma radiation were investigated in the RBL-2H3 rat mast cell line [S56]. Exposure to 0.01 to 0.1 Gy, but not to 0.5 Gy (^{137}Cs source, 0.6 Gy/min) was found to reduce FcεRI-activated mast cell migration. The phosphorylation of PI3K and BTK kinases and activation of Rho GTPases involved in intracellular actin dynamics were also reduced in these conditions, as was expression of the orphan nuclear receptor Nr4a2, induced during mast cell activation. Inhibition of Nr4a2 inhibits PI3K/BTK activation, migration of activated mast cells, and their secretion of MCP-1. Anti-MCP-1 neutralizing antibodies inhibit activated mast cell migration to about the same extent as exposure to 0.05 Gy. Thus, low-dose radiation could modulate mast cell activation by attenuation of Nr4a2-induced MCP-1 production.

75. The putative mechanisms underlying radiation-induced cytokine secretion were also analysed in murine macrophages following whole body X-irradiation [S26]. Peritoneal macrophages were harvested from non-irradiated mice and mice exposed to 0.05–6 Gy at 12.5 mGy/min (0.05–0.2 Gy) or 0.287 Gy/min (0.5–6 Gy), from 4 to 48 hours post-exposure. Radiation exposure results in a dose-dependent increase of IL-18 and IL-1β secretion ex vivo until 4 Gy, with a decline at 6 Gy. This increase lasted for 48 hours after exposure to 0.075 and 2 Gy. In these conditions, the nuclear translocation of p65/p50 and p50/p50 NF-κB dimers increase sharply for up to 8 hours post-exposure and then declines for the next 40 hours. Expression of the TLR4 receptor and associated MD2 and CD14 co-receptors and of MyD88 followed mostly similar patterns, except that maximum induction was observed after 2 Gy. It therefore appears that the whole signal transduction pathway is activated, from expression of membrane receptors able to sense radiation-induced danger signals to adaptor proteins and transcription factors.

76. The level of Nrf2 (redox signalling) and phospho-IκBα (i.e. activation of NF-κB signalling) were analysed by Western blot in spleen cells at day 2 and 7 after exposure of C57BL/6 mice to 0.001, 0.01, 0.1 Gy gamma radiation (total body irradiation) in a single dose or in three fractions delivered on consecutive days. There is an early (day 2) increase in Nrf2 at very low dose (1 mGy) and phospho-IκBα at all doses in mice exposed to three fractions, but no change in mice exposed to a single dose, whereas the pattern is inverted at day 7: increase in Nrf2 for 0.001 and 0.01 Gy and phospho-IκBα at all doses in mice exposed in a single dose, but no changes in those mice exposed to three fractions. Thus, very low and low doses do elicit a response in vivo. The kinetics of this response depends on the dose and dose fractionation [S57].

77. The effects of low-dose radiation fractionation on the activation of the Akt and Nrf2 signalling pathways was also analysed in the kidneys of mice with experimentally induced diabetes exposed to 25 mGy, 75 mGy or 3 times 25 mGy in 3 consecutive days of X-rays delivered at 25 mGy/min, 1 to 12 hours post-exposure. The level of p-Akt and p-Akt2 were increased for 12 hours after 75 mGy, but only for 6 hours after 1 or 3 fractions of 25 mGy, Nrf2 induction was observed transiently, from 1 to 6 hours after 75 mGy, and 3 to 6 hours after 3 fractions of 25 mGy. The expression of Nrf2 target genes NADPH quinone oxidoreductase and heme oxigenase 1 was up-regulated from 3 to 6 and 3 to 9 hours, respectively after exposure to 75 mGy delivered in one or three fractions [X1].

78. To analyse the effects of low-dose gamma radiation and its eventual ability to modulate the response to a further high-dose exposure, either proton or gamma radiation, Rizvi et al. [R9] quantified the level of total and activated proteins belonging to different signalling pathways: NF-κB, JNK, p38MAPK, Lck and ZAP70 in CD4+ T-cells purified from irradiated mice 4 and 21 days post-exposure and activated in vitro. Importantly, the modulation of the total and activated proteins was not always concordant, showing different effects of radiation exposure on protein expression and activation of the signalling pathways. Only activated proteins will be discussed below. At day 4, low-dose radiation induced only a down-regulation of JNK and p38MAPK whereas at day 21, it induced an increase in NF-κB. Thus, different signalling pathways are affected at different times during the response to low-dose radiation [R9]. When mice were exposed to a simulated solar particles event (sSPE; 2 Gy protons, 30 to 201 MeV) after low dose and low-dose-rate exposure, the overall pattern resembled more the one evoked by low dose and low dose rate alone than by sSPE alone, suggesting an imprint of prior low dose and low-dose-rate response, broadly similar to an adaptive response. These observations were largely confirmed in a following study and extended to an exposure of 2 Gy of gamma radiation instead of sSPE after low dose and low dose rate, and from 21 to 56 days post-exposure [G14].

79. In summary, low-dose radiation modulates the activation of intra-cellular signalling pathways in all types of human and animal immune, and non-immune cell types analysed, after in vivo or ex vivo exposure. These effects may be visible after exposure to as low as 1 mGy in murine splenocytes. A given pathway can be activated or inhibited according to the activated or resting status of the cell type analysed. Low dose priming radiation exposure may modify the response to a subsequent high dose challenge. The effects of radiation may follow non-linear dose–response relationships.

D. Gene and protein expression

80. That radiation exposure can lead to altered expression of genes was recognized in the UNSCEAR 1994 Report, annex B "Adaptive responses to radiation in cells and organisms" [U4]. The UNSCEAR 2012 White Paper on "Biological mechanisms of radiation actions at low doses" [U9] noted that gene expression responses at high and low doses had been reported but were inconsistent. The Committee has not previously undertaken a comprehensive review of the area.

81. Over the last several years studies have considered the impact of radiation exposures on gene and protein expression. There are now data available from exposures at high, moderate and low doses as well as more limited information from studies carried out at low dose rate. Here, gene expression is the main focus as methods to assess transcriptional response are better developed and more comprehensive than those for protein expression. It should be noted, however, that gene expression changes per se will not alter cell phenotypes, that requires changes in protein expression.

82. Global changes in gene expression are probably one of the key components of biological responses to radiation exposures. This is especially important in the case of low-dose exposures and their possible long-term consequences, such as cancer. However, almost all work on radiation alteration of gene expression considers only rather short post-exposure time points; as cancer develops only after protracted lag periods of years, relating short term (up to 1 or 2 days) post-irradiation alterations to long term (several years) development of cancer is very challenging. More work on expression changes following exposure and their evolution over time is required. Exposure to ionizing radiation regulates multiple genes as well as signalling pathways in cells. Radiation response at the gene level may depend on radiation type or quality, dose rate and dose. When compared to high-dose radiation exposures, responses to low-dose radiation exposures may differ both qualitatively and quantitatively. Consequently, it is an important

issue to determine how robustly low- and high-dose responses can be considered as different and leading to different long-term consequences. Dose–response relationships have been obtained for some genes over dose ranges of 1 to 3 Gy and 0.5 to 4 Gy and down to doses as low as 20 mGy. Most of the studies have used human blood lymphocytes, and this is often carried out with a view to the development of biodosimetry methods. There are studies conducted on cells in culture as well as 3D skin models that more closely mimic human tissue exposure situations. Transcriptional responses to radiation exposures can be determined by global gene expression profiling or by reverse transcription (RT)-polymerase chain reaction (PCR) for specific genes in question.

83. A number of studies focused on particular pools of genes and types of pathways. Selected genes were generally validated by RT-PCR methods to confirm the differential expression by microarray analysis. Transcriptomic analysis compared well with other modes of validation of specific genes. More robust analysis is required to strengthen the importance of such gene-expression studies and associate them with cancer induction. Research articles published from 2006 to 2019 were reviewed and the results are summarized in table 4.

84. Transcriptomic responses of peripheral blood cells from healthy individuals exposed chronically to low doses of 0.2 to 49 mSv ranging from natural background levels to elevated levels due to contamination accumulated over a period of 11 to 13 years as a result of the Chernobyl accident were determined by complementary DNA cytokine and cytokine receptor microarrays, which revealed specific differential expressions of TGF receptor, *CD40* and *EB13* in more than 50% of the volunteers evaluated. Though such direct studies are rather scarce, in vitro and experimental analysis of gene expression profiling provided crucial information on the genetic pathways involved in tumour development following low doses of ionizing radiation. Some of the most common gene products which were shown to be differentially expressed following low-dose radiation exposure are: *CDKN1A, GADD45, DDB2, BAX* and *PCNA* [K1, S54], i.e. genes known to be involved in carcinogenesis.

85. The majority of the research reviewed here on radiation-induced gene expression changes has been conducted with an objective of developing biomarkers of radiation exposure. It is evident from the research covered in this annex that multiple signalling pathways are modulated by low-dose radiation exposure. Genes that fall under the following pathways are important in maintaining the genome to prevent tumour development – cell death, cellular development, DNA replication, recombination and repair, cell growth and proliferation, cell cycle, cell-mediated immune response. Canonical pathways that were affected by radiation exposure within the range of low to high doses include: DNA damage response, apoptosis and, in general, molecular mechanisms of cancer. Some evidence suggests that low-dose radiation exposure may primarily modulate immune response, whereas high-dose exposure activates the recognized radiation responses of p53 signalling pathway, DNA damage response and apoptosis pathways at transcriptional levels. Genes such as *CCR4*, *GNG11*, and which belong to the cytokine and chemokine signalling pathways, immune-related and inflammation processes were shown to be differentially expressed at doses of 0.05 Gy [S54]. There is convincing evidence that low-dose, moderate-dose and high-dose exposures lead to changes in biological processes within human blood lymphocytes. Detailed analysis of the published data using a bioinformatics approach may help in understanding the different signalling pathways altered after low-dose exposure. The data analysed so far, reveal the complexity of transcriptional changes in human blood cells at low levels of ionizing radiation. Some of these changes appear to be transient. However, long-term consequences of more persistent changes cannot be ruled out.

1. Gene expression changes in cells from occupationally/environmentally exposed individuals and irradiated human blood and other cell types in vitro

86. The city of Ramsar, Islamic Republic of Iran, has one of the highest levels of natural background radiation in the world (doses from radon ranging from 200 Bq/m^3 to 3,700 Bq/m^3). In the study by Bakhtiari et al. [B5] blood samples from 60 individuals from a high background radiation area and from 30 individuals from a normal background radiation area were analysed for two mismatch repair genes, *MLH1* and *MSH2*. There was a significant up-regulation of the *MLH1* gene in residents of high background radiation areas. The association between *MLH1* and *MSH2* gene expression was seen in both the sexes. The increased *MLH1* expression was detected in people aged above 50 years and a decreased expression of *MSH2* was observed in ages under 50 years. The results suggest the activation of the mismatch repair system in individuals living in high natural background radiation areas.

87. Albanese et al. [A9] generated gene expression profiles for mononuclear cells derived from 19 apparently healthy individuals (9 females and 10 males) exposed for 11–13 years to low-level ionizing radiation (doses from 0.18 to 49 mSv, including elevated levels due to exposure as a consequence of contamination of the environment with ^{137}Cs, ^{90}Sr and Pu isotopes) in Belarus following the accident at the Chernobyl nuclear power plant. These individuals did not show any signs of malignancy at the time of analysis. Expression changes were recorded for specific cytokine and cytokine receptor pathways. Multivariate statistical methods – hierarchical clustering analysis, principal component analysis and projection pursuit – were employed in the analysis. The results showed distinct changes in the gene expression pattern in cells from individuals exposed to >10 mSv and <10 mSv. Serine/threonine protein kinase receptor, TGF receptor, EB13 and CD40 ligands were commonly expressed in irradiated samples. The authors reported that they have identified differences in the genes involved in the pathways such as growth factors, cytokine receptors and their cognate ligands as well as apoptosis-modulating proteins in samples from individuals that experienced exposures >10 mSv compared with those of <10 mSv. The authors highlighted the variability in the analytical tools and complexity in the microarray analysis while handling the gene expression data. This is an important consideration and possible limitation of all such studies, the low sample size and potential for confounding due to infection or stress probably also limit the interpretation of this study.

88. A study by Detours et al. [D16] involved a search for the gene signatures in papillary thyroid cancers (PTC) using the sporadic cases as well as samples from thyroid cancers in patients who were exposed to radiation as a consequence of the Chernobyl accident (i.e. samples from the Chernobyl Tissue Bank). Tumours from 14 patients with no history of exposure to radiation and 12 patients from the Chernobyl Tissue Bank were used in the analysis. Gene expression profile data from PTC from adult French patients with no history of exposure to radiation and from Ukrainian patients exposed to radiation from the Chernobyl accident during childhood were obtained in order to identify possible gene signatures for radiation carcinogenesis. The authors confirm their earlier finding that French and Chernobyl Tissue Bank tumours have the same overall expression profiles and have indistinguishable B-Raf proto-oncogene serine/threonine kinase mutation and *RET*/*PTC* frequencies. Globally transcriptional responses of human cells to H_2O_2 and gamma radiation (a dose of 2.5 Gy) were also similar. However, on a finer scale, a 118 gene signature discriminated the gamma radiation and H_2O_2 responses. They were able to classify tumours (such as those from France and the Chernobyl Tissue Bank) with an error of 15–27%. Similar results were obtained with an independent signature of 13 genes involved in HRR. Even though both sporadic and radiation-induced PTC represent the same disease, they can be distinguished with molecular signatures reflecting specific responses to gamma radiation and H_2O_2. Such signatures may reflect the susceptibility profiles of the patients. Unlike most other studies described in this section, this study relates to gene expression changes in tumours that are sampled long after radiation exposure, it is unclear if the alterations observed are a direct or indirect consequence of the exposure.

89. Samples from thyroid cancer tissues from 11 post-Chernobyl PTC and 41 sporadic PTC were analysed for gene signatures for radiation-induced thyroid cancers. Chernobyl radiation exposure in children ranged from 0.15 to 9 Gy. Microarray screening criteria included a change of about 5-fold differences in the gene expression among the groups studied. About 646 up-regulated and 677 down-regulated genes were identified in the tumour samples which discriminate the two PTCs. Seven genes (*SFRP1, MMP1, ESM1, KRTAP2-1, COL13A1, BAALC* and *PAGE1*) were shown to distinctively separated in the two types of PTCs. Gene expression patterns which are reminiscent of aggressive tumour type in older individuals were more pronounced in post-Chernobyl PTCs [P21].

90. Occupationally-exposed individuals from the Mayak Production Association, Ozeorsk, Russian Federation employed since 1949 were selected in the study by Abend et al. [A3]. Plutonium workers were exposed to densely ionizing alpha particles along with external exposure to gamma radiation whereas the reactor workers were exposed to external gamma radiation only. A control group without any exposures was chosen for comparison. This is a study of 146 individuals from the cohort of which 17 were exposed to external gamma rays only (~0.5 Gy), 81 were exposed to both plutonium (~0.7 kBq) and gamma radiation (~1.01 Gy) during their occupation from 1949 onwards and 48 unexposed controls. Blood samples were taken from 150 individuals in 2011 with stringent exclusion criteria such as individuals dying within 6 months of sampling or developing malignant cancer. Individual dose estimations were made through records as well as by biophysical dosimetry. Peripheral blood was taken from workers older than 70 years of age. Using transcriptional (microarray, mRNA) and post-transcriptional analysis (microRNA), the authors identified a set of 376 candidate genes (80 up-regulated and 296 down-regulated) and all appeared to be associated with internal ^{239}Pu exposure and to a lesser extent were associated with external gamma-ray exposure. A similar trend was observed for microRNA expressions as well. Down-regulated genes predominantly associated with ^{239}Pu exposure while up-regulated genes after external gamma-ray exposure. However, no enrichment of genes related to certain biological processes could be found in this study.

91. Fachin et al. [F2] used gene expression profiling on lymphocytes derived from individuals who were occupationally exposed to different doses of ionizing radiation and from unexposed individuals. Peripheral blood samples were taken from 14 donors with a record of occupational exposure to radiation and 9 donors with no radiation exposure history. Physical dosimetry records of these workers revealed that the cumulative doses ranged from around 0.7 to 39 mSv. There were five males and nine females in the occupational group, three males and six females in the control group. The workers included physicians, nurses and radiological technicians. They were exposed during their work for an average of 9.32 ± 5.97 (mean ± SD) years. In addition, the workers were exposed to mixed radiation sources such as X-rays, gamma rays and beta rays. Based on analysis of the "exposed" and "unexposed" groups, gene expression data identified a total of 78 genes (21 up-regulated and 57 down-regulated) involved in several biological processes such as ubiquitin cycles (*UHRF2* and *PIAS1*), DNA repair (*LIG3, XPA, ERCC5, RAD52, DCLRE1C*), and cell cycle regulation/proliferation (*GSTP1, PPP2R5A, DUSP22*). Some of these gene expression changes were validated by an RT-PCR technique. Fachin et al. [F2] speculate that some of these gene markers might be suitable as biomarkers of radiation exposure and as such may be relevant for risk estimation for workers exposed to radiation.

92. Visweswaran et al. [V13] investigated the quantification of entrance surface dose of X-rays from neuro-interventional procedures to various regions (forehead, eyes, shoulders and thyroid) and correlated the doses with the levels of early DNA damage biomarkers measured in the peripheral blood lymphocytes of patients. Fifty-one patients underwent a clinically indicated neuro-interventional procedures (diagnostic – cerebral angiography or therapeutic – embolization, stenting, flow diverter placement, coiling, sclerotherapy and percutaneous transhepatic biliary drainage procedures) and doses received were calculated to be in the range of 4.9–273 mGy (forehead), 14–398 mGy (eyes), 8–433.3 mGy (shoulders) and 4.7–242.5 mGy (thyroid). Blood samples were collected at different time points – pre-

exposure, and post-exposure (within 30 minutes). Phosphorylation of γH2AX and p53ser-15 was increased in samples obtained post-exposure compared to pre-exposure, but the increase was statistically significant only for p53. Selected genes such as *CDKN1A*, *FDXR*, *BAX*, *DDB2*, *SESN1*, *BCL2*, *MDM2*, *TNFSF10B* and *PCNA* showed statistically non-significantly reduced expression while *GADD45A*, *ATM* and *TNFSF9* showed elevated expression. The data suggest that neuro-interventional procedures as explained above produced higher DNA damage and altered gene expression in patients after receiving low doses of X-radiation.

93. Azimian et al. [A29] undertook a study to assess the effects of low doses of ionizing radiation on the expression of specific apoptotic genes such as *BCL2* and *BAX*. Blood lymphocytes from four healthy donors were exposed ex vivo to 20, 50 and 100 mGy of ^{60}Co gamma rays at a dose rate of 13 mGy/min. Expression analysis was carried out at 4, 24, 48, 72 and 168 hours post-irradiation by real-time PCR. They reported that the low doses used in the study can induce early down-regulation of *BAX* with normal levels restored at 168 hours. *BCL2*, the anti-apoptotic gene, was up-regulated. They reasoned that, in view of the transient nature of the observed effects, gene regulation/modification as early as four hours post-irradiation may be due to adaptive response [A29].

94. El-Saghire et al. [E15] exposed blood samples obtained from 10 healthy donors to doses of 0.05 or 1 Gy of X-rays ex vivo at a dose rate of 30 mGy/min. Gene expression profiling was carried out on these samples and gene-set-enrichment-analysis (GSEA) was performed on differentially expressed genes. Functional analysis on these genes exposed to a dose of 0.05 Gy displayed enrichment of chemokine and cytokine signalling. GSEA produced a higher ranking of inflammatory and immune-related gene sets that are responsible for response and secretion of growth factors, chemokines and cytokines. At a dose of 1 Gy, GSEA revealed enrichment of established radiation-response pathways such as p53 signalling, apoptosis, DNA damage response and repair. Subsequent validation with RT-PCR showed the significant induction of specific chemokine-related gene alterations (*PF4*, *GNG11* and *CCR4*) at a dose of 0.05 Gy and DNA damage/repair genes such as *DDB2*, *AEN* and *CDKN1A* at a dose of 1 Gy. Thus, this study suggests a differential response in gene signalling pathways at low and moderate doses of radiation which may be relevant for assessment of risk to health based on the dose of radiation.

95. Fachin et al. [F1] exposed blood samples taken from four healthy individuals to doses of 20, 50, 100, 500 and 1,000 mGy of ^{60}Co gamma rays for cytogenetic analysis. The dose rates used were 47 mGy/min for the first three dose levels (100 mGy and below) and 910 mGy/min in the last two. For gene expression experiments, blood lymphocytes obtained from the four donors were exposed to ^{60}Co gamma rays at doses of 0, 100, 250 and 500 mGy (at a dose rate of 1.18 Gy min^{-1}) and the cells were cultured for 48 hours before conducting gene expression profiling. The study identified 86, 130 and 142 differentially expressed genes of which 25, 35 and 33 genes were exclusively modulated, at doses of 100, 250 and 500 mGy, respectively. Dose-dependent changes, up-regulation (↑) or down-regulation (↓), were seen in some of the genes such as *(a) CYP4X1*(↑), *MAPK10* (↓) and *ATF 6* (↑) (100 mGy), *(b) DUSP16* (↑) and *RAD51L1* (↑) (250 mGy), and *(c) RAD50* (↑), *REV3L* (↓) and *DCLRE1A* (↓) (500 mGy). A set of 34 genes significantly up/down-regulated by radiation was common for all doses. In comparison, chromosome damage was seen at doses >100 mGy for total aberrations and >500 mGy for dicentrics. The biological processes affected based on the transcriptional changes in genes were metabolism, stress response/DNA repair, cell growth/differentiation and transcription regulation. No information was provided on potential confounding factors for the described radiation responses.

96. Ghandhi et al. [G4] exposed human blood to doses of 0.56, 2.23 or 4.45 Gy of X-rays at a dose rate of 1,030 mGy/min (high dose rate) or 3.1 mGy/min (moderate dose rate). Even though the dose range used in this study is from moderate to high doses, the relatively low dose rate study of 3.1 mGy/min is of importance to identify gene expression changes. The cells were subjected to gene expression profiling 24 hours after irradiation. The data showed that 454 genes were differentially expressed after irradiation

with the 3.1 mGy/min dose rate at all doses. However, after acute or high-dose-rate exposure, 598 genes were differentially expressed in cells at all doses. Gene ontology revealed genes related to immune processes and B cell-mediated immunity was enriched in both sets of cells. However, acute exposure produced enrichment of genes with functions related to natural killer (NK) cell activation and cell-to-cell signalling. Further, significant enrichment of the p53 pathway was seen at all doses and both dose rates.

97. Gruel et al. [G18] studied the responses of different lymphocyte subtypes, namely $CD4^+$, $CD8^+$ and $CD56^+$ cells, to ionizing radiation. Human blood lymphocytes from five healthy donors were exposed ex vivo to doses of 0.05 and 0.5 Gy of ^{60}Co (1.25 MeV) gamma rays at a dose rate of 0.45 Gy/min. The different subsets of lymphocytes were subjected to microarray analysis at 3 and 24 hours post-exposure. The data indicated that there was no change in the percentage of the different subpopulations of lymphocytes following irradiation. *BAX*, *PCNA*, *GADD45*, *DDB2* and *CDKN1A* were induced at 24 hours following a dose of 0.5 Gy, but not 0.05 Gy. The number of modulated genes differed between cell types. The number of down-regulated genes was 10 times higher for the $CD4^+$ population than for other cell types three hours after exposure to a dose of 0.05 Gy. Pathway analysis has indicated that most of the down-regulated genes participate in the cellular processes of protein biosynthesis and oxidative phosphorylation. Several biological pathways in $CD4^+$ cells seem to be sensitive to radiation exposure.

98. Knops et al. [K29] exposed human peripheral blood lymphocytes from six healthy donors to low (0.02 and 0.1 Gy), moderate (0.5 and 1.0 Gy) and high (2 and 4 Gy) doses of ^{137}Cs gamma rays. Gene expression analysis using whole human genome DNA microarrays was used on lymphocytes at 6, 24 and 48 hours (with those lymphocytes exposed to moderate and high doses) and 24 and 48 hours (with those lymphocytes exposed to low doses) after irradiation. The authors identified nine genes that could be used to determine low-dose radiation exposure with 96% sensitivity with doses in the range of 0.02 and 0.1 Gy used in the study. Expression alterations increased with increasing dose and time after exposure. Altered genes were assigned to biological processes such as nucleosome assembly, apoptosis and DNA repair response. The authors concluded that it is possible to use gene expression profiling data to determine radiation exposures as low as 20 mGy. At all doses, genes involved in proteolysis and in the positive and negative regulation of apoptosis were significantly altered as determined by one-way analysis of variance ($p<0.05$). This study has identified 9 genes (*MKL2*, *FDXR*, *C10orf39*, *PFKFB3*, *FLJ35379*, *LY6G5C* and 3 other genes with no gene symbols available (Agilent ID A-24_P506680, A_32_P138939, A_32_P20997)) as signature genes which discriminate low-dose exposures.

99. The objective of a study by Manning et al. [M10] was to develop a dose–response relationship for gene expression profiling following low-, moderate- and high-dose ex vivo irradiation of blood lymphocytes. Peripheral blood samples were collected from 26 healthy human donors (17 females and 9 males). Moderate to high doses (0.5, 1, 2 and 4 Gy) and low doses (5, 10, 20, 50, 75 and 100 mGy) of X-rays (250 kV and 0.2 mA) were used in the study at a dose rate of 0.5 Gy/min (for the moderate- to high-dose study) or 4.9 mGy/min (for the low-dose study). Blood aliquots were irradiated, and the samples were studied for cell viability. At 2 and 24 hours after irradiation, multiplex RT-quantitative (q)PCR was used to determine the expression profiles of selected genes. The results indicated that moderate- to high-dose response curves for individual genes (from doses of 0.5 to 4 Gy) were best constructed using a polynomial fit while low-dose response curves used a linear fit. The authors also observed individual variations in the gene expression data. The experiments were conducted more from a biodosimetric point of view. However, the data were obtained for certain genes such as *CDKN1A*, *PCNA*, *MYC*, *DDB2*, *MDM2* etc. that may have roles in carcinogenesis [M10].

100. Paul and Amundson [P6] studied gene signatures that developed in human blood lymphocytes following ex vivo exposure to doses (moderate to high) of gamma radiation of 0.5, 2, 5 and 8 Gy using whole genome microarray expression profiling. Peripheral blood samples were taken from 10 donors (with five independent experiments each at 6 hours post-irradiation and 24 hours post-irradiation using

different donors for each experiment and time-point) and gene expression profiling was generated for biodosimetry purposes. On the basis of a pooled analysis from all donors, the study identified a 74 gene signature which was distinguishable between the four different radiation doses used. More than one third of the genes were regulated by p53. Five selected genes (*CDKN1A, FDXR, SESN1, BBC3 and PHPT1)* were also validated by RT-PCR.

101. Turtoi et al. [T23] exposed blood from a healthy donor to radiation doses of 1, 2 and 4 Gy (^{137}Cs gamma rays, 0.8 Gy/min) and performed gene expression analysis to identify early radiation-response genes. Blood lymphocytes from another two healthy donors were irradiated with similar doses as above and the identified gene markers were validated in these samples. This study was conducted with an objective to develop biomarkers of exposure for use in biodosimetry. Twenty-four of 102 genes identified as markers showed a dose-response pattern. Fifteen genes were up-regulated (*CD69*, *CDK1A*, *EGR1*, *EGR4*, *FLJ35725*, *hCG2041177*, *hCG16434662*, *IFNG*, *ISG20L*, *C-JUN*, *MDM2*, *MUC5B*, *PLK2*, *RND1*, *TNFSF9*). However, the other nine genes were down-regulated (*GRIK3*, *OCLN*, *RPL10A*, *SERHL2*, *SGK3*, *STARD13*, *hCG2038519*, *hCG1998530*, *hCG1985174*). Genes such as *EGR1*, *EGR4*, *IFNG*, *C-JUN* and *TNFF9* produced significant correlations with absorbed radiation dose over the range of 1–4 Gy.

102. Turtoi and Schneeweiss [T24] exposed human lymphocytes obtained from a healthy donor to doses of 1, 2 and 4 Gy (^{137}Cs gamma rays, dose rate of 0.8 Gy/min). Quantitative evaluation of early response proteins and genes was carried out at two hours post-irradiation. A set of early response proteins and genes was identified based on the response at two hours post-irradiation. Most of the proteins and genes indicated alterations of cellular structure (*BACTIN*, *TALIN 1*, *TALIN 2*, *ZYXIN-2*), immune and defence reactions (*MBP2*, *IL17E* and *IFNG*), cell-cycle control (*CDKN1A*, *MDM2*, *ANXA6*, *GADD45A*, *PCNA*, *DUSP*) as well as detoxification processes (*PEX1*) and apoptosis (*BBC3*). The main aim of this study was to identify biomarkers of exposure for biodosimetry purposes.

103. McDonald et al. [M26] exposed primary human prostate fibroblast cells and a prostate epithelial cell line (RPWE-1, human papilloma virus 18 (HPV-18) immortalized) to acute and chronic gamma radiation. Acute radiation exposures were performed using a ^{137}Cs source that gave a dose rate of 0.48 Gy/min. The total doses were 0.1 and 2 Gy. Chronic irradiation employed a ^{57}Co source giving a dose rate of about 24.5 mGy/d (approx. 0.017 mGy/min). The exposure times were about 7,000 and 1,500 minutes. Samples were subjected to clonogenicity assays and gene expression profiling 24 hours after irradiation. A total of 396 genes were shown to be significantly differentially regulated by both acute and chronic doses compared to non-irradiated samples. There was no measurable effect at doses of 100 mGy. There were 106 genes which are common between the samples that had been given an acute dose of 2 Gy compared to those that had been subjected to chronic doses. These genes were down-regulated and were involved in cell cycle or chromosome movement in M-phase. Pathway analysis revealed that the genes which were differentially expressed map to the biological processes such as cell cycle, chromosomal movement, cell survival and DNA replication, recombination and repair as well as predicted activation of transcriptional regulators p53 and Cdkn2a. Most of them showed decreased expression following irradiation. Prostate epithelial cells displayed functional decreases in proliferation and mitotic cells following exposure to a dose of 2 Gy or after chronic irradiation.

104. Zhou et al. [Z20] used normal human fibroblasts (NHF1, NHF3, NHF10) and their hTERT-immortalized versions in an experiment where the cells were exposed to doses of 0.5, 1.5, 4.5 Gy of ^{137}Cs gamma rays at a dose rate of 0.84 Gy/min. A dose of 1.5 Gy reduced the colony-forming ability of the cells, thereby affecting their proliferation. This dose was therefore used in the experiments on gene expression profiling. Fibroblasts displayed severe cell-cycle arrest from G2 to M at two hours post-irradiation and G1 to S at 6 and 12 hours post-irradiation. Global gene expression profiling revealed nine radiation-responsive genes which were commonly affected in all the three different cell types including

a dominant p53-dependent G1 checkpoint response. Many genes regulated by the E2F family transcription factors (*CDK2*, *CCNE1*, *CDC6*, *CDC2*, *MCM2*) were significantly down-regulated at 24 hours after irradiation. Several affected genes fell under processes such as DNA metabolism. The results revealed a highly stereotypic pattern of response to ionizing radiation in human fibroblasts, which could be the result of synchronization of cell cycle following radiation exposure.

105. Mezentsev and Amundson [M31] exposed EPI-200, a 3D tissue model which mimics the construction and function of human epidermis, to high (2.5 Gy) and low (0.1 Gy) doses of low-LET protons. Samples were exposed to 0, 0.1 or 2.5 Gy 4.5 MeV protons (5.5 MV singletron accelerator) and the samples were cultured for 4, 16 or 24 hours before gene expression analysis. Gene ontology analysis revealed that the genes involved in the recovery and tissue repair were altered at the low dose while the high dose affected the genes involved in structural integrity and terminal differentiation. Network analysis of the significantly responding genes indicated that the dose of 2.5 Gy produced a p53-response while Hnf4A, a transcription factor not previously associated with radiation response, was prominent following the low-dose exposure. The authors found that Hnf4A protein levels and its phosphorylation were elevated in tissues and cells after low- but not high-dose irradiation.

106. Tilton et al. [T13] used the reconstituted human skin model EpiDermFT in their experiments. The 3D skin model was exposed to doses of 0.1, 2 and 10 Gy over periods of 3.0, 3.4 and 6 minutes at dose rates of 0.033, 0.6 and 1.6 Gy/min, respectively. Using a systems biology approach, dose- and time-dependent responses in transcripts were determined using the direct RNA sequencing (RNAseq) method. A total of 1,701 genes were significantly affected by high-dose radiation exposure (2 or 10 Gy) with a more pronounced effect at 10 Gy. About 29 genes, including *BBC3*, *PPM1D*, *FDXR*, *GADD45A*, *MDM2*, *CDKN1A*, *TP53inP1*, *CYCSP27*, *SeSN1*, *SESN2*, *PCNA* and *AEN* were differentially expressed at doses of 2 and 10 Gy but not at 0.1 Gy at both 3 and 8 hours timepoints. A higher effect was observed at 10 Gy with a larger set of up-regulated genes. At higher doses, down-regulated genes exhibited a linear dose response and these genes regulate the cell cycle. Very few genes were altered by exposure to a dose of 0.1 Gy. It was seen that genes regulating cell cycle progression and inflammatory responses consistently displayed opposite trends in their regulation compared to the high-dose irradiated groups. The study highlighted the fact that certain biomarkers identified could be useful in predicting future disease outcomes in individuals.

107. A study of the temporal response to radiation exposure of a 3D human skin tissue model using transcriptional profiling by microarray analysis was carried out by von Neubeck et al. [V14]. Skin tissue was exposed to a dose of 100 mGy from X-rays at a dose rate of 6.3 mGy/min. Radiation-induced cell type specific changes in the genes, with >1,400 genes differentially expressed in the dermis while >400 genes were regulated in the epidermis. There were no overlapping genes which showed differential response in both tissue compartments. Pathway analysis showed changes in the processes such as cell-cycle regulation, immune response, hypoxia, reactive oxygen signalling and DNA damage repair. The paper discusses the role of cell proliferation and emphasizes how dysregulation might lead to radiation-induced diseases including cancer. The data from this study are not comparable with Tilton et al. [T13] though both have used similar experimental model. The X-ray dose and dose rate and machine parameters were different in the studies.

108. Skin keratinocytes were exposed to low doses of ionizing radiation (Varian 2100 C linear accelerator) (doses 10 or 100 mGy) at 800 mGy/min [B30]. Expression of selected proteins was analysed 1 or 4 days post- exposure. Proteomic analysis revealed that seven of the 11 proteins (epidermal-fatty acid binding protein (EFABP), alpha-enolase, histidine triad nucleotide-binding protein (HINT-1), heat shock protein (HSP27), lactate dehydrogenase A (LDH-A), protein disulphide isomerase precursor (PDI) and S100A9) showed significant differences for radiation exposure alone. In combination with arsenic,

these effects might have influence on the long-term consequences in the form of skin carcinogenesis from combined exposures.

109. Normal human dermal fibroblasts were exposed to low-dose radiation at 0.1 Gy gamma radiation in the study by Bae et al. [B2] and cellular responses were analysed at 6 and 24 hours post-irradiation. Differential cell viability and cell-cycle status were observed at the two timepoints studied. Collagen type I alpha 1 (*COL1A1*) mRNA expression was up-regulated at 24 hours while matrix metalloproteinase-1 (*MMP1*) mRNA was down-regulated at 24 hours post-irradiation. The authors speculated anti-ageing effects of low-dose radiation based on the regulation of collagen synthesis-related genes. microRNA analysis revealed that some of the deregulated miRNAs are associated with early or late radio-adaptive response. However, the authors did not provide any functional studies to prove their speculation on reverse ageing or adaptive response.

110. Recently, Lowe et al. [L45] published a study on chronic irradiation experiments with primary human cells. Primary cells from human neonatal foreskin, adult facial skin and RPE-1 cells were used in the study. Cells were chronically irradiated by exposing them to ^{137}Cs gamma rays at the following dose rates for 7 days: 6 mGy/h (accumulated dose 1.0 Gy), 8 mGy/h (accumulated 1.3 Gy), 12 mGy/h (accumulated 2.0 Gy) and 20 mGy/h (accumulated 3.4 Gy). Acute exposures were carried out with X-rays at 0.5 Gy/min. Chronic radiation exposure resulted in the reduction of histone levels. Protein analysis revealed specific reductions in the levels of non-histone chromatin associated proteins such as HMGB and SMC as well as levels of certain proteins involved in DNA replication and cell-cycle progression (MCM2-7, SMC1A/3, POLD1). Pathway analysis of proteins indicated that the altered proteins participate in DNA replication, cell cycle, DNA mismatch repair, base excision repair and nucleotide excision repair pathways. In addition, the levels of HMGB2, Lamin B1 and TMPO were reduced in irradiated along with elevated expression of p16 (*CDKN2A*) and p21 (*CDKN1A*) – influencing the activation of cellular senescence in the irradiated cells. Histone reductions were also accompanied by an increase in global transcription including that of pro-inflammatory genes. This study concluded that chronic irradiation, at low dose rates, can promote cellular senescence indirectly possibly via an epigenetic route, limiting the tumorigenic events.

111. Sokolov and Neumann [S54] exposed four different lines of human embryonic stem cells to doses of 0.05 and 1 Gy from ^{60}Co gamma rays (a teletherapy unit was used but no dose-rate information was provided). The authors used one low-dose and one high-dose exposure in their experiments to determine the changes in gene expression at 2 or 16 hours post-irradiation. The data showed that the gene expression profiles are dose-, time- and cell type-dependent. About 50 genes were identified as potential early response signatures of high-dose radiation exposure across all the cell types studied. However, the authors identified substantial differences in the signalling pathways affected by low- and high-dose radiation. *CDKN1A* was shown to be up-regulated in all the cell types and after low-dose exposure (0.05 Gy). Commonly affected pathways are cell-cycle arrest, and p53 signalling in all the cell types.

112. Blood samples were collected from 8 healthy adult volunteers and peripheral blood mononuclear cells were exposed ex vivo to 300 mGy and 1 Gy of gamma rays (^{60}Co) at a dose rate of 0.4 Gy/min. Proteomic analysis was carried out by mass spectrometry after 1 and 4 hours after irradiation. Alterations of 23 proteins were observed in the irradiated cells compared to the control peripheral blood mononuclear cells with +/−1.5-fold change whereas three proteins showed >2.5-fold change. Redox sensor protein (chloride intracellular channel protein 1- CLIC-1), antioxidant protein (peroxiredoxin-6) and pro-survival molecular chaperone 78kDA (glucose regulated protein GRP78) were among the 23 modulated proteins after radiation exposure in peripheral blood mononuclear cells. The response seemed dose dependent where coefficient of variation calculated to be at 33.7% for 300 mGy and 48.3% for 1 Gy. Proteomic responses may help in understanding the cell protective mechanisms following irradiation [N16].

113. The human lymphoblastoid cell line AHH-1 was irradiated with 0.05, 0.2, 0.5, 2.0 and 10 Gy (^{60}Co gamma rays) at 1.7 Gy/min (for doses >0.5 Gy) or 9.6 mGy/min (for 0.2 and 0.05 Gy). Global transcriptional changes were analysed 4 hours after irradiation. It was found that doses as low as 0.05 Gy produced efficient induction of transcriptional changes including up-regulation of 25 genes, some of which are involved in signal transduction pathways (*BMPR2*, *GPR124*, *MAPK8IP2*, *AGGF1*) and the down-regulation of 18 genes. The dose range of 0.05 to 10 Gy induced changes in a number of radiation response genes, i.e. DNA repair gene *XPC, TP53I3* and immediate early response 5 gene in a dose dependent manner. The identification of such early response genes provides opportunity to develop biomarkers of radiation injury as well as to understand the mechanisms of their biological effects [L41].

114. Sanzari et al. [S9] exposed human thyroid epithelial cells (Htori-3) to high-atomic-number and energy (HZE) particles at a dose of 100 mGy from 1.0 GeV/nucleon Fe ions. The experiments were also conducted on cells treated with 5 μM selenomethionine to determine the protective effects of this antioxidant. Two hours after exposure to Fe ions, gene expression profiling was carried out on cells with or without selenomethionine treatment. Radiation exposure up-regulated the expression of certain classes of genes, namely secreted proteins of the chemokine/cytokine gene cluster (*CXCL1*, *CXCL2*, *IL6*, *IL11*, *IL8*, *IL24* and *TGFB2*). Selenomethionine treatment significantly decreased the expression of the above up-regulated genes. Selenomethionine counteracted the radiation-induced expression of several specific genes, *PLAU*, *IGFBP3*, *FOLR1*, *B4GALT1* and *COL1A1*. Validation of the altered expression of certain genes was carried out by qPCR. While the study specifically highlighted up-regulation of the genes belonging to selected pathways (chemokine-cytokine pathways), selenomethionine regulated these altered expressions as a countermeasure for some of the acute inflammatory/immune responses induced by low-dose HZE-particle irradiation.

115. It is clear that one of the key responses to ionizing radiation is the activation of cells to reprogramme gene expression. Previous studies have not distinguished between changes in RNA synthesis and RNA turnover. A recently developed BruDRB-seq (Bru-seq) methodology by Veloso et al. [V6] determines the contribution of synthesis as well as degradation of RNA to transcriptional responses. The technique allows the measurement of rapid changes in transcription rates alone without the influence of pre-existing, steady-state RNA and assessment of transcription originating from different promoters of multi-promoter genes. Exposure to human HF1 and HCT116 cells to 2 Gy X-rays (acute) revealed that ATM and p53 regulate both RNA synthesis and stability. Moreover, many genes in the p53-signalling pathway were coordinately up-regulated by both increased synthesis and RNA stability while down-regulated genes were suppressed either by reduced synthesis or stability. To distinguish between DNA damage and damage to other biomolecules (e.g. proteins) transcriptional responses were measured in cells in which DSBs were induced by restriction enzyme (AsiSI) or alternatively treated with Nutlin-3 to activate p53 [N10]. Comparing the top 200 genes showing induced transcription revealed 39 genes common between the three treatment groups (ionizing radiation, restriction enzyme, p53 activator) many of which were known as p53 target genes. A functional annotation analysis of the database for annotation, visualization and integrated discovery revealed that DSBs by restriction enzyme and p53 induction by Nutlin-3 lead to transcriptional changes of genes belonging to similar groups as 2 Gy ionizing radiation. This also suggests that it is p53 activation in response to DSBs that drives the transcriptional reprogramming after radiation exposure at moderate doses. This technique has the potential to define more precisely the effects of low-dose exposures on de novo gene transcription.

116. There are several reports of gene expression changes which have used moderate to high doses of radiation in their studies; these are summarized in table 4 below. Although high doses are used in these studies, the changes observed in the genes in response to radiation exposure may be relevant for low-dose exposures and are important in terms of understanding dose–response relationships. Currently there is no agreed gene expression pattern unique to either high- or low-dose exposures. Many of the genes

belong to the signalling pathways which have major implications in cancer development. Data obtained from radiotherapy patients are summarized as well.

2. Gene expression changes in vivo in experimental organisms and in cells from radiotherapy and cancer patients

117. Work with animals exposed to whole-body irradiation has revealed numerous gene expression changes, with many groups of genes affected by radiation in ways unique for specific animal genotype, tissues and organ(s), doses and dose rate. Similar to in vitro cell assays – even within the same tissue, no gene was found to be universally modulated by same or similar low-dose or low-dose-rate radiation exposures in all cases.

118. For example, the study conducted by Lee et al. [L13] used gamma rays at total dose of 0.2 Gy delivered at dose rate of 3.81 Gy/min for whole-body irradiation of 7-week-old female C57BL/6 mice. Several different tissues (brain, heart, lung, spleen and intestine) were collected one or three days after exposure and evaluated by immunohistochemistry for presence of p53 and p21 proteins and for numbers of apoptotic cells (TUNEL assay). At day 3, all tissues showed increased staining for p53 and p21 including those tissues (brain and heart) where apoptosis was not evident. Expression of 23 genes was explored by RT-PCR in all organs at day 3; the genes neogenine, *Apo1*, nuclease-sensitive element binding protein 1, syntaxin, cyclin G1, *hNOP56*, paraoxonase and glutathione peroxidase were found to be increased in all tissues, albeit less than twofold in most cases.

119. A study by Meadows et al. [M28] sought to determine gene signatures in peripheral blood of mice and humans and to determine the specificity of these signatures under different conditions. Mice of C57BL and BALB/c strains (5–10 mice per group 10–11-week old) were exposed whole body to ^{137}Cs gamma rays at doses of 0.5, 2 and 10 Gy. Human blood samples were obtained from healthy volunteers, patients who were exposed to 1.5 to 2 Gy (as part of pre-transplantation screening for stem cell transplantation and patients prior to chemotherapy – bloods from 18 healthy donors, 47 patients prior to haematopoietic stem cell transplantation, and 41 patients who had undergone alkylator-based chemotherapy only). DNA microarray analysis was carried out on these samples at specific timepoints. A clear pattern of gene expression was identified in mice without any influence of strains used in the study. However, sex did influence the accuracy of the assessment of radiation exposure at the lowest level of dose of 0.5 Gy. Gene signatures were able to distinguish the radiation exposure and sepsis conditions. Comparison of lymphocyte gene expression profiles of radiation-exposed individuals (1.5–2 Gy) and chemotherapy patients distinguished radiation exposure from chemical exposure. The study was more focused on distinguishing gene signatures than predicting cancer risk using such data [M28].

120. Meadows et al. [M29] identified gene signatures in peripheral blood lymphocytes following partial-body irradiation of mice. C57BL/6 mice were exposed to doses of X-rays from 0.5, 2 to 10 Gy (hardened by 0.1 mm Cu/2.5 mm Al filtration). The dose rates used were 1.4 Gy/min (anterior and posterior region) and 1.25 Gy/min (hindlimb region). Gene expression analysis indicated the radiation status with a high level of accuracy compared to the non-irradiated mice. The differential gene expression profiles for whole-body and partial-body irradiation were different – different sets of genes were affected. Since the gene profile changes are different for partial-body and total-body irradiation, partial-body signatures from total-body irradiated mice failed to completely predict the radiation status of partially irradiated animals or non-irradiated controls. However, the study can be applied in biomarker identification and biodosimetry in the case of partial-body exposure during therapy or accidents as they produce unique gene signatures, but authors suggest the use of multiple signatures [M29].

121. In a different study, 8-week-old male C57BL/6J mice were exposed to chronic whole-body gamma-ray radiation in two separate experiments. In the first one, dose rates used were 16.6, 0.858 or 0.042 mGy/d for 485 days, accumulating to total doses of 8, 0.4 and 0.02 Gy, respectively. In the second experiment, dose rates were 20.0, 1.00 or 0.050 mGy/d for 401 days accumulating to total doses of 8, 0.4 and 0.02 Gy, respectively [U1]. Gene expression profiles in the liver of six animals from each exposure group were compared individually with pooled sham-irradiated animals using microarrays; findings were confirmed by RT-PCRs. Numbers of genes modulated more than 1.5-fold in mice irradiated with total dose of 8, 0.4 and 0.02 Gy were 20, 11 (nine of them shared with 8 Gy mice) and 3 (all of them shared with 0.4 Gy and 8 Gy mice), respectively. Most of these genes were down-regulated (*Usp2*, *Dbp*, *St3gal5*, *Zbtb16*, *Lcn13*, *Herpud1*, *Rapgef4*, *Slc22a5*, *Hspa1b*, *Serpina4-ps1*, *Angptl4*, *Slc25a30*, *Socs2*, *Alas1*, *Pim3*, *Lpin2*, *Gne*, *Acot1*) and only two in the 8 Gy group (*Camk1d* and *D9wsu90e*) and one (Pde4b) in the 0.4 Gy group were increased compared to controls. The reported bioinformatics analysis suggested that these genes may be involved in obesity and tumorigenesis.

122. Seven-week-old C57BL/6 mice were X-ray irradiated at high-dose rate of 1.03 Gy/min or continuously exposed with a dose rate of 3.09 mGy/min to total doses of 1.1, 2.2 or 4.4 Gy and their blood drawn 24 hours later for RNA isolation [P8]. After microarray analysis and RT-PCR confirmation of select transcripts, mRNAs with similar or opposite patterns of expression dependent on dose rate were identified; a group of 164 genes could be used as a classifier for different exposures.

123. Six- to seven-week-old female Institute of Cancer Research (ICR) mice were exposed to 0.25, 0.5 or 1 Gy of gamma rays delivered at dose rate of 500 mGy/h or 500 mGy/min and sacrificed after four hours [M13]. Skin samples were collected, and RNA used for RT-PCR analysis. A group of 80 genes participating in regulation of extracellular matrix or acting as adhesion molecule genes was tested; in most cases gene expressions did not follow dose rate or dose differences. *MMP2* and *MMP15* showed most change in response to 500 mGy/h dose rate and decreased with the increase of dose, while *MMP1* and *MMP9* responded best to 500 mGy/min dose rate and increased with total dose. This study suggests that exposure to high-dose-rate photon radiation induced oxidative stress and extracellular matrix-associated alterations in gene expression profiles.

124. Expression of DNA repair genes in thymi of low dose rate irradiated AKR/J mice was the focus of a study by Bong et al. [B50]. This work compared AKR/J mice housed in a low-dose-rate/high-total-dose (^{137}Cs, 0.7 mGy/h, 2.1 Gy) gamma irradiation facility with sham or high dose rate (^{137}Cs, 0.8 Gy/min, 4.5 Gy) irradiated mice. The thymi were isolated, at the conclusion of irradiation in low dose rate exposed mice, or 130 days after acute irradiation in high dose rate mice. Genes including *MSH2*, *MSH3*, *ERCC8*, *ERCC6*, *GTF2H2*, *OGG1*, *LIG4*, *NEIL3*, *UNG*, *LIG1*, *RAD51L1*, *MBD4*, *TERF2*, *H2AX*, *ATM*, *TFRC*, *FTH1*, *RRM1*, *RRM2*, *RRM2B, P53*, *BAX* and *PARP1* were up-regulated according to RT-PCR evaluation in both groups of mice (albeit to different degrees, and with most of the genes over- or under-expressed by less than threefold. Low dose rate exposed animals developed fewer thymic lymphomas and lived longer; moreover, an increased expression of DNA repair-associated genes, *LIG4*, *RRM2*, *H2AX* and *ATM*, was noted at 130 days after the start of low-dose-rate irradiation, although no functional DNA repair data were presented.

125. Mammary tissue differences, both up-regulation and down-regulation in expression of lncRNAs were studied in BALB/c and SPRET/EiJ mice, known to have different susceptibilities to breast cancer due to non-homologous-end-joining of DSBs differences between the strains, were studied by Tang et al. [T10] using microarray analyses. Baseline inter-strain differences between non-irradiated controls in this work were significant with nearly 300 lncRNAs and 600 mRNA differentially expressed. X-ray irradiation to a single dose of 100 mGy was carried out with animals aged 8 to 9 weeks and mammary tissues were collected for analyses 2, 4 and 8 weeks after exposure for BALB/c mice and at four weeks for SPRET/EiJ mice. Several interesting considerations were employed in this work – for example,

lncRNA expression was internally verified by correlating it to expression of a dependent mRNA (1,337 of 1,338 lncRNAs identified to be differentially expressed in response to 100 mGy exposure in either strain were verified in this manner); gene groups associated with changes of expression compared to controls were "subtracted" for genes regulated by the oestrus cycle. Importantly, more than 3,000 genes are modulated by oestrus cycle, so after this subtraction groups of about 300 lncRNAs and mRNAs were found to be "specific" outcomes of radiation exposure. Several gene expression pathways were associated with low-dose exposure in each strain; interestingly, enrichment for inflammatory response genes was noted in both strains [T10].

126. A study by Cassie et al. [C7] used C57BL/6 mice divided into controls, acutely exposed group (500 mGy at a dose rate of 2 mGy/s), and a group subjected to protracted exposure (50 mGy/d for 10 days). At the start of the experiment, animals were 45 days old and they were sacrificed three hours after the final exposure given to the mice exposed to protracted radiation. Spleens from irradiated animals showed signs of both apoptosis and cell division with some differences between two sexes. Apoptotic index of spleen cells was greater in males regardless of the means of exposure, and as a consequence cell proliferation was greater in males as well. Differences between the two types of exposure were noted as well. The most pronounced sex differences were found in expression of *Ki67* in spleen of mice acutely exposed to 500 mGy and expression of *PCNA* in spleens of mice exposed to 500 mGy over 10 days.

127. Snijders et al. [S50] compared BALB/c mice (breast cancer prone) with C57BL/6 mice (resistant by comparison), for gene expression in mammary tissue and blood cells. Exposures of 75 mGy and 1.8 Gy were given weekly, for 4 weeks (total doses of 300 mGy and 7.2 Gy). Strain and exposure differences were numerous but with substantial overlaps; the more resistant mice showed down-regulation of *SOX9*, which is associated with breast cancer. The sensitive strain showed early transcriptional responses in pathways involving immune response (diminished), cell stress (increased) and altered TGFβ signalling and abnormal expression of developmental genes. The study provides evidence for cancer-risk prediction based on the appropriate gene expression changes following radiation exposure.

128. Yi et al. [Y8] studied proteins by 2D gel electrophoresis in samples taken from chronically exposed C57BL/6J male mice (6 weeks of age, 40 mice in 4 groups). The exposures took place over 180 days (22 hours per day); the dose rates used were <50, 50–500 and 500–1,000 μGy/h from ^{137}Cs gamma rays. The higher dose rates produced pathological changes in the liver. 2D analysis showed that with increasing dose rates 20, 21 and 28 proteins had 1.5-fold changes in their expression compared to control. More stringent analysis showed that about 22 proteins had >2.5-fold changes in their expression. These proteins belong to the pathways such as cytoskeleton, cell metabolism, biological defence, mitochondrial damage, detoxification and tumorigenesis. The authors validated the expression of one protein, calreticulin, which is involved in stress reactions in the mouse liver as a marker of low-dose-rate exposure. They concluded that calreticulin may be a marker of exposure and may be used to predict the risk of radiation-induced liver tumorigenesis in mice.

129. Hurem et al. [H41] used the vertebrate model of zebrafish to determine the toxic effects as well as the transcriptomic changes of radiation exposure. Fish embryos were exposed to gamma rays over 3 to 92 hours beginning with 2.5 hours post-fertilization, with dose rates varying between 0.4 and 38 mGy/h for toxicity assays and 0.54, 5.4 and 10.9 mGy/h for transcriptomic analysis. Total doses ranged between 1.6 to 3,496 mGy after 43.8, 92 and 3 hours exposures. The survival of fish following irradiation at a dose rate of 38 mGy/h was significantly reduced, and viable embryos showed developmental changes following exposures at all dose rates. RNA sequencing analysis was carried out at 5.5 hours post-fertilization. There was a dose–response relationship in the total number of differentially expressed genes in the exposed groups starting at a dose rate of 0.54 mGy/h. Pathway analysis indicated retinoic acid receptor activation, apoptosis and glutathione mediated detoxification signalling as the most affected pathways at low dose rates, while eif2 and mTOR involved in the modulation of angiogenesis were

affected at high dose rates. Altered expression of *Myc*, *Tp53*, *Tnf*, *Hnf4a*, *Tgfb1* and *Cebpa* were identified as the most radiation responsive genes that also affect the developmental processes in zebrafish.

130. Zhikrevetskaya et al. [Z18] exposed fruit flies to low and moderate doses of gamma rays (50 to 400 mGy) at a dose rate of 36 mGy/h. Flies were exposed for 1 hour 23 minutes, 2 hours 47 minutes, 5 hours 34 minutes and 11 hours 8 minutes, resulting in doses of 50, 100, 200 and 400 mGy, respectively. Changes in lifespan and expression of stress-sensitive genes were identified. The authors found extended lifespan for males exposed to 50 mGy and in females exposed to 400 mGy but a lifespan decrease in females at lower doses. Changes in stress gene expression were not dose-dependent. All in all, the study showed a non-linear response to low-dose radiation exposure and sex-specific radiation resistance in *Drosophila melanogaster*.

131. In a study by Berglund et al. [B29] skin biopsies from five prostate cancer patients were taken from the patients before and over a 24-hour period after radiotherapy to determine the radiation response after a single exposure ex vivo to a dose of 100 mGy. Previously known gene groups and pathways were selected in the expression analysis. Significant transient transcriptional changes in the human tissue samples were identified in nine gene groups which returned to baseline by 24 hours post-exposure. Genes involved in DNA repair and tissue remodelling, cell-cycle transition and inflammation show significant changes in expression with variability between patients. The authors concluded that the data may serve as a reference for the temporal response dynamics following low-dose radiation exposure.

132. Albrecht et al. [A10] carried out ex vivo irradiation of skin from two adult female patients undergoing abdominoplasty. Skin samples were viable for six weeks in culture under appropriate conditions. Skin was exposed to low (0.05 Gy) or high (5 Gy) doses of X-rays at a dose rate of 4 Gy/min. Microarray analysis was carried out on irradiated skin samples at 0, 2, 8 and 30 hours post-exposure and also on non-irradiated skin samples. The study revealed qualitative and quantitative differences in the responses to low- and high-dose radiation. Three different categories of changes were identified: *(a)* unique genes responsive to doses of either 0.05 or 5 Gy but not both, and also showing dependence on time in culture; *(b)* specific genes that respond to doses of either 0.05 or 5 Gy but not both and not dependent on time in culture; and *(c)* dose-dependent responsive genes. While exposure to a dose of 0.05 Gy induced transient changes in gene expression, the higher dose at 5 Gy resulted in persistent gene expression changes. Of all the changes, the authors highlighted that neither p53- nor TGFβ-targeted genes were modified at low dose and this claim suggests a p53-dependent DNA damage response.

133. Using the whole genome gene exposure approach, Abend et al. [A4] have identified and selected genes for further disease association in the cohorts from Chernobyl and Mayak. Dose–response relationships (dose-to-gene) and clinical outcome relationships (gene-to-effect) were analysed to determine whether such information can be utilized to understand mechanism of cancer progression, to develop radiation protection measures or for clinical purposes. The authors report that performing molecular radiation epidemiological studies is more challenging and expensive than doing conventional epidemiological studies, however, such studies have the potential for improving our understanding of biological mechanisms underlying health effects of radiation and identification of new and clinically relevant molecular targets for diagnosis and/or therapeutic intervention.

134. Pernot et al. [P13] highlight the importance of using established radiation biomarkers to identify health risks. This approach will be helpful in determining the magnitude of long-term health consequences of low dose and low dose rates (below 100 mSv and/or 0.1 mSv/min). Most of these biomarkers have been in use to investigate exposure, effects and susceptibility to ionizing radiation. The authors under the framework of DoReMi (Low Dose Research towards Multidisciplinary Integration) propose a classification of biomarkers that are relevant for molecular epidemiology with special reference to disease manifestation late in life.

135. The literature considered in this section and the table 4 below provide strong evidence that ionizing radiation at high and low doses, chronic or acute exposure and of varying qualities provide changes in gene expression in many cell types. These studies have yielded some reliable quantitative biomarkers of radiation exposure that have been applied for biodosimetry. While some studies provide evidence of specific responses to low and high doses, and low and high dose rate, there is no consensus on a gene expression profile to distinguish low- from high-dose exposure nor low-dose-rate from high-dose-rate exposure. Cancers have gene expression patterns different from those of normal cells, and many of the genes, the expression of which can be modulated by ionizing radiation, are among those dysregulated in cancers. Therefore, some of the gene expression changes following low-dose radiation exposure may relate to carcinogenesis; however, as noted, many studies consider only 24–48 hours post-exposure and this makes it difficult to relate the observed effects directly to cancer development. It should be noted that a range of analytical methods have been used in these studies, and the methods vary in sensitivity and range of genes assessed. There continue to be developments and refinements in the available methods to quantify gene expression, and it may be that these refined methods are able to define expression profiles characteristic of specific exposure scenarios. This will require consideration of inter-individual variations in response and cell type dependence of responses.

Table 4. Summary of gene/protein expression literature considered in this evaluation

Cell type/model	*Irradiation conditions*	*Outcomes recorded*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*	*Reference*
Human Blood lymphocytes	X-radiation Dose: 2, 4 Gy Dose rate: 0.7 Gy/min	Gene expression analysis at 2 and 24 hours post-exposure	The study has identified genes which are consistently up-regulated following exposure to 2 or 4 Gy at different timepoints. Down-regulated genes included cyclins, centromeric and mitotic checkpoint with direct relevance to chromosome instability and cancer	Sestrin 1 (*SESN1*), growth arrest and DNA damage inducible 45 alpha (*GADD45A*), cyclin dependent kinase inhibitor 1A (*CDKN1A*), cyclin G1 (*CCNG1*), ferredoxin reductase (*FDXR*), p53 up-regulated mediator of apoptosis (*BBC3*) and Mdm2 p53-binding protein homolog (*MDM2*)	[K1]
Human whole blood	Natural background radiation (200 to 3 700 Bq/m^3)	Changes in mismatch repair genes	Activation of mismatch repair system	*MLH1* and *MSH2*	[B5]
Human Mononuclear cells from blood	Chernobyl Nuclear Power plant area Exposed to 0.18 to 49 mSv over 11–13 years	Cytokine and receptor arrays mRNA expression especially TGF receptor, EB13 and CD40 ligands	Variable expression patterns for genes and cytokines in the low-dose-irradiated individuals. More frequent expression of serine/threonine protein kinase receptor, TGF receptor, EB13 and CD40 ligands in the exposed individuals. Authors suggest appropriate use of statistical tools to evaluate the data generated	Cytokines, mRNA, receptor arrays	[A9]
Human Thyroid cancer samples	Patients with sporadic thyroid cancers Patients who were exposed to radiation in Chernobyl	Gene expression	Molecular signatures reflecting specific responses to gamma radiation in patients were identified; about 118 gene signature discriminated radiation exposure		[D16]
Human Thyroid cancer tissues	Thyroid cancer patients from Chernobyl area Dose: 0.15–9 Gy	Gene expression analysis	646 up-regulated and 677 down-regulated genes were identified in discriminating thyroid cancers which have history of radiation exposure	*SFRP1, MMP1, ESM1, KRTAP2-1, COL13A1, BAALC* and *PAGE1*	[P21]
Human whole blood	Occupational exposure at Mayak Product Association Gamma radiation Dose: ~0.5–1.01 Gy ^{239}Pu: ~0.7 kBq	Gene expression profiling – microarray as well as microRNA	A set of 376 candidate genes – 80 up-regulated and 296 down-regulated – associated with ^{239}Pu exposure Down-regulation in ^{239}Pu exposure; up-regulation in gamma ray exposed	mRNA and microRNA	[A3]

Cell type/model	*Irradiation conditions*	*Outcomes recorded*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*	*Reference*
Human Blood lymphocytes from radiation workers	Occupational exposure Absorbed dose: 0.696–39.088 mSv	Gene expression profiling	A total of 78 genes (21 up-regulated and 57 down-regulated) involved in several biological processes such as ubiquitin cycle, cell cycle regulation/proliferation and stress response. Alterations in these gene products may have implication on long-term genetic effects on these workers	*UHRF2, PIAS1, LIG3, XPA, ERCC5, Rad52, DCLRE1C, RHOA, CABLES2, TGFB2, IL16, GSTP1, PPP2R5A, DUSP22*	[F2]
Human Exfoliated oral epithelial cells	X-radiation Dose: 23.4 mGy during dental examination	Selected markers H2AX and pCHK2	Both the markers were up-regulated after dental X-ray exposure	*H2AX* and *pCHK2*	[Y11]
Human peripheral blood lymphocytes	Diagnostic procedures – computed tomography, angiography	Selected marker γH2AX	Significant γH2AX positivity in exposed individuals Multiple CT scans produced higher DNA damage	γH2AX	[K23]
Human peripheral blood lymphocytes	Diagnostic neuro-interventional procedures Dose: – forehead 4.9 to 273 mGy – eyes 14–98 mGy – shoulders 8–433.3 mGy – thyroid 4.7–242.5 mGy)	γH2AX and p53 Selected gene expression profiling	Increased phosphorylation of γH2AX and p53 in exposed samples Differential regulation of selected genes	*CDKN1A, FDXR, BAX, DDB2, SESN1, BCL2, MDM2, TNFSF10B, PCNA GADD45A, ATM* and *TNFSF9*	[V13]
Human Mononuclear cells	Gamma radiation (^{60}Co) Dose: 20, 50, 100 mGy Dose rate: 13.7 mGy/min	Expression of apoptotic genes at 4, 24, 48, 72 and 168 hours following irradiation	Low doses of gamma radiation induced down-regulation of Bax immediately after irradiation which is restored to normal level by 168 hours. Up-regulation of Bcl-2 was seen in most of the doses	*BAX* (pro-apoptotic) *Bcl-2* (anti-apoptotic) Bcl	[A29]
Human Peripheral blood lymphocytes	X-radiation Dose: 0, 0.5, 1 Gy Dose rate: 30 mGy/min	Global gene expression profiling	p53 signalling and DNA damage response was evident at high doses, leading to the activation of cell-cycle arrest and apoptosis processes. However, at low doses there appears to be a mixed response – in addition to DNA damage, the innate and adaptive immune responses are induced through the activation of MAPK, NFκB, chemokines and cytokines	DNA damage, p53 signalling, apoptosis	[E15]

Cell type/model	*Irradiation conditions*	*Outcomes recorded*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*	*Reference*
Human Blood lymphocytes	Gamma radiation Dose: 100, 250, 500 mGy	Gene expression profiling	Dose specific changes in specific gene pools – *CYP4X1*, *MAPK10* and *ATF6* (100 mGy) *DUSP1* and *RAD51L1* (250 mGy) and *RAD50*, *REV3L* and *DCLRE1A* (500 mGy) The authors report that they have identified a set of 34 significant genes which are common for all the doses – some up-regulated and some down-regulated	*SERPINB2* and *C14orf104* – up-regulated *CREB3L2*, *DDX49*, *STK25* and *XAB2* – down-regulated	[F1]
Human Blood lymphocytes	X-radiation Dose: 0.56, 2.23, 4.45 Gy Dose rates: – acute: 1.03 Gy/min – low-dose-rate: 3.1 mGy/min	Gene expression at 24 hours post-exposure	There were 454 genes which were differentially expressed for low-dose-rate exposure whereas 598 genes were differentially expressed after acute exposure. Genes related to immune processes and B cell-mediated immunity were shown to be affected. Study concluded that low-dose-rate exposure produced distinctive gene expression patterns compared to high dose rate	Immune processes and B cell-mediated immunity; NK cell activation and cell-to-cell signalling; p53 pathway was enriched at both low- and high-dose-rate exposures	[G4]
Human Blood lymphocytes	Gamma radiation (^{60}Co) Dose: 0.0, 0.05, 0.5 Gy Dose rate: 0.45 Gy/min	Gene expression analysis at 3 and 24 hours post-exposure	Genes *BAX*, *PCNA*, *GADD45*, *DDB2* and *CDKN1A* were induced at 24 hours. $CD4^+$ cells displayed down-regulation of genes even at 0.05 Gy – genes involved in protein biosynthesis and oxidative phosphorylation. Conclusion: $CD4^+$ cells may provide useful information in the biological processes affected after irradiation	Different subgroups of lymphocytes such as $CD4^+$, $CD8^+$, $CD56^+$ cells	[G18]
Human Blood lymphocytes	Gamma radiation (^{137}Cs) Dose: 0.02, 0.1, 0.5, 1.0, 2.0, 4.0 Gy Dose rate: 0.7 Gy/min	Gene expression at 24 and 48 hours (0.02, 0.1 Gy) and 6, 24 or 48 hours (0.5, 1.0, 2.0 and 4.0 Gy)	Expression alterations increased with increasing dose and time after exposure. The authors concluded that it is possible to use gene expression profiling data to determine radiation exposures as low as 20 mGy	Differentially (positively or negatively) expressed genes were assigned to pathways such as nucleosome assembly, apoptosis and DNA repair response; nine signature genes were identified (*MKL2*, *FDXR*, *C10orf39*, *PFKFB3*, *FLJ35379*, *LY6G5C* and 3 other genes with no gene symbols available (Agilent ID A-24_P506680, _A_32_P138939, A_32_P20997))	[K29]
Human Blood lymphocytes	X-radiation Low dose: 5, 10, 20 50, 75, 100 mGy exposed at dose rate of 4.9 mGy/min Moderate to high dose: 0.5, 1, 2, 4 Gy exposed at dose rate of 0.5 Gy/min	Dose response analysis for gene expression changes	Dose response curves for high doses (0.5 to 4 Gy) were constructed for gene expression changes. Individual variation was noticed in both high- and low-dose exposures	*FDXr*, *DDB2* and *CCNG1*	[M10]

Cell type/model	*Irradiation conditions*	*Outcomes recorded*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*	*Reference*
Human Blood lymphocytes	Gamma radiation Dose: 0.5, 2, 5, 8 Gy	Gene expression profiling at 6 and 24 hours post-irradiation	The study identified 74 gene signature which was distinguishable between the four different radiation doses – where more than ⅓ genes were regulated by p53	Validated genes – *CDKN1A, FDXR, SESN1, BBC3, PHPT1* Full list of 74 genes (see table 1 in [P6])	[P6]
Human Blood lymphocytes	Gamma radiation (^{137}Cs) Dose: 1, 2, 4 Gy Dose rate: 0.8 Gy/min	Gene expression profiling	24 of 102 genes identified as markers of radiation showed dose response pattern. 15 genes (*CD69, CDK1A, EGR1, EGR4, FLJ35725, hCG2041177, hCG16434662, IFNG, ISG20L, CJUN, MDM2, MUC5B, PLK2, RND1, TNFSF9)* up-regulated, and 9 genes (*GRIK3, OCLN, RPL10A, SERHL2, SGK3, STARD13, hCG2038519, hCG1998530,* hCG1985174) down-regulated	*EGR1, EGR4, IFNG, CJUN* and *TNFF9* – correlated with absorbed dose of 1 to 4 Gy	[T23]
Human Blood lymphocytes	Gamma radiation (^{137}Cs) Dose: 1, 2, 4 Gy Dose rate: 0.8 Gy/min	Gene expression profiling at 2 hours post-irradiation	Pathways affected – alterations in cellular structure (*Bactin, Talin 1, Talin 2, Zyxin-2*), immune and defence reactions (*MBP2*, *IL17E* and *IFNG*), cell cycle control (*CDKN1A, MDM2, ANXA6), GADD45A, PCNA, DUSP)*, detoxification process *(per1)* and apoptosis *(BBC3)*		[T24]
Human Fibroblasts (primary) and a prostate epithelial cell line	Acute: Gamma radiation (^{137}Cs) Dose: 0.1 and 2 Gy Dose rate: 0.48 Gy/min Chronic: Gamma radiation (^{57}Co) Dose: 10–24.5 mGy Dose rate: 17.0 µGy/min	Clonogenicity assay and gene expression profiling at 24 hours	No measurable effects at 100 mGy 396 genes were differentially expressed for both acute and chronic irradiation. 106 genes were down-regulated at acute 2 Gy exposure	Pathways affected were cell cycle, chromosomal movement, cell survival and DNA replication, recombination and repair as well as *CDKN2A* and *P53*	[M26]
Human Fibroblasts and immortalized cells	Gamma radiation (^{137}Cs) Dose: 0.5, 1.5, 4.5 Gy Dose rate: 0.84 Gy/min	Colony formation and gene expression profiling	Inactivation of colony forming ability at 1.5 Gy. DNA metabolism affected; p53-dependent G_1 checkpoint response altered	*CDK2*, *CCNE1*, *CDC6*, *CDC2*, *MCM2* - down-regulated at 24 hours	[Z20]
Human 3D tissue model mimicking human epidermis	Low-LET protons Dose: 2.5, 0.1 Gy	Gene expression	The genes involved in the recovery and tissue repair were altered at the low dose while the high dose affected the genes involved in structural integrity and terminal differentiation	*TP53* for high dose and *HNF4A* after low-dose exposure	[M31]

Cell type/model	*Irradiation conditions*	*Outcomes recorded*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*	*Reference*
Human Skin model	X-radiation Dose: 0.1, 2, 10 Gy Dose rate: 0.033, 0.6, 1.6 Gy/min, respectively	3 or 8 hours post-exposures transcript analysis by RNA sequencing	1 701 genes were significantly affected by high doses and 29 genes at doses 2 and 10 Gy but not at 0.1 Gy at both 3 or 8 hour timepoints. Very few genes were altered at 0.1 Gy	*BBC3, PPM1D, FDXR, GADD45A, MDM2, CDKN1A, TP53inP1, CYCSP27, SeSN1, SESN2, PCNA* and *AEN*	[T13]
Human 3D skin tissue model	X-radiation Dose: 100 mGy Dose rate: 6.3 mGy/min	Gene expression profling	>1 400 genes differentially expressed in the dermis while >400 genes in the epidermis. Processes such as cell cycle regulation, immune response, hypoxia, reactive oxygen signalling and DNA damage repair were affected. Points out to the role of dysregulated cell proliferation in inducing cancer by radiation		[V14]
Human skin keratinocytes	Varian 2100 C linear accelerator Dose: 1 cGy or 10 cGy Dose rate: 80 cGy/min	Proteomic analysis of selected proteins on 1 or 4 days after exposure	Seven proteins showed significant changes after radiation exposure	EFABP, Alpha-enolase, HINT-1, HSP27, LDH-1, PDI and S100A9	[B30]
Human dermal fibroblasts	Gamma radiation (^{137}Cs) Dose: 0.1 Gy	microRNA analysis	De-regulated microRNAs are associated with early or late radioadaptive response Regulation of collagen-synthesis related genes	*COL1A1, MMP1*	[B2]
Human neonatal foreskin, adult facial skin cells, RPE-1 cells	Gamma radiation (^{137}Cs) or X-radiation Dose: 1–3.4 Gy Dose rate was 6–20 mGy	Histone analysis, protein expression studies	Reduction of histone levels, reduction in the levels of non-histone chromatin associated proteins, cell cycle, DNA replication, proinflammatory genes	HMGB, SMC, MCM2-7, CDKN2A (p16), CDKN1A (p21)	[L45]
Human Embryonic stem cells	Gamma radiation (^{60}Co) Dose: 0.05 and 1 Gy	Gene expression profiling at 2 or 16 hours post-exposure	Gene expression profiles were dose, time and cell type-dependent. 50 early responsive genes for high dose were identified. Substantial differences in the pathways affected at low- and high-dose exposures	*CDKN1A* increased at low-dose exposure; common pathways – cell-cycle arrest, p53 signalling	[S54]
Human peripheral blood mononuclear cells	Gamma radiation (^{60}Co) Dose: 300 mGy and 1 Gy Dose rate: 0.4 Gy/min	Proteomic analysis	Alterations in 23 proteins in irradiated samples with 1.5-fold change and 3 proteins showing 2.5-fold change	GRP78, Peroxiredoxn-6, CLIC-1	[N16]
Human lymphoblastoid cell line	Gamma radiation (^{60}Co) Dose: 0.05, 0.2, 0.5, 2, 10 Gy Dose rate: 1.7 Gy/min	Gene expression profiling at 4 hours after irradiation	Transcriptional changes in 25 genes at 0.05 Gy	*BMPR2, GPR124, MAPK8P2, AGGF1, XPC, TP53I3*	[L41]

Cell type/model	*Irradiation conditions*	*Outcomes recorded*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*	*Reference*
Mouse Brain, heart, lung, spleen and intestine	Gamma radiation Dose: 0.2 Gy Dose rate: 3.81 Gy/min	Immunohistochemistry for selected genes and gene expression profiling	Increased expression of p53 and p21 at day 3 Out of 23 genes tested, 8 genes were found to be increased in all tissues at day 3	p53 and p21; neogenine, Apo1, nuclease-sensitive element binding protein 1, syntaxin, cyclin G1, hNOP56, paraoxonase and glutathione peroxidase	[L13]
Human and mouse Blood lymphocytes	Gamma radiation (^{137}Cs) Dose: 0.5, 2, 10 Gy for whole-body irradiation Dose: 1.5–2.0 Gy for pre-transplantation screening or patients prior to chemotherapy (human samples)	Gene expression profiling	A clear pattern on gene specific changes without any influence of mouse strains used but sex of the animals did influence the outcome of radiation exposure in mice. Though the goal of the human lymphocyte experiments is to distinguish gene signatures rather than predicting cancer risk		[M28]
Mouse Peripheral blood lymphocytes	X-radiation Dose: 0.5, 2.0, 10 Gy Dose rate: – 1.4 Gy/min for anterior/posterior region – 1.25 Gy/min for hindlimb region	Gene expression for partial-body and whole-body irradiation	Gene expression analysis was useful in comparing irradiated and non-irradiated mice but not helpful in discriminating whole-body and partial-body irradiation in mice		[M29]
Mouse	Chronic whole-body gamma irradiation Dose: 8 030, 416, 20.6 mGy Dose rate: 16.6, 0.858, 0.042 mGy/day for 485 days Dose: 8 015, 401, 20 mGy Dose rate: 20, 1, 0.05 mGy/day for 401 days	Gene expression profiling in liver	Bioinformatics analysis revealed that the genes which are differentially expressed may be involved in obesity and tumorigenesis	*Usp2, Dbp, St3gal5, Zbtb16, Lcn13, Herpud1, Rapgef4, Slc22a5, Hspa1b, Serpina4-ps1, Angptl4, Slc25a30, Socs2, Alas1, Pim3, Lpin2, Gne, Acot1, Camk1d, D9Wsu90e* and *Pde4b*	[U1]
Mouse	Gamma radiation Dose: 1.1, 2.2 or 4.4 Gy High dose rate: 1.03 Gy/min Low dose rate: 3.09 mGy/min	Gene expression profiling in blood after 24 hours	Same genes were differentially expressed in both low- and high-dose-rate exposures		[P8]

Cell type/model	*Irradiation conditions*	*Outcomes recorded*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*	*Reference*
Mouse	Gamma radiation Dose: 0.25, 0.5, 1 Gy Dose rate: 500 mGy/h, 500 mGy/min	Gene expression on skin samples at 4 hours post-exposure	A group of 80 genes in regulation of extracellular matrix (adhesion molecule genes) were tested. No dose rate or dose differences were observed. *MMP2* and *MMP15* displayed most changes in expression with a decrease at 500 mGy/h in a dose-dependent manner. *MMP1* and *MMP9* increased with dose at 500 mGy/min		[M13]
Mouse	Gamma radiation Dose: 2.1, 4.5 Gy Dose rate: 0.7 mGy/h, 0.8 Gy/min	Gene expression in thymus	Genes involved in repair in thymus - Lig4 and RRM2 are overexpressed 130 days after low-dose-rate exposure. Many additional genes including MSH2, MSH3, ERCC8, ERCC6, GTF2H2, OGG1, LIG4, NEIL3, UNG, LIG1, RAD51L1, MBD4, TERF2, H2AX, ATM, TFRC, FTH1, RRM1, RRM2, RRM2B, P53, BAX and PARP1 were up-regulated according to RT-PCR	Including *MSH2, MSH3, ERCC8, ERCC6, GTF2H2, OGG1, LIG4, NEIL3, UNG, LIG1, RAD51L1, MBD4, TERF2, H2AX, ATM, TFRC, FTH1, RRM1, RRM2, RRM2B, P53, BAX* and *PARP1*	[B50]
Mouse	X-rays Dose: 10 cGy	mRNA and lncRNA 2, 4, and 8 weeks post-exposure	Genes involved in tissue injury inflammatory responses and mammary gland development. Influence of genetic background in the response		[T10]
Mouse	X-rays acutely exposed group Acute: Dose: 500 mGy Dose rate: 2 mGy/s protracted exposure (50 mGy/d for 10 days)	Selected gene/protein expression	Gender differences in the expression of genes important in cell proliferation	PCNA, RB, RBBP9	[C7]
Mouse Mice with differential susceptibility to breast cancer development	X-radiation Dose: 75 mGy, 1.8 Gy for 4 weeks Dose rate: 196, 783 mGy/min	Gene expression profiling	Differential expression of genes involved in breast cancer tumorigenesis in these strains; study provides evidence to predict breast cancer risk following changes in gene signatures	*SOX9* down-regulation	[S50]
Mouse	Chronic gamma radiation (^{137}Cs) Dose rate: 50–1 000 µGy/h (22 hours per day for 180 days)	Protein expression	69 proteins – 1.5-fold changes and 22 proteins with >2.5-fold changes were identified. Pathways affected are cytoskeleton, cell metabolism, biological defence, mitochondrial damage, detoxification and tumorigenesis. They propose calreticulin as may predict radiation-induced liver tumorigenesis in mice	Calreticulin	[Y8]

Cell type/model	*Irradiation conditions*	*Outcomes recorded*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*	*Reference*
Zebrafish	Gamma radiation (^{60}Co) Dose: 1.62–3 496 mGy Dose rate: 0.4, 3.9, 15, 38 mGy/h	Survival and RNA sequencing	Survival reduced at 2 to >10 mGy/h Dose–response relationship in the number of differentially expressed genes in the exposed group starting at a total dose of 1.62 mGy; low dose rates affected retinoic acid receptor activation, apoptosis and glutathione mediated detoxification signalling whereas modulation of angiogenesis (eif2 and mTOR) at high dose rates	*myc, tp53, TNF, hnf4a, TGFB1* and *cebpa* – most relevant downstream targets affected	[H41]
Fruit fly	Gamma radiation (^{226}Ra) Dose: 50, 100, 200, 400 mGy Dose rate: 36 mGy/h	Lifespan and gene expression profiling	Changes in lifespan and stress sensitive genes were identified. They report "hormesis" after exposure to doses of 50, 100 and 400 mGy		[Z18]
Human Skin biopsies from prostate cancer patients	X-radiation Dose: 100 mGy Dose rate: 6.0 Gy/min before therapy	Gene response to 100 mGy as well as after radiotherapy (3, 8 and 24 hours)	Transient transcriptional changes in previously known radioresponsive genes	DNA repair, tissue remodelling, cell-cycle transition and inflammation pathways	[B29]
Human Skin from patients undergoing abdominoplasty	X-radiation Dose: 0.05, 5 Gy Dose rate: 4 Gy/min	Gene expression at 0, 2, 8, and 30 hours	Transient changes in gene expressions at low-dose and persistent changes at high dose. No changes in *TGFB* or *TP53* at low dose		[A10]
Human Thyroid epithelial cells	Fe ions Dose: 100 mGy from 1.0 GeV/n	Radiation protection by selenomethionine and gene expression profiling	Up-regulation of genes in the chemokine/cytokine gene cluster (*CXCL1, CXCL2, IL6, IL11, IL8, IL24* and *TGFB2*)		[S9]
Skin biopsies from prostate cancer patients	Radiation type not given in paper, but prior publications suggest 11 and 15 MV photon fields) Dose: 0.015 to 1.10 Gy fractionated radiotherapy treatments for 7 days	γH2AX and 53BP1 staining before radiation exposure and at 30 min, 2 to 72 hours post-exposure	Dose-dependent activation of γH2AX and 53BP1 in skin biopsies Persistence of hyper-radiosensitivity	γH2AX and 53BP1	[Q2]

E. DNA repair and effects on somatic cells

136. The repair of radiation damage to DNA has been considered in detail in the UNSCEAR 2000 Report, annex F, "DNA repair and mutagenesis" [U5]. This report concluded that DNA repair and related damage response functions were of crucial importance for radiation carcinogenesis, and that mutations in DNA repair/response genes play an important role in the maintenance of genome stability. In particular, the importance of the response to DNA double-strand breaks and complex lesions in determining dose, dose rate and radiation quality effects was noted. No evidence was found that DNA repair functions were entirely error-free at low exposure levels, and thus any exposure, however low, carries a risk, albeit low, of mutation in genes critical for the development of cancers. The ability of radiation to lead to cellular consequences such as mutation and chromosomal rearrangements was noted, and the importance of these for carcinogenesis.

137. Ionizing radiation-induced DSBs are repaired by two distinct pathways: NHEJ and HRR. The principal difference between these pathways is that HRR occurs in late S and G_2 phase of the cell cycle and requires an undamaged template to guide relatively error-free repair, whereas NHEJ is capable to repair DSBs throughout the cell cycle, with the exception of the mitotic phase, as no undamaged template copy is required. DSB repair by HRR is dependent on sequence and generates stretches of 3′-overhanging single-stranded DNA (end resection) as a priming end that copies the undamaged template. End resection is mainly carried out by the nuclease activity of the MRE11, RAD50, and NBS1 complex (MRN complex). NHEJ is a sequence independent process driven by DSB end binding and protection by the heterodimer Ku70-Ku80 as well as their ligation by the XRCC4-DNA ligase IV complex. However, alternative endjoining pathways exist as residual end-joining activity was observed in NHEJ deficient cells. These alternative noncanonical endjoining pathways are characterized by their dependence on small stretches of repetitive DNA sequences up- and downstream of DSBs (so-called microhomology) and are termed alternative end joining (Alt-EJ) and microhomology-mediated end joining [S14, S20]. Microhomology-mediated end joining is driven by DNA polymerase theta and may occur in S-phase when an undamaged template is not available.

138. Ionizing radiation induced oxidative base damage repair is carried out by base excision repair that operates throughout the cell cycle. Base excision repair is initiated by DNA glycosylases, which recognize and remove damaged bases, forming so called apurinic/apyrimidinic sites. These are then cleaved by an AP endonuclease. The resulting single-strand break can then be processed by either short-patch (where a single nucleotide is replaced) or long-patch base excision repair (where 2–10 new nucleotides are synthesized, often additionally associated with single-strand break repair).

139. The main molecular mechanism that initiates repair of radiation-induced DSBs is signalling, i.e. post-translational modification of specific proteins. This includes the modification of chromatin flanking the DSB and core repair proteins. Antibodies raised against these proteins have been successfully employed to measure induction and removal of DSB in cultured cells, human primary lymphocytes and in vivo in mouse tissues. These assays also revealed inter-individual variation in DSB foci parameters in primary fibroblasts exposed to doses of ionizing radiation (50–250 mGy, linear induction with dose) in a small survey (25 persons) of apparently normal people suggesting that hypomorphic genetic variants in DNA damage response may contribute to differential susceptibility to cancer after exposure to ionizing radiation [W12]. In vitro systems with reporter genes have been applied to measure repair of single enzymatically engineered DSB in mammalian cells. These systems in combination with defined repair deficient cells have allowed to elucidate the role of NHEJ and HRR after X-rays, including some low-dose exposures or enzymatically-induced single DSB. The consequences of incomplete repair (or misrepair) have been assessed by measuring induction of cancer-related genetic damage such as loss of

heterozygosity (LOH), chromosomal aberrations and micronuclei as well as cancer induction employing irradiated normal and (genetically modified) repair deficient organisms.

140. The number and complexity of chromosomal aberrations is critically related to the structure of DSBs. Whereas the majority of DSBs induced by low-LET radiation of mammalian cells are rejoined in less than 60 minutes, a small (variable) fraction of DSBs (<20%) requires much more time for repair (up to >24 hours) or are left unrepaired [D3, L40]. The proportion of the slowly repaired DSBs rises with increased LET of radiation and largely reflects the increasing proportion of complex/clustered damage sites, i.e. DSBs that have a high frequency of oxidized base modifications and abasic (AP) sites directly adjacent their ends. The base excision repair pathway is compromised close to DSB ends. This poor repair of base lesions and apurinic/apyrimidinic sites close to ends of DSB drastically reduces the repair of DSB by inhibition of ligation of ends [D4]. A minority of low-LET radiation induced DNA damage represents clustered damage sites of varying structural and chemical complexity, whereas densely ionizing radiation generates primarily clustered damage sites including DSBs. The complexity of the clusters, reflecting the number of lesions present in non-DSB clusters (oxidative bases) and DSB clusters (DSB and oxidative bases), increases with the LET of the radiation. Although dose–response relationships for the induction of complex DNA lesions are not well characterized, it is highly likely that complex lesions are induced by low dose (<100 mGy) photon radiation as oxidative base damages and DSBs are induced linearly by X-rays down to 100 and 1.5 mGy, respectively (see section III.A). Kinetics of repair of DSBs after low- or high-LET irradiation revealed that rejoining of DSBs consists of a fast and a slow component and that free radical scavenging by dimethyl sulfoxide reduces mainly the fast component of DSB-repair, while slow repair is related to repair of complex DSBs, particularly by HRR. The relative involvement of NHEJ, HRR and base excision repair in repair of complex DNA lesions induced by low- or high-LET radiation (0.05–18 Gy) has been studied with normal and NHEJ, HRR and base excision repair deficient hamster cells. The data revealed that DSBs induced by the indirect effect of radiation (sensitive to dimethyl sulfoxide), are more efficiently repaired by NHEJ and base excision repair compared to HRR [B3].

141. Repair of DSBs can be followed over time by quantifying DNA damage repair focus numbers (γH2AX and phospho-ATM), and these measurements also indicate some possible differences between high- and low-dose exposure. Although the number of DSBs induced may be low and less than one per cell, at low doses of the order of a few milligray, repair as monitored by loss of repair foci has been observed to be slower or even incomplete, leading to long-term persistence of residual foci over time after irradiation [G17, O14, R17]. Similar observations of slower and/or incomplete repair have been made in stem cells (see section III.F). Assessment of DSB repair following CT scans however leads to a different conclusion, that repair following the lowest dose exposures can be complete within 24 hours [L39].

142. The findings of incomplete repair following low-dose exposure are suggestive of an inducible component to DSB repair processes. Grudzenski et al. [G17] showed following a dose of 10 mGy, that cells failed to show any foci loss. However, pre-treatment with 10 μM H_2O_2 led to efficient removal of γH2AX foci induced by 10 mGy. Moreover, 10 μM H_2O_2 up-regulated a set of genes that was also up-regulated after moderate (200 mGy) but not after low (10 mGy) radiation doses. This suggests that low levels of oxidative damage induce an oxidative response required for the repair of radiation-induced DSBs. The data also suggested that a threshold for endogenous stress may be required to activate the cellular response to radiation-induced DSBs. A notable issue is that persistence of DSB at low dose solely depends on foci analysis and lacks physical quantification of DSB and that other explanations might be possible.

143. Some studies of γH2AX foci and chromosome aberration induction by radiation suggest that DNA repair activity changes with age. Higher yields of dicentric chromosome aberrations have been found in adult blood samples by comparison with umbilical cord blood and blood from under 5-year old children, this difference is observed at only 1 Gy and not at the low dose of 41 mGy [G7]. The chromosome aberration results were not reflected in γH2AX foci assays, i.e. γH2AX foci frequencies did not differ

significantly among age groups. Automatic scoring of dicentric chromosomes improved the sensitivity of similar assays. It was found that under 5-year-old children and newborns have lower background dicentric frequencies and higher yields of induced dicentrics were observed in the younger groups than adults at both 41 mGy and 1 Gy X-rays delivered to blood in vitro in a clinical CT scanner [O8].

144. Hernández et al. [H26] explored the DNA damage response of normal human mammary epithelial cells obtained from women 28, 50 and 58 years of age. Cells were exposed once, twice or ten times to 10 mGy X-rays delivered by a CT scanner (X-rays molybdenum-anode tube with 28 keV voltage at a dose rate of 0.67 Gy/min). Two 10 mGy exposures led to delayed resolution and accumulation of double-stranded DNA breaks and a significant increase in micronuclei frequency in human mammary epithelial cells from old but not young donors. In addition, cells of all three donors went both through 24–29 population doublings or through 32–38 doublings for "aging in cell culture". Accumulation of γH2AX foci was greater in cells from later passages in all three cases, but the increase of the number of foci was always the most prominent in cells from the 58-year-old. Note that this study included only one donor of each of the given ages and this limits the ability to generalize from the conclusions drawn.

145. Sakane et al. [S8] investigated DNA and chromosomal damage in lymphocytes from 209 patients undergoing chest CT examinations. Significantly elevated γH2AX foci and unstable chromosomal aberrations were observed in those having standard dose CT (5 mSv effective dose) but not in those receiving low dose (1.5 mSv effective dose) CT examinations. The latter result may be a reflection of the limited statistical power of the study, especially in light of the modest effect observed at the higher exposure (5 mSv); this issue is discussed in more detail by Brenner [B55].

146. Impaired DSB repair has been suggested as a cause of aging of human oocytes based on the observation that DSBs (γH2AX foci) accumulate in primordial follicles (2–28 years) and oocytes (23–24 years) with age [T14]. In parallel, expression of key DSB repair genes *BRCA1*, *MRE11*, *Rad51*, and *ATM* decline in single human oocytes. Also, ovarian reserve was impaired in young women with germline *BRCA1* mutations compared to controls. In line, (eyelid) fibroblast cell cultures (in total 50) isolated from healthy donors at the age of 16–75 years, revealed that the efficiency of NHEJ and HRR declines with age leading to increased ionizing radiosensitivity in cells (0–8 Gy X-rays) isolated from old donors [L26]. Whereas decreased expression of NHEJ factors with age was observed, the key HRR-related factor Rad51 did not change expression with age but exhibited slow kinetics of recruitment to DNA damage sites in aged fibroblasts. In contrast to the above-mentioned results, 20 (eyelid) adipose-derived stem cell cultures from healthy individuals (ages ranging 17–59 years) revealed that the efficiency of base excision repair but not NHEJ or HRR is impaired in aged human stem cells [Z11].

147. The role of HRR and NHEJ in X-ray induced tumorigenesis (medulloblastoma) has been assessed in heterozygous Patched1 (*Ptc1*$^{+/-}$) mice; exposure of newborn mice to X-rays increases the frequency and shortens the latency of medulloblastoma. In *Ptc1*$^{+/-}$ mice, medulloblastoma is characterized by loss of the normal remaining *Ptc1* allele. To test the role of defective HRR or NHEJ in tumorigenesis, control and irradiated (2 Gy) *Ptc1*$^{+/-}$ mice with two, one or no functional *Rad54* (HRR) or *DNA-PKcs* (NHEJ) alleles were monitored for medulloblastoma development [T11]. It was found that although HRR and NHEJ collaborate in protecting cells from DNA damage and apoptosis, they have opposite roles in medulloblastoma tumorigenesis. In fact, although *Rad54* deficiency increased both spontaneous and radiation-induced medulloblastoma development, *DNA-PKcs* disruption suppressed medulloblastoma tumorigenesis. The data provided the evidence that Rad54-mediated HRR in vivo is important for suppressing tumorigenesis by maintaining genomic stability. In radiation-induced medulloblastoma tumours the pattern of *Ptc1* LOH was distinct from spontaneous tumours: radiogenic medulloblastomas showed large interstitial chromosome 13 deletions, suggesting different molecular mechanisms for

spontaneous or radiation-induced *Ptc1* loss. Within the sample size analysed, no obvious difference in the pattern of *Ptc1* LOH was observed following genetic inactivation of *Rad54* or *DNA–PKcs*.

148. Exploring the same mouse genotypes as described in the previous paragraph, different types of repair were found to be engaged differently in vivo dependent on stage of development of embryonal mice. $Ptc1^{+/-}$ mice lacking one *Ptc1* allele were crossed with $Rad54^{-/-}$ (lacking HRR) and $DNA\text{-}PKcs^{-/-}$ (lacking NHEJ) mice on C57BL/6 background [T12]. F1 mice used for experiments came from further crosses of genotypes $DNA\text{-}PKcs^{+/-}/Ptc1^{+/-}$ × $DNA\text{-}PKcs^{+/-}/Ptc1^{+/+}$ and $Rad54^{+/-}/Ptc1^{+/-}$ × $Rad54^{+/-}/Ptc1^{+/+}$. F2 mice were X-ray irradiated in utero at embryonic day E13.5 or E16.5 with 0.25 or 3 Gy and followed up until development of medulloblastoma, which depends on loss or mutation of remaining wild-type allele of *Ptc1*. An exposure of 3 Gy induced medulloblastoma sooner in mice irradiated at E13.5 while 0.25 Gy exposure caused medulloblastoma development sooner in mice irradiated at E16.5. In addition, apoptosis was followed in sacrificed embryonal animals and this study noted that loss of HRR (absence of *Rad54*) drives apoptosis in mice irradiated at E13.5, while compromised NHEJ (absence of *DNA-PKcs*) leads to more apoptosis in mice irradiated at E16.6. Thus, a more efficient if erroneous repair may be more important for cell survival at higher doses, while at the lower dose HRR, more accurate but less efficient, ensures development of fewer medulloblastomas. Medulloblastoma incidence pattern changes at different ages for different doses.

149. Tsuruoka et al. [T22] exposed *Ptch1* heterozygotes to a dose of 100 mGy and this resulted in LOH following the same deletion pattern as LOH mutations in non-irradiated mice. A portion of the chromosome arm from the telomere to beyond the *Ptch1* gene occurred most often as a result of faulty recombination which was different from the LOH pattern that followed a dose of 500 mGy. In a study that used newborn one day old $Ptc1^{+/-}$ mice ($Ptc1^{neo67/+}$ with disrupted exons 6 and 7 on C57BL/6JCrl background were crossed with C3H/He females) [I10] total-body irradiation was carried out by X-rays and with radiation doses ≤100 mGy (at 100 mGy/min) or >100 mGy (at 650 mGy/min). Life shortening followed increase of dose, beginning with 50 mGy. As in most other studies using heterozygotes for tumour suppressor genes, one of the endpoints in this work was LOH. Importantly (and again, similarly to studies with p53 heterozygotes), LOH patterns elicited by radiation were different than LOH patterns that occurred spontaneously in the absence of radiation and LOH due to faulty recombination was observed at 50 mGy. In the case of the latter, loss of the entire chromosome arm carrying *Ptc1* gene predominated, while "local" DNA loss with the remainder of the chromosome present was most frequent for doses above 1 Gy. Low doses showed a mix of the two LOH patterns.

150. An animal model of accelerated aging was used by Nomura et al. [N17] to evaluate lifespan radiation effects of radiation exposures. Four-week-old homozygous mutant female klotho knockout mice and wild-type littermates were exposed to ^{137}Cs gamma rays at dose rates of 0.35, 0.7 or 1.5 mGy/h over a period of 23 hours a day. While non-irradiated knockout mice lived 80 days at most, daily irradiation with either 0.35, 0.7 mGy/h dose rate (but not with 1.5 mGy/h dose rate) extended the lifespan in more than 10% of the animals; the eldest klotho knockout animal died at 119 days (it belonged to 0.70 mGy/h group). Very few differences between irradiated and non-irradiated wild-type littermates were registered in this study. With regard to antioxidant enzymes, catalase activity in klotho knockout animals irradiated for 21 days was the most significantly increased.

151. Penninckx et al. [P12] examined ex vivo repair of radiation-induced damage in non-immortalized skin fibroblasts from 15 strains of mice (5 inbred reference strains and 10 collaborative-cross strains) of both sexes. Radiation exposure was low-LET (X-rays, (0.1, 1 and 4 Gy)) and high-LET (1.1 or 3 particles per 100 μm^2 of 350 MeV/nucleon ^{40}Ar or 600 MeV/nucleon ^{56}Fe ions). As the doses increased, strain to strain differences became more apparent suggesting that the lowest doses of exposure may not trigger DNA damage repair programmes for which these animals had genetic differences as effectively. This phenomenon was especially pronounced for high-LET exposures.

152. Transgenic mice with repair deficiency mutations were used to study the effects of low- and moderate dose radiation on embryonic brain. Animals carrying a mutation for DNA ligase IV (*Lig*), coding for an enzyme involved in NHEJ, were crossed with animals with a knockdown of the ATM gene which is critical for all DNA double-strand break repair. Animals of all three genotypes (*Lig4* mutants, *ATM* knockouts and double-mutants) were exposed to radiation as embryos at E13.5 as pregnant mice were exposed to X-rays between 50 and 200 mGy. DNA double-strand breaks in embryos were studied as γH2AX or 53BP1 foci up to 60 minutes post-exposure. More foci and less ongoing repair-associated DNA synthesis were found in mutant embryos, and more apoptosis triggered by DNA double-strand breaks. Overall the numbers of spontaneous DNA damage lesions and frequency of apoptosis in embryonic mutant animals matched those in wild-type embryonic mice exposed to 100 mGy. After birth, in 2–3 month-old mice endogenous DNA damage in *Lig4* mutants is reduced and irradiation of age-matched wild-type mice with dose of 50 mGy causes a twofold increased number of DNA damage foci and TUNEL staining when compared to the spontaneous foci and TUNEL staining in Lig4 mutants [B15].

153. If radiation carcinogenesis is largely driven by the induction of gene mutations and chromosome aberrations, then DNA repair deficiencies should modify radiation cancer risk. There is evidence that genes involved in the two main DSB repair pathways, NHEJ and HRR, modify radiation cancer risk in mice with indications for some tissue specificity [D13, H5, H6, T12]. Also, a single nucleotide change or polymorphism in *CtIP*, a factor that affects repair pathway selection, has been demonstrated to lead to increased radiation leukaemogenic sensitivity in mice [P5]. These studies have however involved moderate to high doses (0.5–3 Gy) and therefore relate only to situations where DSBs and their consequences are of prime importance. Functional assays similarly indicated that DSB repair after chronic gamma-ray exposure correlated with lung cancer risk in mice [O6].

154. Several studies explored chromosomal changes in bone marrow cells from wild-type and mutant animals. One such study using fluorescence in situ hybridization analysis on 400–1,000 scored bone marrow cells isolated from severe combined immunodeficiency (SCID) mice (loss of NHEJ) irradiated in groups of 20 [R8] found that exposures to 50 and 100 mGy (unlike exposure to 1,000 mGy) did not increase the numbers of altered chromosomes one or six months after irradiation, although earlier timepoints one and four hours did show chromosomal changes. At most timepoints the complexity of chromosomal alterations was smallest in 50 mGy exposed animals. At the time of exposure to ^{137}Cs gamma rays (dose rate 750 mGy/min) mice were 10 to 12-weeks old. Similarly, 10 to 12-weeks old male BALB/cJ and C57BL/6J mice were irradiated and screened in the same way [R6]. Chromosomal aberrations were increased at late timepoints only in mice exposed to 1,000 mGy. Exposure to 50 mGy was found to be "protective" where animals had fewer than control numbers of chromatid and chromosome breaks one and six months after radiation exposure.

155. In another study, DSB foci were scored in four different genotypes of mice exposed to 10 mGy per day for 10 or 20 days, sacrificed four hours after final exposure [S13]. Animals used in this study included repair competent C57BL/6 mice, repair deficient SCID mice (loss of NHEJ) and $ATM^{-/-}$ mice (checkpoint and HRR deficient) as well as their repair proficient $ATM^{+/-}$ littermates (both generated by crosses between $ATM^{tm1Awb/+}$ animals). Exposures of 10 mGy per day, five days a week for 2, 4, 6, 8 or 10 weeks were carried out; mice exposed only once were sacrificed at 0.5, 24 and 72 hours and animals exposed to higher doses were sacrificed at 24 or 72 hours after final irradiation. DSBs were detected by immunofluorescence with antibodies against 53BP1. In lung, heart and brain of repair proficient mice modest but statistically significant increase in number of foci was found (this was even more pronounced at 0.5 hours after single irradiation). When all four genotypes were compared, SCID mice had the greatest number of foci regardless of tissue, while $ATM^{-/-}$ mice showed more foci than repair proficient animals only in brain cortical neurons but not other tissues.

156. While development of cataracts was the primary focus of a study by Dalke et al., many other endpoints relevant for cancer were also explored [D1]. Both F1 mice from the cross between C3H wild-type males with C57BL/6J wild-type females and mutant F1 heterozygotes from a cross between male homozygous *ERCC2* knockout on C3H background and C57BL/6J wild-type females were used; 10 week-old mice were irradiated with doses of 63, 125 and 500 mGy delivered at a dose rate of 63 mGy/min from a ^{60}Co gamma irradiator. At 4 and 24 hours after irradiation and at 12 and 18 months after irradiation bone marrow cells were isolated from three mice of each group and examined for chromosomal aberrations and telomere length. No statistically significant differences were found for chromosomal aberrations or telomere length. Moreover, cumulative survival of mice exposed to 63 mGy was the longest, while 125 and 500 mGy exposed mice showed life shortening. No tumour type was found to have increased frequency in the 63 mGy irradiation category, ovarian and pituitary adenoma increased in 125 and 500 mGy groups, and other tumours and inflammation also increased in 500 mGy mice. Heterozygotes had slightly higher rates of ovarian cancers but lower rates for all other cancer types. Radiation had different effects on the two genotypes: a higher risk of chromosomal aberrations, particularly dicentrics, in the heterozygous mutants was observed 12 and 18 months particularly after 500 mGy irradiation. Because non-symmetric cytogenetic aberrations such as dicentric chromosomes show a loss of 50% per cell division, with increasing time since irradiation the number of such radiation-induced cytogenetic defects decreased.

157. Studies with zebrafish treated with anti-Ku80 antibody (inducing loss of NHEJ) support the notion that functional repair is necessary as protection from even lowest doses of radiation. TUNEL staining was used to identify apoptotic cells in wild-type zebrafish (Danio rerio) [B37]; to modulate repair capacity Ku80 gene expression was suppressed by injecting an antisense phosphorodiamidate morpholino oligonucleotide into the yolk of one-cell embryos. Irradiations used two approaches: control embryos were divided into 12 groups and received total doses of 0, 50, 100, 200, 300 etc. (increasing by 100 mGy) to 1,000 mGy, delivered in 10 mGy increments at dose rate of 1 Gy/min produced by a ^{137}Cs gamma-ray source. Also, 6 MeV X-rays were used to deliver total doses of 1, 3, 8, 20 and 50 mGy to anti-Ku80 injected fish embryos. Fish were exposed as shield-stage embryos and allowed to develop for additional 18 hours before harvesting for TUNEL assay. Anti-Ku80 injected fish were irradiated six hours after fertilization and fixed at 24 hours for TUNEL processing. In both groups of animals, apoptotic cell numbers increased linearly with dose; in zebrafish without functional Ku80 apoptotic cells were 34-fold more numerous than in control injected embryos and dose response could be seen already at total doses between 1–50 mGy.

158. Gene mutation studies have been carried out in mammalian cells, in fruit flies and mice. However, mutation assays at low doses are challenging due to the low frequency of induction, unless mutant animals are used as described in the case of *Ptch*$^{-/-}$ mice [T22]. LOH mutations are particularly relevant in terms of cancer as studies of mutations in both spontaneously arising and radiation-associated cancers have shown that LOH is a common feature, and furthermore, LOH can be generated through a variety of mechanisms including chromosomal translocation events that are known to be induced by radiation exposure. Studies using an HLA-A2 gene loss system in human TK6 or derived *p53*$^{-/-}$ WI-L2-NS cells reported a linear dose–response for gene loss by LOH in the 50–500 mGy range [B44], *p53* status did not affect mutation yields. Using the human TK6 cell thymidine kinase locus mutation assay, Manesh et al. [M9] observed no dose-rate dependence of mutation induction at 0.5 or 1 Gy doses accumulated at 1.4, 5, 15 mGy/min or 24 Gy/h. These results from TK6 cells and derivatives suggest dose rates above 1.4 mGy/min are broadly similar with respect to LOH induction.

159. Studies using the fruit fly *Drosophila melanogaster*, argue against a similarity in mutational effect of the dose level, as 200 mGy X-ray radiation reduced the somatic mutation frequency below "spontaneous" levels [K33]. This reduction of somatic mutation frequency by X-rays is supported by previous studies such as the 2007 study by Koana et al. [K31] that used fruit flies with a sex-linked

recessive lethal mutation; irradiation of immature spermatocytes and spermatogonia (achieved by irradiation of fly embryos) was carried out at dose rates of 0.05 or 0.5 Gy/min, for total doses of 0.2 or 10 Gy. While mutation frequencies at 10 Gy were increased in both cases, exposure to 0.2 Gy at higher dose rate had no effect on mutation frequency. Exposure to 0.2 Gy at lower dose rate (0.05 Gy/min) had a protective effect and decreased mutation frequency and lethality in fruit fly progeny. In other words – spontaneous mutation frequency was decreased at the lower-dose and low-dose-rate exposure because it increased innate error-free DNA repair functions. In subsequent studies [K32, O11] experiments were extended to include irradiation of immature wild-type flies. When irradiation was carried out with a total dose of 0.2 Gy either at a dose rate of 0.0224 Gy/h [O11] or at a dose rate of 0.05 Gy/min [K32] mutation frequency was reduced compared to the sham-irradiated groups. It is important to note that when total dose was dropped to 40 μGy delivered at dose rate of 1.5 mGy/h, the mutation frequency was once again approaching value found in the control, though the data were not shown in the publication just cited as "data not shown" [O11]. Finally, repetition of some of the same experiments (exposure to 0.2 Gy total dose at dose rate of 0.5 Gy/min) in the presence of anti-apoptotic *p35* gene introduced by baculovirus, reconfirmed that the mutation frequency is reduced in irradiated flies [K33]. However, this was not true for *Drosophila melanogaster* mutants with deficient single-strand DNA repair.

160. Okudaira et al. [O15] investigated deletions of a $red^{+}gam^{+}$ bacteriophage lambda transgene in the spleen and liver of gamma irradiated SWR mice. Deletion induction was assessed at dose rates of 920 mGy/min, 1 mGy/min and 12.5 μGy/min with total accumulated doses of 2–8 Gy. Mutation rates were found to be about fivefold elevated at the high dose rate compared with the low dose rate. Dose–response relationships at all dose rates and in both tissues were linear; however, mutation induction at the 12.5 μGy/min dose rate was fivefold lower than for 920 mGy/min for equal total doses and for the dose rate of 1 mGy/min spleen and liver had different responses. Larger, multi-locus deletions appeared less frequently in low-dose-rate irradiated tissue.

161. The induction of micronuclei is a sensitive approach to measure genetic damage in vitro and in vivo at the few milligray range. Results of recent studies are not fully consistent with a complete loss of cells harbouring micronuclei. Moreover, pulverized DNA from micronuclei might be reintegrated into the main genome thereby inducing locally clustered rearrangements in single or a few chromosomes. Using a sensitive system to evaluate the induction of micronuclei in primary human skin fibroblasts, a linear dose–response was reported in the 0–100 mGy range with statistical significance at 20 mGy and above [B44]. S-phase cells were more sensitive to the induction of micronuclei by ionizing radiation than those in other cell-cycle phases. Induction of micronuclei in reticulocytes after in vivo irradiation of mice was also reported to be linear over the 10–100 mGy X-ray range with no evidence for a threshold above 10 mGy [M10]. These studies demonstrate the induction of micronuclei at doses in the region around 10 mGy both in vitro and in vivo. The in vitro studies with fibroblasts do not appear consistent with the finding from γH2AX staining studies of DSB repair that suggest breaks in non-dividing cells are not repaired in the mGy region and cells may be lost to apoptosis [R17]. The reticulocyte assay results would seem particularly relevant as they relate to in vivo exposure and the cells, in which micronuclei were scored, are derived from exposed haematopoietic stem/progenitor cells. Analysis of micronuclei induction in human blood lymphocytes irradiated in vitro indicated that at a dose rate of approximately 0.3 Gy/min induction is linear with dose, while at an approximately 1 Gy/min dose rate a linear quadratic relationship applies [B32]. Reduced micronuclei yields in human fibroblasts were observed following low-dose-rate exposure (100 mGy delivered over 48 hours) and this reduction could be attributed to up-regulation of antioxidant defences [D10]. Consistently, in vivo under conditions of chronic exposure to low doses and dose rates (0.88–35 mGy/y) no increase of micronucleus by natural background radiation was observed [K16]. A plausible mechanism for the linear non-threshold induction of micronuclei by X-rays is based on the recent observation that in repair proficient cells mitosis inhibits DNA damage checkpoint functions and DSB repair. Shut down of DSB repair is proposed to be a guarding mechanism

against telomere fusions [C9, L25, O18]. Neither the DNA damage checkpoint nor DSB repair occur by virtue of blockage of recruitment of two crucial repair factors RNF8 and PB531 to DSB due to their phosphorylation that prevents checkpoint activation and DSB repair. As a consequence, DNA fragments generated by ionizing radiation linearly with dose, will be processed into micronucleus ultimately leading to a linear dose–response relationship for micronucleus.

162. Another recent study focused on reticulocytes was carried out with human and animal samples [K24]. Six to eight-week-old male C57BL/6N mice were exposed to X-rays at dose rates equivalent to CT scans and their blood was collected 48 hours later and processed to evaluate percentages of micronucleated CD71+ reticulocytes. Doses of 8.3 to 1,333 mGy were tested, and a statistically significant increase in micronuclei was noted for all doses, although it should be mentioned that a dose of 243 mGy was necessary for doubling the frequency of micronuclei.

163. A different study was carried out by Manning et al. [M11]; their objective was to evaluate effects in vivo caused by exposure to Fluorodeoxyglucose-radiolabelled with ^{18}F, a positron emission tomography imaging agent. In this work wild-type mice 47–48 weeks of age were injected with different quantities of imaging agent, corresponding to bone marrow doses between 7 and 370 mGy. To provide external beam controls 18 weeks old mice were irradiated with X-ray doses between 5 and 300 mGy. Curves describing the frequency of micronucleated reticulocytes had a similar slope although fewer micronuclei were found in the external beam exposure cohort of mice. BBC3 and Cdkn1a mRNA expression was checked in these animals and found to be congruous for internal emitter and external beam exposures. A notable increase in gene expression was found only in samples exposed to 300 mGy or equivalent.

164. In vivo irradiated spleen cells from female C3H/HeN mice eight weeks of age exposed for 5 to 400 days to low dose rate of 20 mGy/d or high dose rate of 200 mGy/d of ^{137}Cs gamma rays were screened for chromosomal aberrations by Giemsa staining [T5]. These included, dicentric chromosomes, centric ring chromosomes, acentric ring chromosomes and fragments, as well as minutes, deletions, hyperploids and chromatid-type aberrations such as gaps, breaks and exchanges. In both dose rate groups serially sacrificed mice that received increasing doses showed linear increases in numbers of dicentrics. Moreover, micronuclei in bi-nucleated cells were counted in splenocytes, while centromere probes of chromosomes 5, 15 and 18 were used in bone marrow cells for interphase fluorescence in situ hybridization. Again, dicentrics and ring chromosomes increased linearly with total dose, reaching maximum for a total dose of 8 Gy. That maximum was 4 chromosomal aberrations per 100 cells for 20 mGy/d and 12 chromosomal aberrations per 100 cells for 200 mGy/d dose-rate exposures. The maximal numbers of aberrations per cell did not go above 1.6 per cell but this maximum was reached promptly, already at total dose of 800 mGy for low dose rate exposed mice. Numbers of micronuclei did not significantly differ from controls, possibly due to loss of micronucleus carrying cells.

165. A recently developed approach to study the effects of radiation on haematopoietic cells is to use "humanized mice". Such animals were used in a study by Hoehn et al., who engrafted 6–8 weeks old NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice with commercially available human cord blood CD34+ cells. Host mice were irradiated (2 Gy) before transplantation of the human cells. Such humanized mice were irradiated with 0.4 Gy 350 MeV/nucleon ^{28}Si ions (with a calculated 1.2 particles traversing each cell of the body) or 1 Gy X-rays (250 kVp) [H30]. In this work, heavy ion exposure (at doses used) consistently had fewer effects on human and mouse cell populations isolated from exposed mice, regardless of the cell surface marker examined; moreover, human cells from X-ray exposed mice had two times more dicentrics compared to Si ion exposed mice. It will be interesting to use humanized animals for low-dose irradiation studies.

166. Exposure of actively cycling cells to ionizing radiation activates DNA damage signalling cascades and complex cellular responses including activation of apoptosis. Reduction in cell numbers through apoptosis might be compensated by repopulation through stem cell proliferation and differentiation. Recent studies suggest that a majority of mutations observed in human cancers may have their origin in DNA replication errors [T17, T18], and thus any factor that leads to increased stem cell replication could enhance cancer risk through replication-associated mutation. However, some stem cell populations appear to have properties that serve to reduce the burden of replication-associated mutations (see [I3] for review (chapter II)). Additionally, it should be noted that the views of Tomasetti and Vogelstein have been challenged by Little et al. [L37] who failed to find a correlation between stem cell proliferation and risk of radiation-associated cancer. Further challenge to the proposition of Tomasetti and Vogelstein comes from the experimental evaluation of stem cell mutation rates in organoid cultures from human colon, liver and small intestine. Blokzijl et al. [B42] found that mutations accumulate at similar frequency in colon, liver and small intestine organoid cultures, despite differences in cancer rates between these tissues. The mutational spectra observed suggest that different mechanisms may act in liver as opposed to colon and small intestine, but spectra are similar to those observed in cancer driver genes, and thus intrinsic mutational processes can contribute to carcinogenesis in humans; furthermore, little interindividual variation in mutational frequency or spectra were observed. Thus, the relationship between stem cell division and cancer risk remains uncertain and environmental carcinogenic exposures are still likely to be a major source of mutagenesis.

167. One in vivo study that ties in spontaneous generation of cancer and the subsequent role that low-dose radiation exposure may play in cancer progression is the following: extremely low doses and dose rates were used in work with B-cell lymphoma prone SJL/J mice [L1, L2]. The dose rate delivered was 40 mGy/month by gamma irradiation from thorium nitrate (or 1.3 mGy/d) and exposures were life-long. This type of exposure did not hasten or delay onset of lymphoma, however in mice that did develop lymphoma chronic low-dose radiation exposures slowed down disease progression and diminished proliferation index in lymphomatous lymph nodes as measured by Ki67 [L2], potentially indicating an association of cell proliferation and cancer progression.

168. It has long been held that chromosomal aberrations provide a mechanistic link between direct cellular effects of radiation and cancers, which are frequently characterized by chromosomal aberrations, yet the specific type of aberration(s) induced by radiation leading to cancer is generally unknown. Translocations are frequently observed in leukaemias and deletions in solid cancers but making direct links is challenging. In a mouse model of radiation-induced acute myeloid leukaemia there is strong evidence for aberrations directly induced by radiation to be causally linked to disease [V7, V8]. Also, several human population studies have suggested that chromosomal aberrations in peripheral blood act as an indicator for cancer risk [B47, B48]. So, while there is evidence for an association between chromosome aberrations and cancer, it is likely that a range of aberration types will be involved. Transmissibility of aberrations through cell division is of course essential and likely to be more common for genuinely simple aberrations.

169. Chromosomal aberrations following chronic irradiation (at low dose rate and accumulating to moderate or high total dose), were studied in normal tissues in lymphomas. Some of the chromosomal aberrations were radiation-induced. A systematic investigation of DNA damage caused by chronic radiation exposure at different dose rates and with different total doses of radiation was reported in detail by Tanaka et al. [T6]. In this work, chromosomal changes in splenocytes were counted as numbers of dicentric and ring chromosomes following pan-centromere staining. These chromosomal aberrations can be seen only as proxy for cancer induction. Cells with dicentric and ring chromosomes are expected to go through mitotic catastrophe while cells with chromosomal translocations (not included in this study) are the ones with a significant likelihood to develop into neoplasia. Details of this study are the following: 8-week-old female C3H/HeN mice were exposed to low-dose-rate (1 mGy/22 h each day or 20 mGy/22 h

each day) or high-dose-rate (200 mGy/22 h each day or 400 mGy/22 h each day) chronic gamma ray ^{137}Cs radiation. Fine gradation of low-dose-rate exposures between 100 and 8,000 mGy was delivered over 5–615 days. High-dose-rate chronic exposures varied between 200 and 8,000 mGy for 1–40 days. At the conclusion of the final irradiation, spleens were harvested and splenocytes cultured for 48 hours before chromosomes were imaged and scored; dicentric chromosomes, centric ring chromosomes, acentric ring chromosomes and fragments were scored in 500–1,000 metaphases after Giemsa staining, dicentrics were also evaluated by fluorescence in situ hybridization using a centromeric probe. For dose rates of 20, 200 and 400 mGy/d numbers of aberrant chromosomes increased linearly with total dose, however, in mice exposed to 20 mGy/d total number of Giemsa evaluated aberrant chromosomes never exceeded a total of four dicentrics and rings per 100 cells analysed. Evaluation of dicentrics by fluorescence in situ hybridization was also used for 1 and 20 mGy/d comparisons; in this case the maximal total dose for lowest dose rate was 615 mGy and at that dose numbers of dicentrics had a value of less than one per 100 cells analysed. Using fluorescence in situ hybridization, less than six dicentrics per 100 cells were found at total dose of 8,000 mGy for dose rate of 20 mGy/d. A similar study using 8-week-old C3H/HeN mice for 1 mGy/d daily exposures with total doses of 125–700 mGy and 20 mGy/d chronic exposures with total doses of 500–8,000 mGy were evaluated lymphocyte translocations using multiplex fluorescence in situ hybridization [T7]. Translocation frequencies showed similar results as dicentric chromosome counts: Overall linear increase with increase of total dose was noted, with less than two translocations per 100 cells for total dose of up to 800 mGy regardless of dose rate, increasing to 10 translocations per 100 cells for 20 mGy/d chronically exposed mice for total dose of 8,000 mGy. Finally, chromosomes from lymphomas from B6C3F1 mice chronically exposed at dose rates of 21 mGy/d to a total dose of 8,000 mGy were characterized by Takabatake et al. [T1]. Chromosomal aberrations in 82 malignant lymphomas from irradiated and non-irradiated mice were compared by microarray-based comparative genomic hybridization. In both groups, loss of the Igh region on chromosome 12 suggested B cell origin lymphomas. Gain of whole chromosome 15 occurred predominantly in lymphomas from the irradiated group. Partial gains on chromosomes 12, 14 and X were found in tumours from non-irradiated animals, while irradiation induced lymphomas showed partial losses on chromosomes 4 and 14. These findings suggest that lymphomagenesis under the effects of continuous low-dose-rate irradiation is accelerated by a mechanism different from spontaneous lymphomagenesis that is characterized by the unique spectrum of chromosomal aberrations.

170. The prevailing hypothesis regarding cancer evolution is that driver mutations are acquired over years to decades. However, accumulating evidence suggests that somatic mutations and genomic rearrangements may in fact accrue in relatively short periods of time through several mechanisms that might be relevant for ionizing radiations of different kinds and levels of dose. Next-generation sequencing of tumours revealed a novel form of genomic instability in cancer cells characterized by thousands of locally clustered rearrangements affecting one or a few chromosomes [S62]. This phenomenon, termed "chromothripsis", is likely to emerge from a single catastrophic event inducing locally clustered multiple DSBs, which are repaired by error-prone mechanisms. These tumours include osteosarcoma for which the only established exogenous carcinogen is ionizing radiation [G6]. While detected in a wide range of tumour entities [R10], the prevalence of chromothripsis in tumours remains unclear due to different methodologies and definitions used in the various studies.

171. Mechanisms underlying the formation of chromothripsis are now starting to be explored, but a detailed picture of the process remains elusive. One likely model to explain how complex chromosomal rearrangements involve only certain chromosomal regions, points to the formation of micronuclei as key intermediates in this process. Micronuclei are markers of genomic instability [K45] and can arise in damaged cells during mitosis either from lagging chromosomes or chromosomal fragments. Crasta et al. [C30] showed that mis-segregated chromosomes are entrapped in micronuclei and that the DNA content

of micronuclei can be pulverized (highly fragmented) due to aberrant (reduced) replication during the S-phase of the cell cycle.

172. A key step forward for understanding relevance of chromothripsis in carcinogenesis was the demonstration of re-integration of pulverized DNA into the main genome. Zhang et al. [Z10] used a new technique combining live-cell imaging and single-cell sequencing to demonstrate that chromosomes mis-segregated to micronuclei, frequently undergo chromothripsis-like rearrangements in the subsequent cell cycle by incorrect reassembly of fragments through erroneous DNA repair. How generally this mechanism operates over all cell types, both normal and immortalized, requires clarification.

173. This mechanism may be relevant for low-dose radiation exposure, but also other dose levels. Clastogenic chemicals and ionizing radiation are very potent inducers of micronuclei in cultured cells and animals [H29]. Cultured human cells exposed to X-rays and mice treated with ^{18}F in the form of fluorodeoxyglucose, display the formation of micronuclei even at doses below 50 mGy [B44, M11]. Moreover, ionizing radiation promotes recombination and integration of plasmid or viral DNA in the main genome dependent on dose but independently of dose rate (low 0.95–1.0 Gy/h and high 1.60–1.78 Gy/min) at doses >1 Gy [S63]. Importantly, recent studies show an efficient genomic integration of extrachromosomal DNA by low and moderate doses of ionizing radiation. An investigation by Zelensky et al. [Z4] provided evidence that low doses of ionizing radiation (10–100 mGy) stimulated random integration of transfected and viral episomal DNA in human and mouse cells with an extremely high efficiency. Likewise, moderate doses of 400 mGy enhanced DNA integration (gene targeting) through HRR in human induced pluripotent and embryonic stem cells by 30-fold [H12]. Microbeam irradiation of cancer cells [M41] combined with sequence analysis of single-cell clones revealed chromothripsis-like complex chromosomal alterations. Furthermore, induction of clustered DSBs by meganuclease in Chinese hamster ovary cells [S15] in concert with sequence analysis of cell clones generated evidence for multiple clustered DNA alterations. Currently, studies relating chromothripsis to ionizing radiation are scarce and key experiments that would demonstrate the re-incorporation of radiation-induced micronuclear DNA in the main genome (such as was carried out by Zhang et al. [Z10] for micronucleated chromosomes) are lacking.

174. The lack of a threshold in micronuclei formation raises the question on repair efficiencies, although variation in background spontaneous micronucleus levels can present difficulties for the quantification of low dose effects [V15]. As pointed out in paragraph 161 mitosis inhibits DSB repair and DNA damage checkpoints as a mechanism protecting against telomere fusions [O18] and explaining the linear non-threshold induction of micronuclei by X-rays. This demonstrates that DNA damage response is not active in all phases of the cell cycle. Another important question is whether or not DNA damage response is equally efficient after low and high doses of ionizing radiation. At low doses (<100 mGy), repair of DSBs and base damage by end-joining mechanisms and excision repair, respectively, might require a threshold of cellular stress to trigger activation [G17, S6]. If this is the case, low-dose radiation might generate an excess of chromosomal damage/micronuclei and oxidative-induced mutagenesis. Indeed, oxidative radicals generated by low-dose radiation might induce micronuclei [D10], but the observations of linear no threshold dose-responses for radiation-induced micronuclei, in vitro and in vivo, suggests that the excess or the impact of low dose induced oxidative damage on chromosomal damage/micronuclei is minor. The situation is different for base damage mediated mutagenesis.

175. It is unclear if DNA repair is equally efficient after low and high doses. Cellular stress mediated by endogenous processes poses a major source of mutations and genomic instability. Saintigny et al. [S6] showed that incorporation of [^{3}H]-thymidine into DNA provides a model to analyse cellular responses to endogenous oxidative species. Incorporation of [^{3}H]-thymidine in mammalian cells generates oxidation-induced mutagenesis (mostly base substitutions) but with a peak at low to moderate doses (20–250 mGy) even though the initial exposure was high (1.85–185 kBq/ml). The results point to involvement of redox

homeostasis and DNA damage signalling pathways and reveal dose-dependent induction of base excision repair. Collectively, the data reveal that a threshold of endogenous stress must be reached to trigger cellular detoxification and DNA repair pathways and, as previously shown, repair foci and HRR [S5]. The authors suggest that below this threshold, the consequences of endogenous stress escape cellular surveillance, leading to high levels of mutagenesis and genomic instability. However, the peak of mutagenesis at 20–250 mGy was not observed when using external radiation exposure.

176. In summary, antibodies raised against signalling proteins (γH2AX, ATM, 53BP1, etc.) have been employed to measure induction and removal of DSB in cultured cells and in vivo human tissue at low doses (<100 mGy). In vitro systems with reporter genes allow the measurement of repair of single enzymatically engineered DSB in mammalian cells by the principle DSB repair pathways NHEJ and HRR. Defined repair deficient cells elucidated the role of NHEJ and HRR in DSB repair after low dose X-rays or enzymatic induced single DSB. In general, repair of DSB in mammalian cells is efficient and complete. Only in case of DSB induced in confluent human fibroblasts, a threshold of repair has been observed by lack of activation of repair due to insufficient ROS. For low dose induced base damage, evidence points also to a threshold of repair due to insufficient ROS to up-regulate the repair. The genetic consequences of ionizing radiation induced DNA damage have been assessed by measuring induction of cancer related genetic damage such as LOH, chromosomal aberrations and micronuclei. Slowly repaired complex DSBs are particular good inducers of chromosomal damage. No threshold is found for induction of micronuclei at low dose (10–100 mGy) in cultured cells and mice. Reduced micronuclei yields observed following low-dose-rate exposure (100 mGy delivered over 48 hours), can be attributed to up-regulation of antioxidant defences indicating a role for ROS. Induction of tumours in high dose X-irradiated NHEJ and HRR deficient mice revealed the importance of HRR and NHEJ for suppressing radiation-induced cancer but their relative roles depend on tumour types. There is good evidence from tumours induced (at high dose) in irradiated mice, that the spectrum of radiation-induced mutations is different from the spontaneous mutation spectrum.

F. Genomic instability, bystander effects, damage/effects on non-nuclear cellular components, adaptive response and hyper-radiosensitivity

177. A range of different approaches and strategies have been used to explore how low-dose and low-dose-rate radiation could cause effects through damage to non-nuclear components of the cell and also trigger either "non-targeted" effects, beneficial or harmful responses in adjacent cells or daughter cells of cells exposed to radiation, at organ level or on the level of the whole organism. The Committee published a comprehensive review of non-targeted effects of radiation exposure in 2006 [U7] that concluded that no specific roles for genomic instabilities or bystander phenomena in radiogenic disease causation had been identified. The UNSCEAR 2012 White Paper [U9] to consider the impact of the non-targeted effects, including genomic instability, bystander phenomena and adaptive responses [U9] noted *(a)* the apparent inter-relationships between these phenomena, including possible mechanistic similarities; *(b)* the expressed concerns on the inherent variability and on reproducibility; *(c)* the increased body of relevant literature; *(d)* an emerging consensus on a lack of observations of transmissible instability following low-LET exposures below 0.5 Gy; and *(e)* the absence of a coherent body of knowledge that can be applied to assessment of low dose health risk. An earlier report on adaptive responses is also available [U4].

1. Effects on mitochondria

178. The possible outcomes of low-dose radiation exposure include changes in non-nuclear compartments of the cell. For example, changed performance of mitochondria may be an outcome of a direct impact on the function of these organelles, or a mutation in genomically-encoded or mitochondrially-encoded genes that encode proteins important for mitochondrial function.

179. There is considerable evidence that mitochondria can be an important target for radiation effects. Respiratory complexes I and III of the mitochondria of haematopoietic progenitor cells have been identified as a non-nuclear target of high doses (10 Gy and above) [P9]. Belikova et al. [B28] identified a role for mitochondrial cytochrome c in mediating radiation-induced apoptosis at high doses (>5 Gy). Furthermore, inhibition of mitochondrial cytochrome c peroxidase reduces radiation-induced cell death by apoptosis at 10 Gy [A24]. Using cells derived from mice deficient in the FancD repair factor it has been possible to demonstrate that radioprotectors targeting mitochondria can ameliorate the effects of high doses even where DNA repair is defective [G10]. FancD2 and its related and interacting proteins are clearly multifunctional having both nuclear (DNA repair) and mitochondrial roles [J13, S69, Z14].

180. At lower dose levels mitochondrial fusion and increased expression of complexes I and III has been observed in 0.2 Gy 6 MeV X-ray-irradiated rat hippocampal neurons [C23]. This was interpreted to indicate that neuronal cells modify energy metabolism in response to 0.2 Gy irradiation; no effects on survival, ROS levels, mitochondrial DNA levels or synaptic function were observed. By contrast, a fractionated X-irradiation protocol (31 twice daily exposures of 0.01 or 0.05 Gy) has been found to increase mitochondrial mass and mitochondrial ROS levels followed by nuclear accumulation of cyclin D1 and consequent growth retardation and senescence in normal human lung fibroblasts (MRC5 and TIG3) [S36]. However, in *ATM*- and *NBS1*-deficient cells, similar fractionated exposures led to accumulation of abnormal mitochondria; this was determined by mitochondrial fragmentation and decreased mitochondrial membrane potential [S35]. Differential effects of X-rays and C ions have been reported in mitochondria of HeLa cells; lower doses (0.2 and 0.5 Gy) of X-rays lead to mitochondrial fusion, with higher doses leading to mitochondrial fission while C ion irradiation at all doses examined (0.2–3 Gy) caused mitochondrial fission [J20]. The role of mitochondria in ROS production following low-dose irradiation has been reviewed recently [K20].

181. The importance of the mitochondrial target has been demonstrated with low-dose alpha particle exposure also. For example, the bystander induction of γH2AX foci by 10 mGy alpha particle exposure in medium transfer experiments is reduced in cells with fewer mitochondria or inhibited respiratory chain function [C16]. Similarly, radon-induced apoptosis is reduced in bronchial airway cells with fewer mitochondria [L20]. Direct damage to mitochondria and mitochondrial function has been shown following precisely targeted microbeam alpha irradiation (2 or 10 alpha particles per cell, a high dose of high-LET radiation) [Z6]. Cytoplasmic irradiation with alpha particles can lead to nuclear damage as assessed by γH2AX foci and micronucleus formation [Z7]. Additionally, cytoplasmic gamma irradiation leads to a metabolic shift to a more glycolytic energy metabolism mediated by Pim1 expression, notably Pim1 was not observed to be induced by nuclear irradiation [W15].

182. Persistent induction of ROS as a consequence of low-dose (20 mGy gamma ray) radiation exposure have been observed to lead to autophagy and mitochondrial dysfunction in haematopoietic stem cells [R14] with the effects apparently restricted to irradiation in vitro and having effects on cell survival.

183. Direct connections to cancer are difficult to ascertain from differences in mitochondrial function, protein expression or metabolism over extended periods of time. Nevertheless, it is conceivable that these effects can be associated with modulation of cancer incidence, for example through persistent elevation of ROS. When wild-type animals were used for low-dose-rate experiments, some lifespan experiments

revealed protein expression differences to be more noticeable than the DNA/RNA differences. For example, at ages of six months and two years, cardiac tissue from mice exposed to radiation in utero presented a number of protein expression differences. The differences between controls and mice exposed to a dose of 1 Gy were only two times higher than the difference between controls and mice exposed to a dose of 100 mGy [B9]. More importantly, of all possible broad cellular functions, the most substantial and consistent differences were those for mitochondrial proteins. However, the relevance of these changes to cancer are not clear but they provide evidence of low-dose radiation effects.

184. The literature outlined above clearly demonstrate an effect of radiation at high and low dose on mitochondria. Not all studies are entirely consistent in their reported effects, but one can conclude that mitochondria represent an important non-nuclear target for the effects of radiation.

2. Genomic instability

185. In the context of studies of radiation effects, genomic instability is defined as a persisting and transmissible (through cell generations) increase in observed frequencies of mutations, chromosome aberrations or other similar endpoint following exposure to radiation. It has been described largely in in vitro studies where cells are exposed to radiation, grown for several cell generations and then assessed for chromosome aberrations, mutation, etc.

186. Zyuzikov et al. [Z23] used C57BL/6 mice, relatively resistant to development of radiation-induced myeloid leukaemia, as well as CBA/Ca mice, susceptible to radiation-induced myeloid leukaemia and chromosomal instability in the bone marrow [Z23], to explore potentially adverse bystander effects of low doses of radiation. At 8–12 weeks of age mice were either sham-irradiated or exposed to X-rays at a dose rate of 1 mGy/s to total doses of 1.7, 5, 12.1, 25, 50 or 100 mGy; dose rate of 7.5 mGy/s was used for delivery of high doses of 0.5, 1 and 3 Gy. Regardless of dose rate, doses below 1 Gy did not cause genomic instability in bone morrow cells of either strain. Percentage of $p53^{+}$ and $p21^{+}$ cells in C57BL/6 and C57BL/6 mice stayed below 7.5 and 2, respectively, and no evidence of bystander effect via p53 signalling was detected at low-dose exposures up to 100 mGy.

187. Rithidech et al. [R6] similarly reported that doses of 50 and 100 mGy gamma rays were incapable of inducing genomic instability as assessed by late forming chromosome aberrations after in vivo exposures of BALB/c mice. By contrast, a 1 Gy dose did induce instability in the BALB/c strain, none of the doses led to persisting instability in C57BL/6. However, 50 mGy led to a reduced frequency of aberrations in C57BL/6 mice at 1 month after irradiation. Similar levels of instability were reported to be induced in BALB/c mice irradiated in vivo with 1 Gy 100 MeV protons and ^{137}Cs gamma rays [R7], no dose dependence between 0.5 and 1 Gy was observed for proton exposures.

188. Cho et al. [C24] reported that doses of 200, 500 and 1,000 mGy X-rays did not induce persistent elevation of micronucleus frequencies up to 10 days (5 passages) in normal human fibroblasts (hTERT immortalized) but level of aneuploidy of chromosomes 1 and 4 were elevated at 10 days post-exposure.

189. Genomic instability specifically investigated in the T-cell receptor rearrangements of irradiated CBA mice were not observed following 0.1 and 1 Gy in vivo exposures [C4]. High-dose-rate exposures to X-rays at 0.5 Gy/min and lower dose-rate exposures to gamma rays at 0.43 or 2.1 Gy/h ^{137}Cs were used, and no difference between dose rates was reported.

190. Using microsatellite instability as a readout of persistent genomic instability it has been possible to examine mouse tumours induced by radiation and compare to spontaneously arising tumours to gauge the role of induced instability in radiation carcinogenesis [H4]. A mixture of acute myeloid leukaemias,

intestinal tumours in *Apc*^{min} mice, medulloblastomas and basal cell carcinomas in *Ptch* mice were considered. This study found that 16% of spontaneously arising tumours had the microsatellite instability phenotype, while 23% of tumours arising in X-irradiated mice were microsatellite instability-positive, and 83% of tumours in neutron-irradiated (fast neutrons with a mean energy of 1 MeV) mice were microsatellite instability-positive. It was concluded that microsatellite instability may play a role in radiation tumorigenesis, particularly after high-LET exposures.

191. In breast cancers arising among those irradiated for haemangioma, a dose-dependent elevation in DNA copy number variants has been described [B34]. While this finding may indicate transmissible instability as having a role in radiation-associated carcinogenesis in human, the paper does not make clear if the DNA copy number variants have increased in number over time since exposure. A higher level of genomic re-arrangement would be anticipated in tumours exposed to higher doses.

192. The more recent genomic instability studies considered in the preceding paragraphs do not substantially alter previous conclusions drawn on the role of genomic instability in radiation carcinogenesis. There is more evidence indicating the existence of a threshold of around 100 mGy low-LET radiation being required to trigger instabilities.

3. Bystander and abscopal effects

(a) *In vitro studies*

193. Functional studies of different types of bystander and abscopal effects have focused on different cell lines, cell densities and qualities of radiation using in vitro models. Abscopal effects operated over substantial distance between organs while bystander phenomena relate to effects observed over the range of a few cell diameters. A range of endpoints have been utilized to assess bystander effects, some involving 'omics methods. The relatively slow advance of computational analyses for 'omics studies is likely to have limited progress in bystander effect studies.

194. Furlong et al. [F25] explored directly irradiated and bystander human keratinocyte HaCaT cells with regard to gene expression in radiation-induced bystander effects. Direct irradiation or conditioned media from cells exposed to 0.05 and 0.5 Gy gamma rays was used to treat HaCaT cells and induction of gene expression was frequently found to include the same genes (*p53*, *Bax*, *Bcl-2*, *JNK* etc.) regardless of direct or "indirect" exposure. Exceptions were *ERK* and Caspase 9 – induced only by direct irradiation and Caspase 3 – induced only by exposure to conditioned medium.

195. Fournier et al. [F17] irradiated several different normal skin fibroblast cell lines using microbeams with C and Ar ions (4.8 MeV nucleon; LET 250 keV/µm and 1,950 keV/µm, respectively). 53BP1 foci were detected only in cells hit with the beam while p21 expression was found in surrounding cells including cells relatively remote from the site of microbeam impact. Ion energies were not found to be important for induction of response. While increased p21 expression was documented, no indication of terminal differentiation of cells was found. Subsequently, the same research team [F18] continued to use different ion beams to explore bystander effects in normal fibroblast cells. In this work, primary focus was on γH2AX and other markers of DNA damage. The authors found no increase in DNA damage in bystander cells either by evaluating γH2AX foci, numbers of sister chromatid exchanges or development of micronuclei when microbeams were used. In control experiments, use of X-rays or ion broad beam caused DNA damage as extensive as expected. A study by Groesser et al. [G16] corroborates these findings – 20 MeV/nucleon nitrogen ions (LET 150 keV/µm) or 968 MeV/nucleon (LET 151 keV/µm)

or 575 MeV/nucleon (LET 176 keV/μm) used to irradiate cells in culture carrying repair interfering mutations caused no detectable DNA alterations in bystander cells.

196. Yeles et al. [Y6] conducted bioinformatics analysis of molecular pathways associated with radiation-induced bystander effects – GEO datasets [N15], and of many endpoints most applicable to all datasets (universal for radiation-induced bystander effects) were the following: the negative regulation of growth, cellular response to Zn^{2+}-Cd^{2+}, and Wnt and NIK/NF-κB signalling.

197. Sekihara et al. [S21] exposed U937 cells mimicking skin-infiltrating monocytes and human primary keratinocytes taken from newborn humans to a 0.1 Gy dose of 4 MeV X-rays delivered at a dose rate of 2.0 Gy/min. Conditioned media were harvested at 24 hours and used to produce bystander cells. Gene expression and protein expression were checked in directly irradiated and bystander cells and potential transcription regulators explored by pathway analyses. Of these, only prolactin was surmised to be a gene expression regulator both in directly irradiated and bystander cells. A similar situation was encountered in directly irradiated and bystander human primary keratinocytes cells. Only few (e.g. c-Myc) genes were found to be activated in both cell lines following the same irradiated cell-bystander cell pattern.

198. Le et al. [L9] described a role for p53 in bystander responses in five cell lines with different *p53* status (HaCaT: *p53* mutated; SW48 *p53* wild-type; HT29 *p53* mutated; HCT116+/+ *p53* wildtype; HCT116–/– *p53* null). Cells were irradiated with beta particles from tritium and UV-mediated bystander response evaluated and found to be associated with *p53* status such that presence of wild-type *p53* correlated with the presence of a radiation-induced bystander effects.

199. Hagelstrom et al. [H1] found that DNA dependent protein kinase catalytic subunit (DNA-PKcs) and ATM are necessary for a bystander signal production but not cell signal reception in normal human cells (with wild-type *p53*) following 1 Gy gamma-ray exposure. In addition, this study found that directly irradiated cells do not respond to simultaneously experienced bystander signals. Sister chromatid exchange was the endpoint of the study; normal human fibroblasts and normal mouse kidney cells were used for the analyses.

200. Studies focusing on ROS and enzymes involved in their production include work from Abdelrazzak et al. [A1] where 208F rat fibroblast cell lines were exposed to less than one to a few hundred mGy of alpha particles (3.0 MeV, LET of 127 keV/μm produced from a ^{238}Pu source), gamma rays (^{60}Co) or ultrasoft X-rays (1.49 keV AlK characteristic X-rays) while non-irradiated bystander cells were v-src-transformed 208Fsrc3 cells. The successful induction of a bystander effect depended on at least 10% of 208F cells being irradiated (i.e. traversed by one or more alpha particles, with one particle traversal equating to a mean dose of 26 mGy). Radiation caused production of enzymes such as peroxidase and this effect could be replaced by exogenous addition of TGF-β [A2]. The importance of TGF-β in bystander effects was also explored in [M25]. In this study, human mammary epithelial cells were clonally analysed to evaluate genomic instability. A threshold for instability was >100 mGy but it could be reduced by exposure to TGF-β1. Work by Wang et al. [W4] on the other hand, indicated that TGF-β1 given on its own or when it is induced by heavy ion irradiation (e.g. delivery of 0.4 Gy of Si or Fe nuclei to human oesophageal epithelial cells (EPC2-hTERT)) causes epithelial-to-mesenchymal transition (EMT). EMT process has been implicated in wound healing, fibrosis, tumour invasion and metastasis, all included under the concept of "stemness". In addition, EMT can be caused by mutations, epigenetic events, and inappropriate signalling.

201. Yang et al. [Y4] found that the frequency of DNA break foci in irradiated and bystander AG01522 human cell fibroblasts doubled after exposure to 0.47 mGy of Fe ions (about 0.02 Fe ions/cell, 1 GeV/nucleon, LET 151 keV/μm) and that delivery of higher doses to targeted cells does not increase damage to bystander cells. The situation was similar with DNA damage doubling both in irradiated and

bystander cells at 70 μGy protons (about 2 protons/cell, 1 GeV; LET 0.24 keV/μm). With further increase of dose of either type of radiation DNA damage did not change in bystander cells. In the irradiated population, irrespective of radiation type, the fraction of damaged cells was constant from the lowest damaging fluence (0.02 Fe ions/cell or 2 protons/cell) to about a 10 mGy dose and then the damage increased linearly with dose. Overall, the bystander effect was less variable across doses and dominated effects at low doses.

202. Lam et al. [L4] observed a "reversed bystander effect" where protection from radiation damage was achieved by use of media harvested from non-irradiated HeLa and NIH/3T3 cells that were previously temporarily sharing media with the irradiated cells. Irradiations were carried out with 50 mGy by alpha particles using an ^{241}Am irradiation source (diameter of active area = 12 mm, average alpha particle energy = 5.16 MeV, activity = 5.02 μCi, dose rate = 180 mGy/min). Incubation of designated bystander cells with irradiated cells that was necessary to trigger the bystander effect was prevented in the presence of NF-κB activation inhibitor BAY-11-7082.

203. Maeda et al. [M5] reported that synchrotron-generated low energy photons (5.35 keV, X-ray microbeam focused to 10 × 10 μm) were used to irradiate the nuclei of, or (5.35 keV, X-ray microbeam focused to 50 × 50 μm) was used to irradiate the whole of, V79 Chinese hamster lung cells. The endpoint was clonogenic survival of the bystander cells. In both cases, NO mediated increased cell death since use of carboxy-PTIO as a scavenger obviated this response. It should be noted that cells were not touching each other in this experiment.

204. Klammer et al. [K27] explored the adaptive response and its induction in bystander cells as well as the ability of cells to respond better to high doses after exposure to low doses. This work used plasmids to monitor DNA repair by NHEJ and HRR in mouse embryo fibroblasts and human fibroblast cell lines HF12 and HF19 for paired exposures to 10 mGy and 5 Gy X-rays. Bystander effects were induced by medium-transfer technique in mouse embryo fibroblasts but not human cells.

205. Heuskin et al. [H28] studied lung adenocarcinoma A549 cells irradiated with 100 keV/μm alpha particles or 5 keV/μm protons at a dose rate of 0.1 Gy/min and showed hyper-radiosensitivity below 0.1 Gy. Because of the low fluence of radiation, it is possible to attribute this response to a bystander effect. Addition of Lindane at a concentration of 40 μM abolished gap junctions and increased cell survival after low- dose exposures.

206. Much work on gap junctions comes from the laboratory of Edouard Azzam. For example, Autsavapromporn et al. [A26] evaluated bystander mechanisms in AG1522 normal human diploid skin fibroblast cell cultures exposed to ^{137}Cs gamma rays, 3.7 MeV alpha particles, 1,000 MeV protons or 1,000 MeV/nucleon Fe ions. Mean adsorbed doses ranged between 0.25 and 2 Gy and numbers of bystander cells with micronuclei were higher in dishes where cells were grown to confluency. Radiation exposure of HeLa cells engineered to express connexin 26 or connexin 32 showed more bystander cells with micronuclei when connexin 26 was expressed.

207. Autsavapromporn et al. [A27] exposed confluent normal human skin fibroblast NB1RGB cells to 0.05 to 0.37 Gy adsorbed dose to a target cell from monochromatic 5.35 keV X-rays (LET, 6 keV/μm), 18.3 MeV/μm C ion (LET, 103 keV/μm), 13 MeV/nucleon Ne ion (LET, 380 keV/μm) or 11.5 MeV/nucleon Ar ion (LET, 1,260 keV/μm) microbeams in the presence or absence of 18-aglycyrrhetinic acid, an inhibitor of gap junction intracellular communication. Variable effects were obtained – in the case of X-ray exposures in the presence or absence of 18-aglycyrrhetinic acid equal numbers of micronuclei were found in bystander cells suggesting a role for secreted factors (not gap junctions) in signal mediation. In cells exposed to heavy ions bystander cells had fewer micronuclei in the presence of 18-aglycyrrhetinic acid suggesting that gap junctions in this case lead to stress increase.

Similar results were reported by [D11] who worked on apparently normal AG1522 human diploid skin fibroblasts expressing connexins 26, 32 and 43, and communicating by gap junctions and secreted factors. These cells were exposed to 3.7 MeV alpha particles, originating from 7.4 MBq ^{241}Am-collimated source (corresponding to a dose rate of 20 mGy/min), 1,000 MeV/nucleon Fe ions, 600 MeV/nucleon Si ions, or ^{137}Cs gamma rays. Experiments included 18-aglycyrrhetinic acid – a reversible inhibitor of connexin channels and hemi-channels, or $LaCl_3$, an inhibitor of hemi-channels. Bystander effects evaluated as a number of cells with micronuclei were prominent after all three types of radiation and they were decreased by application of 18-aglycyrrhetinic acid inhibitor.

208. Buonanno et al. [B58] used C3H 10TK mouse embryo fibroblasts to evaluate modulation of the spontaneous frequency of neoplastic transformation in the progeny of bystander mouse embryo fibroblasts that had been in co-culture 10 population doublings earlier with mouse embryo fibroblasts exposed to moderate doses (0.25 Gy) of densely ionizing Fe ions (1 GeV/nucleon) or sparsely ionizing protons (1 GeV). An increase ($p<0.05$) in neoplastic transformation frequency, likely mediated by intercellular communication through gap junctions, was observed in the progeny of bystander cells that had been in co-culture with cells irradiated with Fe ions, but not with protons.

209. A different focus on gap junctions comes from Ramadan et al. [R2]. Two human endothelial cell lines immortalized to permit telomere maintenance were used: hTERT telomerase immortalized human coronary artery endothelial cells and telomerase immortalized human dermal microvascular endothelial cells. Exposure to single 0.1 and 5 Gy doses and three fractions (0.033 or 1.67 Gy/fraction/d) of X-ray irradiation modulated gene expression of atheroprotective connexins 37, 40 and proatherogenic connexin 43. Further, irradiations with acute but not fractionated 0.1 Gy caused increase in gap junctional communication at 72 hours as indicated by exchange of 6-CF dye. This effect was abolished by carbenoxolone.

210. Ojima et al. [O13] used shielding and a change in mAmps to modulate X-ray delivery from total doses of 1.2 to 200 mGy. Gap junctions were inhibited by Lindane, an inhibitor of gap junction intercellular communication. Phosphorylated ATM foci in cells were imaged and quantified. Foci were found at 1.2 mGy and increased supralinearly or, after addition of Lindane linearly. Primary normal human lung fibroblasts MRC-5 were used in this study.

211. Jelonek et al. [J16] carried out a review of exosomes indicating that radiation induces increased exosome release in a dose- and time-dependent manner due to the activation of stress-inducible pathways of exosome secretion: increased expression of *TSAP6* (transmembrane protein tumour suppressor-activated pathway 6), which is stimulated by *p53* transcription factor as noted in human epithelial lung H460 cell line and aneuploid immortal keratinocyte HaCaT, human breast adenocarcinoma MCF7, and human prostate cancer cell lines, where this effect was additionally related to senescence. In human glioblastoma cell lines increase in exosome release following irradiation was observed in *p53*-mutated/overexpressed cell lines (LN18 and U251) in comparison to U87 cell line with wild-type *p53*.

212. Freudenmann et al. [F19] studied exosomes from PC3 (prostate adenocarcinoma) or A549 (lung adenocarcinoma) cells released into conditioned medium following radiation. Conditioned medium from either cell line when no irradiation was carried out induced proliferation of self, one another or HSF7 (normal skin fibroblasts). This effect was decreased when conditioned media was harvested from cells previously exposed to 2 Gy. Exosomes from all cells had no effect on proliferation of any of the three cell lines. While irradiated exosomes from PC3 cells did not have the capacity to induce cell proliferation, conditioned media from non-irradiated cells did. Exosomes of non-irradiated PC3 cells contain L-Plastin: this protein was absent after 2 Gy irradiation.

213. Jella et al. [J15] irradiated HaCaT cells with 0.005, 0.05 and 0.5 Gy gamma rays from a ^{60}Co instrument and harvested exosomes isolated after 1 hour. While complete conditioned media had

extensive effects on non-irradiated cells, exosome free conditioned media did not cause any reduction in viability, calcium influx or production of ROS.

214. Zhang et al. [Z13] explored skin tissue secretome – protein secretion in a reconstituted 3-dimensional skin tissue model (human skin tissues, EpiDerm Full Thickness 400) that was evaluated 48 h after exposure to 30 or 100 mGy (both at a dose rate of 600 mGy/min) and 2 Gy (dose rate 600 mGy/min) of X-rays using a targeted irradiation of mitochondria. Twelve replicates each were carried out; different proteins showed a variety of responses to radiation including dose response and biphasic response.

(b) *In vivo bystander studies*

215. Blyth et al. [B43] employed transplantation of sham or tritium loaded or X-ray irradiated splenocytes into animals not exposed to radiation (genotypes in this work were C57BL/6J mice donors and $pKZ^{-/-}$ and $pKZ^{-/+}$ recipients). Donor splenocytes were exposed to total doses of 0.1 or 1 Gy (dose rate 5 Gy/min) or labelled with similar amounts of [methyl-^{3}H]-thymidine (specific activity 3.18 TBq/mM), about 0.3 mBq per cell. Regardless of the approach, however, adjacent splenocytes from recipient mice showed no changes for endpoints under consideration such as apoptosis or proliferation at 22 and 72-hour timepoints post-transplantation.

216. A very different study design was used to investigate effects of cell-free serum from gamma ray irradiated mice [S67]. Eight-week-old female C3H/HeN and B6C3F1 mice were either irradiated, 22 hours a day by a dose rate of 0.0181 Gy/h for 10 or 20 days (to a total accumulated dose of 4 or 8 Gy), or acutely exposed to equal doses at high dose rate of radiation 0.9 Gy/min. Serum was collected and added to primary cells in culture (mouse embryonic fibroblast cells or hepatocytes) that did not encounter radiation. After 24 hours gene expression in these cells was checked and increased expression of LCN2 protein was found in mouse embryo fibroblasts exposed to sera from acutely exposed mice. This effect was removed when antibodies against interleukin (IL)-1α were added to the mix. At the same time, LCN2 was not altered in primary hepatocytes cultured in the presence of the irradiated serum of the mice. A different LCN2 expression status was discovered in liver tissue isolated from irradiated mice: While LCN2 expression increased in livers of acutely exposed animals, it was unchanged in mice exposed for 10 days and significantly decreased in animals irradiated for 20 days.

217. Mancuso et al. [M7] irradiated neonatal *Ptch* $^{+/-}$ mice with whole- or partial-body exposure to study abscopal effects. Control animals had the highest frequency of medulloblastoma and lowest numbers of apoptotic cells in the cerebellum. Test mice were irradiated with whole body X-ray irradiation (doses 0.036 to 3 Gy) or with partial body irradiation of 3 Gy delivered to different parts of the body. The greatest number of apoptotic cells in cerebellum was found in whole body or partially irradiated mice where 3 Gy was delivered. The same experimental groups of mice had the highest incidence and the speediest onset of medulloblastoma as well. Conversely – mice exposed to 0.1 Gy had fewer medulloblastomas than the control mice. Importantly, connexin 43 induction was the greatest in situations where abscopal apoptosis in non-irradiated cerebellum was the greatest (i.e. 3 Gy partial body exposure).

218. Szatmari et al. [S74] found that extracellular vesicles mediate systemic bystander effects at 24 hours post-injection when they are isolated from BM and spleen of 9–14-week-old male C57BL/6 mice at 24 hours after exposure to 0.1, 0.25, 2 Gy (THX-250 therapeutic X-ray source). While DNA damage found as chromosomal aberrations and γH2AX increases with dose in irradiated animals (maximum at 2 Gy), bystander mice exposed to extracellular vesicles from irradiated mice show more DNA damage after injection with extracellular vesicles from mice exposed to 0.25 Gy.

219. Szatmari et al. [S75] continued exploration of directly irradiated (0.1 or 2 Gy) and bystander mice and screened both the miRNA expression in extracellular vesicles and proteins from whole blood focusing on cytokines. A complex situation arose. Among increased proteins the only dose-dependent direct radiation and bystander radiation effect that overlapped was an increase in lipocalin 2 at 2 Gy. M-CSF and pentraxin 3 were increased in all cases: i.e. directly irradiated and bystander samples after either 0.1 or 2 Gy exposure. miRNAs isolated from extracellular vesicles from directly irradiated animals suggested activation of acute myeloid leukaemia and TGF-β regulatory pathways.

220. The work considered in this section continues to highlight the variability of bystander phenomena. There is no consistently identified mechanism for bystander signalling, and thus the effects may represent a range of effects at the cellular or tissue level, the importance of which for carcinogenesis at low doses is not yet clear. Tomita and Maeda [T19] provide a detailed and extensive review of bystander phenomena and similarly note the mixed results reported. They conclude by concurring with the position on bystander phenomena stated in the UNSCEAR 2012 White Paper [U9], that there is little of the coherence required of robust data among bystander studies that can be used confidently for risk assessment.

4. Adaptive response

221. A number of studies relating to adaptive responses have been published in recent years. These studies include in vitro studies, in vivo studies usually utilizing rodent models and a few studies relating to human populations. Some studies concern the optimal adapting dose (also referred to variously as priming dose, adaptive dose or pre-exposure in certain studies) to adapt cells to respond differently to the subsequent, higher, challenge dose.

222. Azimian et al. [A29] studied the effects of low doses of ionizing radiation on the expression of specific apoptotic genes such as *Bcl-2* and *Bax*. Blood lymphocytes from four healthy donors were exposed to 20, 50 and 100 mGy of ^{60}Co gamma rays at a dose rate of 13 mGy/min. Expression profiling analysis was carried out at 4, 24, 48, 72 and 168 hours post-irradiation by real-time PCR. It is reported that low doses used in the study can induce early down-regulation of *Bax* with normal levels restored at 168 hours. *Bcl-2*, the anti-apoptotic gene, was up-regulated. They argue that gene regulation/modification as early as 4 hours post-irradiation may underlie adaptive responses, due to their transient nature. All expression levels returned to normal within one week after exposure.

223. A study by Saini et al. [S4] combined both acute exposures to low doses of gamma radiation and adaptive response of human lymphocytes by measuring expression levels of selected DNA damage response markers. Blood samples were collected from 24 healthy human volunteers and exposed to doses of 0.1, 0.3, 0.6, 1.0 and 2.0 Gy from ^{60}Co gamma rays at a dose rate of 0.7 Gy/min. Both DNA damage measured by comet assay and γH2AX expression showed dose-dependent increases. There was dose-dependent up-regulation of *GADD45A*, *CDKN1A* and *TP53* from doses up to 1 Gy at five hours post-irradiation. Adaptive responses (adapting doses of 0.1–0.6 Gy, challenge dose of 2 Gy) at the level of mRNA expression were seen for *CDK2*, *CYCLIN E* and *TP53* (i.e. these transcripts showed higher levels of expression 1 and 5 hours after exposure to adapting doses followed by a 2 Gy challenge than a single 2 Gy exposure), whereas other genes such as *ATM*, *ATR*, *GADD45A* and *MDM2*, did not display any adaptive response (i.e. transcript levels were similar following a 2 Gy exposure with or without an adapting dose) [S4]. In a further study of gene expression modifications correlating with adaptive response in G_0 human lymphocytes, Shelke and Das [S29] found that Ku70, Ku80, XLF and Ligase IV were significantly up-regulated at 4 hours after exposure to an adapting dose of 0.1 Gy, suggesting the involvement of the NHEJ pathway of repair in adaptive response.

224. Kumar et al. [K48] investigated DNA breakage assessed by alkaline comet assays in peripheral blood lymphocytes drawn from subjects living in the Kerala province of southern India. Blood samples were from one of three groups: *(a)* 43 from a "low dose group" living in an area of elevated natural background radiation receiving an average annual effective dose of 2.69 mSv, *(b)* 34 from a "high dose group" living in areas of elevated natural background radiation receiving on average a 9.62 mSv annual effective dose and *(c)* 37 from those living in areas of normal background radiation levels, <1 mSv per year average. The initial induction of breakage by 2 and 4 Gy ^{60}Co irradiation was found to be lower in the high dose group samples that either of the other groups. Repair of breakage over a 30 minute time course was marginally more rapid initially in the high dose group samples, but all samples had repaired the same proportion of breaks at 30 minutes post-exposure. The lower initial level of breakage observed in the high dose group is contrary to the usually observed equal initial damage induction irrespective of cell type and may suggest some inconsistent dosimetry or sample handling. Nonetheless, the authors suggest that the study may indicate that exposure to elevated levels of background radiation induces an adaptive response in blood lymphocytes. In broadly similarly exposure groups, a reduced initial frequency of micronuclei were observed in those samples from persons aged 40 years or greater, but not in those of less than 40 years of age; basal micronucleus frequencies were statistically indistinguishable among groups though somewhat higher in the samples from those living in the higher background radiation areas [R1]. Assessment of stable (translocations, inversions) and unstable (dicentrics, rings) chromosomal aberrations among the Kerala populations using standard Giemsa stained metaphases of peripheral blood lymphocyte revealed no differences in the background frequencies of these aberrations between high and normal natural background residents by Karuppasamy et al. [K17]. Premature chromosome condensation assays, again on samples from broadly similar groups, though not distinguishing between the two elevated levels of natural background exposure, found similar basals and 2 Gy ^{60}Co irradiation induced premature chromosome condensation fragments, though initial (1 hour post-irradiation) repair of premature chromosome condensation fragments was observed to be greater in samples from those living in areas of elevated natural background radiation [K49]. Nishad and Ghosh [N16] conducted a proteomic analysis of peripheral blood lymphocytes identified some differences in protein levels between samples collected from high natural background residents and normal natural background area residents, and increased responsiveness of some pro-survival proteins following challenge with 2 Gy gamma irradiation in samples from high natural background area residents. Collectively these results have been interpreted in the cited papers as providing evidence of an adaptive response having been induced in blood cells of individuals inhabiting areas of substantially elevated natural background radiation.

225. In a study by Jain and Das [J10], gene expression profiling was carried out on individuals living in high natural background radiation areas in Kerala, India. Peripheral blood lymphocytes were taken from 36 individuals. The natural background radiation dose ranges from <1.0 to 45 mGy in a year. Global transcriptomic analysis was carried out on the blood lymphocytes to find out the significance of chronic exposure. The results point to a dose-dependent increase in the number of differentially expressed genes with respect to different natural background radiation dose levels. Gene ontology analysis indicated that majority of differentially expressed genes fall under pathways responsible for DNA damage response, DNA repair, cell-cycle arrest, apoptosis, histone/chromatin modification and immune response. The authors report the identification of 64 genes which could be considered as biological signatures of exposure to natural background radiation. They link differential expression of genes in DNA damage response and DNA repair pathways to radiation adaptive response and normal or reduced cancer incidence reported [N4] in the population in the high-natural background radiation area. There are, however, no adaptive response data presented in the publication, but the findings may explain the observed efficient repair of DNA damage among persons inhabiting high natural background radiation areas of Kerala using alkaline comet or γH2AX assays [J11, K48].

226. Studies have also been carried out on blood samples drawn from the inhabitants of areas in the Ramsar region of the Islamic Republic of Iran living in elevated natural background radiation regions. Alkaline comet assays are reported to show elevated basal levels of DNA damage in those living in areas with average 10.2 mSv per year effective doses. The induction of damage by 0.3–4 Gy ^{60}Co irradiation was reduced in control (normal background radiation level) groups by comparison with samples from donors living in areas of high natural background radiation. Breaking the samples from those inhabiting elevated natural background radiation areas into <10.2 mSv per year average effective dose and >10.2 mSv per year groups revealed that incomplete repair was observed in the highest background exposed groups but in the <10.2 mSv per year group repair was elevated compared to control exposure groups [M20]. Zakeri et al. [Z3] observed elevated levels of unstable (chromatid-type and chromosome-type) chromosomal aberrations in those inhabiting areas of elevated natural background radiation in the Ramsar area. Mohammadi et al. [M38] report that blood lymphocytes drawn from those living in areas of Ramsar with elevated natural background radiation show reduced micronucleus induction, increased apoptosis induction and increased DNA damage repair assessed in alkaline comet assays, all after 4 Gy ^{60}Co irradiation. Unlike in the Kerala groups described in the previous paragraph, comet assays revealed an elevated level of basal damage and elevated induction of damage by 4 Gy ^{60}Co irradiation in the samples from those living in elevated natural background radiation conditions.

227. Basri et al. [B22] carried out a small study of DNA damage among those living under elevated natural background radiation conditions in a region of Sulawesi, Indonesia. Elevated background levels were an average of 7 mSv per year effective dose and normal background regions 2 mSv per year. Though not statistically significant due to small sample size (8 from high background areas, 29 from normal background areas), an elevated level of γH2AX foci was observed among lymphocyte samples from those in the high background regions. Looking at mitotic index in cultured peripheral blood lymphocytes from persons living in high (n=35, average 2.52 mSv/y, range 1.99–3.17 mSv/y) or normal (n=25) background radiation areas of Botteng village, Indonesia, Ramadhani et al. [R3] found no difference in proliferation characteristics of lymphocytes taken from elevated or normal background radiation areas.

228. To explore the effects of low- and high-dose radiation in human immune cell lines, cell sensitivity was tested following irradiation with 0.05, 0.1 or 2 Gy of ^{137}Cs gamma rays (dose rate – 0.0173 Gy/s) in normal cell lines as well as in tumour cell lines [P3]. The survival after exposure of different normal and tumour human immune cell lines correlated AKT expression, which seems to be under the control of PP2A. Exposure to a low dose of 0.01 or 0.05 Gy 4 hours before a challenge dose of 2 Gy protects IM9 (B lymphoblast) from cell death, and decreases cleavage of PARP-1, caspase-3 and caspase-9. These effects seem to be mediated by ERK activation which may have a role in the adaptive response to radiation at low doses.

229. The study by Hou et al. [H36] investigated the adaptive response induced in human fibroblasts (AG01522) exposed to a 50 mGy of adapting dose (X-rays, 60 kV at 105 mGy/min) and then to 2 Gy of X-rays as a challenge dose (220 kV, 2 Gy/min). Transcriptomic analysis revealed that low-dose X-ray exposure produced an alert, triggering and altering cellular responses (particularly p53-mediated responses) potentially to protect against high dose-induced damage. Changes in gene expression levels were associated with reduced chromosomal changes (micronuclei formation) in these cells through induction of adaptive response. Also using human fibroblasts in culture, Yim et al. [Y10] studied the expression of phosphoproteins following radiation exposure. Cells were exposed to 0.05, 0.1, 0.2, 0.5, 1, 2 or 4 Gy of ^{137}Cs gamma rays at a dose rate of 1.03 Gy/min. Cell proliferation was not affected and cells continued to proliferate without genomic instability (γH2AX foci formation and micronuclei induction) at low dose of gamma irradiation (50 mGy). Phospho-antibody microarray analysis and western blotting revealed the elevated expression of two phosphoproteins - phospho-NFκB (Ser536) and phospho-P70S6K (Ser418) at 8 hours after irradiation with 50 mGy. It is concluded that activation of phospho-NFκB (Ser536) and phospoho-P70S6K (Ser418) and absence of genomic instability after low-dose

radiation exposure (50 mGy) may indicate their involvement in subsequent DNA damage response and repair processes. The up-regulation may contribute to adaptive responses.

230. Vares et al. [V4] demonstrated that adapting doses of heavy ion radiation (0.01 Gy, 290 MeV/nucleon C ion and 400 MeV/nucleon Ne ion beams, with LET values of 20, 40 (C ion) or 150 keV/µm (Ne ion)) induce an adaptive response to a subsequent 6 hours later challenge dose (1–4 Gy) of high-LET radiation (C ion: 20 and 40 keV/µm, Ne ion: 150 keV/µm) in TK6, AHH-1 and NH32 cells. A significant decrease of micronuclei was found in the first two cell lines. Similarly, γH2AX foci repair was more rapid when adapting exposures were applied only in TK6 and AHH-1 cells.

231. Effects of low-dose radiation on the proteome in both human fibroblasts and stem cells were studied by Hauptmann et al. [H13]. Cells were irradiated with a ^{137}Cs source with a dose rate of 26,400 and 50 mGy/h. Cells were exposed to a 40 mGy adapting dose followed by challenge dose of 100 mGy after two-hour incubation. Challenge doses of 40, 100 and 140 mGy were also given. Non-linear dose–response relationships were observed for the up- and down-regulated proteins. Primarily, the structural and stress-response proteins were affected. The responses observed could be on/off effects involving different proteins, which depended on dose and dose rate. Exposure to an adapting dose significantly altered the proteome regulation by a challenge dose later.

232. The existence of an adaptive response was also investigated in human aortic endothelial cells (HAoECs) pre-exposed to high-dose gamma radiation (2 Gy) delivered at high (1 Gy/min) or low (6 mGy/h) dose rate as an adapting dose before receiving a challenge dose of 2 Gy delivered at 1 Gy/min. Overall, low-dose-rate priming increased the transcription of genes involved in anti-oxidative stress response, vascular growth factors and cytokines when compared to 2 Gy challenge dose only, while high-dose-rate priming by comparison either reduced the expression of these genes or had no effect [V10]. In this case, it appears that radiation promotes a protective response of irradiated endothelial cells.

233. In addition to the human cell studies, two relevant non-human cell studies have been published. Fan et al. [F7] exposed mouse epidermal cell line JB6P+ to low doses of 50 or 100 mGy of X-rays and a challenge dose of 2 Gy gamma rays (^{60}Co gamma radiation, 2.3 Gy/min). The low doses activated stress-sensitive genes *NF-κB*, *MnSOD*, *pERK*, *14-3-3eta* and *cyclin B1*; activation of these genes increased radioresistance to higher radiation dose (2 Gy). In order to study the immune adaptive response, Cheng et al. [C19] investigated expression levels of selected DNA repair proteins in murine lymphoma cells (EL-4) following irradiation. Adaptive low-dose exposure to X-rays (0.075 Gy at 0.0125 Gy/min; 200 kVp 10 mA) significantly prevented cell death and cell-cycle arrest in EL-4 cells exposed to high dose/high dose rate (1 to 2 Gy, 0.287 Gy/min). Low-dose radiation exposure prevented the increase of p53 expression in response to high-dose radiation. It is observed that these effects require PARP-1 activity in the cells. Therefore, PARP-1 and p53 might play important roles in adaptive response induced by low-dose irradiation.

234. Beneficial effects of an adaptive dose of 100 mGy to X-rays (0.028 Gy/min) of BALB/c mice were studied with the mice irradiated at 6–8 weeks to a challenge dose of 5 Gy alone or to the adaptive dose followed by the challenge dose [J19]. Pre-exposure was found to be beneficial for mouse skin in animals exposed to 100 mGy. *MnSOD* activity was higher, as well as oxygen consumption and ATP production, suggesting improved mitochondrial resilience.

235. Another study used in utero exposures to low doses of gamma radiation [H38] followed by acute exposure as a challenge dose. Pregnant BALB/c mice were sham-irradiated or exposed to an adaptive dose of 10–13 mSv/d for 10 days during organogenesis and weaned pups, 16–18 days old, were given an acute challenge dose of 2.4 Sv. Comparisons of DNA damage, white blood cell counts, and gene expression suggest that an adaptive response was elicited in pups that experienced in utero exposure.

With regard to gene expression, RT-PCR of liver mRNAs showed that genes involved in DNA damage response *(Brca1*, *Egr1*, *Ercc6*, *Gjc1*, *Gpx1*, *Hif1a*, *Hist3h2a*, *Hspa1b*, *Mlh1*, *Mos*, *Parp1*, *Polb*, *Rad51*, *Raf1*, *Sod1*, *Tnf*, *Trp53* and *Xpa*) had a more moderate suppression of expression in mice exposed to an adaptive dose prior to challenge dose than animals exposed to challenge dose only.

236. The induction of an adaptive response by low-dose and low-dose-rate exposure on the modulation of immune parameters by high dose low- or high-LET radiation was also investigated in C57BL/6 mice irradiated in vivo and ex vivo. In particular, Gridley et al. [G13] showed that protracted exposure to 0.01 Gy of gamma radiation delivered at 0.18 mGy/h did not modulate the response of mice, and especially their $CD4^+$ T-lymphocytes, to a subsequent proton exposure representing a simulated solar particle event (sSPE, 1.7 Gy at 30 to 210 MeV delivered in 36 hours). A following study showed that in this model, low dose and low dose rate and sSPE exposures independently activate different intracellular signalling pathways in $CD4^+$ T-cells purified and activated in vitro 4 and 21 days post-exposure, as revealed by phosphorylation of NF-κB, p38 MAPK, JNK, and T-cell receptor-associated signalling adaptors Lck and ZAP-70. When low-dose and low-dose-rate exposure was applied before sSPE exposure, most of the effects of proton exposure were inhibited, demonstrating that low-dose and low-dose-rate gamma radiation induces radio-adaptation able to modulate intracellular signalling induced by proton exposure [R9]. Despite this modulation of intracellular signalling pathways in activated $CD4^+$ T-cells, protracted low-dose and low-dose-rate exposure was found to have only a marginal effect on the homeostasis of leukocyte populations, platelets and erythrocytes in mice 4 and 17 days post-proton exposure (2 or 3 Gy, 210 MeV) [L51].

237. In line with these findings, the response of mice and their $CD4^+$ T-cells to 2 Gy gamma or proton radiation exposure was largely unaffected by prior low-dose and low-dose-rate exposure to gamma rays (0.01 Gy delivered at 0.3 mGy/h). Only a limited number of subtle changes were observed at day 21 and 56 (higher frequency of splenic $CD4^+CD25^+Foxp3^+$ regulatory T-cells (Tregs) in low-dose and low-dose-rate exposure + gamma radiation compared with gamma radiation only at day 21; inhibition of increase in the number of splenic Tregs and of the increase in vascular endothelial growth factor (VEGF) secretion in low-dose and low-dose-rate exposure + protons compared with protons only at day 56). Thus, the radio-adaptive ability of low-dose and low-dose-rate exposure to a challenge dose of high-dose gamma or protons radiation is quite limited at the level of cellular homeostasis [G15]. The expression of apoptosis-related genes was analysed in the liver of mice exposed in similar conditions. Low-dose and low-dose-rate exposure alone had no effects, but pre-exposure to low dose and low dose rate adapting doses modulates gene expression in mice exposed to 2 Gy gamma rays and protons. While only two genes are down-regulated in 2 Gy gamma-irradiated mice, 11 are modulated (up- and down-regulated) in mice previously exposed to low-dose and low-dose-rate exposure. Similarly, six and 12 genes are modulated (up or down) in proton compared with low-dose and low-dose-rate exposure + protons exposed mice. This study does not allow a conclusion as to whether this radio-adaptation is beneficial (increased apoptosis of exposed cells) or potentially detrimental (increased survival of possibly exposed damaged cells) [G15].

238. The main focus of the study by Lopez-Nieva et al. [L43] was to investigate the adaptive response in mouse thymocytes. C57BL/6J mice were exposed to an adaptive dose of 0.075 Gy of X-rays (100 kV/15 mA). This adaptive dose was known to induce an adaptive response in mouse thymocytes. After 6 hours, mice were subjected to a challenge dose of 1.75 Gy of gamma rays (^{137}Cs source). Levels of caspase-3 mediated apoptosis and global transcriptional expression in isolated thymocytes were determined in both male and female mice. Appropriate controls of mice exposed to a dose of 0.075 Gy alone, or a dose of 1.75 Gy alone were used for comparison. Male mice displayed lower levels of caspase-3 mediated apoptosis than females: a differential sex effect on adaptive response was observed. In the transcriptomic profiles of radioadapted thymocytes, 17 transcripts among the 1,944 genes involved in apoptosis signalling exhibited differential expression among both sexes. Dlc1 and Fis1 closely related

genes in p53 mediated apoptosis response were up-regulated and this up-regulation correlated with the increased accumulation of phosphoserine-18-TRP53 and caspase-3 in radio-adapted thymocytes of female mice. In contrast, down-regulation of Fis1 and phosphoserine-18-TRP53 was found to be associated with the protection from thymocyte apoptosis mediated by caspase-3 in males. It is concluded that transcriptomic analysis has revealed for the first time a sex-specific differences in gene expression influenced by radio-adaptive response in mouse thymocytes.

239. A series of publications have utilized the $pKZ^{Tg/+}$ transgenic mouse that allows assessment of chromosomal inversions. $pKZ1^{Tg/+}$ heterozygotes maintained by backcrosses with C57BL/6J mice were used for evaluation of chromosomal rearrangements in prostate tissue [D6]. Exposure to a priming dose of 0.001 mGy X-rays (250 kV, 15 mA at 0.0014 mGy/min), reduced frequency of the inversions that would have been induced by a single 1,000 mGy dose (dose rate 180 mGy/min) as well as the portion of inversions for spontaneous background frequencies. The study shows that adaptive responses produced from priming doses of X-rays protected against the formation of inversions induced by high doses of X-rays (1,000 mGy). This adaptive response has been shown to require only a single *ATM* copy. Day et al. [D7] investigated the influence of heterozygous mutation of *ATM* on radiation-induced adaptive response. Furthermore, in prostate an adapting dose of 0.001 mGy delivered several hours after a challenge dose of 1,000 mGy was observed to lead to a similar adaptive response as when the priming doses are delivered before challenge doses [D8].

240. Bannister et al. [B13] tested 20 mGy low dose (0.5 mGy/min), 100 mGy dose (dose rate 10 mGy/min) and 500 mGy dose (dose rate 10 mGy/min) as priming doses. When priming doses were delivered 4 or 24 hours ahead of a challenge dose of 2 Gy, there were no effects on micronucleus frequencies in splenocytes following in vivo or in vitro irradiation in C57BL/6 or BALB/c mice. A subsequent report confirmed and extended these findings [B14].

241. Collectively, these studies illustrate the ability of low-dose and low-dose-rate gamma radiation to modulate the response of immune cells in mice to a subsequent high dose of gamma or proton radiation. This modulation has apparently no discernible effects of the homeostasis and/or phenotype of immune cells, but is able to affect intracellular signalling pathways and gene expression. Potential differential response between males and females has been reported.

242. To conclude, there continue to be adaptive-response studies reported using both in vivo and in vitro approaches. There are both positive and negative findings reported, some indication of potential sex-dependent differences, and some endpoints showing adaptive response while others do not. A range of adapting doses have been reported, and different time schedules for the adapting and challenge exposures, including delivery of the adapting dose after the challenge dose. The gene expression studies have yet to provide a consensus on the pathways potentially up-regulated by adapting dose exposures, though several studies suggest p53 and/or NF-κB-dependence while ATM is apparently not involved. These reported findings are not consistent with current knowledge of DNA damage response signalling, which shows linear response consistently.

5. Examples of DNA repair modulation in cellular and animal models

243. Some animal models, with an innately high rate of spontaneous mutations showed decreased mutation burden after low-dose radiation exposures. These studies may be considered as providing evidence for the concept of an adaptive response, though there is no coherence between the animal and cellular data considered to date.

244. Low-dose-rate and/or low-dose exposures were found to act as deterrents for cancer development in animal models that harbour mutations making them predisposed to cancer development. Examples of such mouse models include *(a)* AKR/J mice that carry murine leukaemia viral oncogenes, known to develop thymic lymphoma during later periods of life [M43, S38]; *(b)* different strains and strain combinations of mice with knocked-out or mutated *p53* gene and their heterozygous counterparts, developing different cancers from lymphomas (e.g. [M12]) to a broad spectrum of solid tumours (e.g. *p53* delta proline mice described by Adams et al. [A6]); *(c)* animals with mutated or knocked-out transcription factor gene, *Ptch1*, regulator of the hedgehog pathway and acting as a tumour suppressor leading to skin cancers and medulloblastomas [A23].

245. AKR/J mice are a well-known model for thymic leukaemia; these animals are viraemic from birth and express the ecotropic retrovirus AKV in all tissues. Shin et al. [S38] exposed female AKR/J mice to gamma-ray radiation beginning with eight weeks of age for a total of 4.5 Gy delivered at low dose rate (0.7 mGy/h) or high dose rate (800 mGy/h). Thymic lymphoma incidence was lowest in low-dose-rate irradiated mice compared to either non-irradiated or high-dose-rate irradiated mice by 10 and 20%, respectively ($P<0.01$). Correspondingly, low-dose-rate irradiated animals lived 243 days, compared with 230 days for non-irradiated mice and 208 days for high-dose-rate irradiated animals. This study also included gene expression analysis. Thymic gene expression studies used animals 130 days after irradiation, 8–9 per group. Six hundred ninety-five genes increased and 1,726 decreased twofold in expression in high-dose-rate irradiated mice. In low-dose-rate irradiated mice 410 genes increased and 198 decreased twofold. Considering that low-dose-rate exposure extended life expectancy of these mice and high-dose-rate exposure decreased it, it is not surprising that only few genes had the same expression pattern in both irradiated groups of mice. These genes were *Cd5l*, *Pycard*, *Fcgr3*, *Lilrb3*, *Igh6, Fcgr2b* and *Mgc60843* and are involved in apoptosis and T- and B-cell activation.

246. Additional studies in AKR/J mice were carried out by the same group [B49, B50, B51]. Eight-week-old female AKR/J mice, carrying murine leukaemia viral oncogenes that lead to spontaneous development of thymic lymphoma, were exposed either to gamma rays produced by ^{137}Cs sources, one delivering dose rate of 800 mGy/min for an acute exposure, or 0.7 mGy/h for 24 hours per day low-dose-rate exposure. In one of these studies, animals were sacrificed and their thymi extracted 130 days after acute 4.4 Gy exposure or after cumulative dose of 2.1 Gy delivered at low dose rate [B49]. In this case, thymus weights were lowest and apoptosis most frequent in low-dose-rate exposed mice. Up-regulated and down-regulated genes numbered 55 and 186 in acutely irradiated or 29 and 8 in low-dose-rate exposed mice. Among these, *Cds1* (CDP-diacylglycerol synthase 1) was increased only in low-dose-rate mice, while *ItgA4* (integrin-α4), *ItgB1* (integrin-β1) and *Myc* showed increased expression in both groups of irradiated mice.

247. A further study used both AKR/J (inbred) and ICR (outbred) strains mice with slightly shorter exposures to 0.7 mGy/h ^{137}Cs gamma rays reaching a total dose of 1.7 Gy, but with a post-irradiation period of 100 days before animal sacrifice and thymus extraction [B51]. This work showed after pathway analyses that three pathways overlapped between two different animal genotypes: PPAR signalling pathway, calcium signalling pathway and systemic lupus erythematosus. Unique to ICR mice were pathways for: cancer, neuroactive ligand-receptor interaction, insulin signalling pathway, extracellular matrix receptor interaction and adipocytokine signalling pathway. Unique to AKR/J mice were pathways for: T-cell receptor signalling pathway, haematopoietic cell lineage, chronic myeloid leukaemia, Notch signalling pathway, primary immunodeficiency and circadian rhythm. Similarly, as before, low dose rate exposed AKR/J mice showed the most thymic apoptosis. This was an important finding considering that in this case low-dose-rate exposure was concluded 100 days prior to animal sacrifice and thymus harvest. TUNEL staining of thymi of ICR mice showed that the highest apoptosis (though not nearly as great in extent as that in AKR/J animals) was evident in high dose acutely exposed animals.

248. A long-term low-dose-rate exposure of *p53*$^{+/-}$ and wild-type mice was carried out by Mitchel et al. [M36]. The exposure periods lasted 30, 60 and 90 weeks. Animals were irradiated for five days a week and the daily dose was 0.33 mGy delivered at a low dose rate (0.7 mGy/h). Thus, the total doses fell into low and moderate dose ranges: 48, 97 or 146 mGy, respectively. Life shortening, and increased cancer incidences were found in wild-type mice exposed for 30 or 60 weeks, but not in those exposed for 90 weeks. At the same time, no differences in cancer incidence were found between non-irradiated or any of the irradiated heterozygote animals.

249. To summarize, the animal model used is important for exploration of effects of irradiation at low doses and low dose rates. In many cases, animals with genetic alterations that lead to development of different diseases benefited from exposures to low doses of radiation. While a direct connection to cancer outcomes is difficult to make in such cases, majority of these studies indicate that low-doses or low-dose-rate exposures to radiation lead to mild stress responses such as increased expression of *MnSOD* [T21] while in mice heterozygous for *p53* the quantity of p53 protein increased after low-dose radiation exposures [L18, L19]. In other words, low doses of radiation mobilize animals' gene expression resources in ways that may be, in the context of the underlying genetic status, beneficial.

6. Hyper-radiosensitivity

250. Low-dose hyper-radiosensitivity describes an effect of extreme cell death due to excessive sensitivity to small single doses of ionizing radiation but a more resistant phenotype (per unit dose) to larger single doses. The underlying molecular mechanism for hyper-radiosensitivity is linked to a dose-dependent pre-mitotic cell-cycle checkpoint that is specific to cells irradiated in the G2 phase and the lack of activation of this checkpoint at very low doses [M17].

251. The adaptive effects of low-dose and/or low-dose-rate exposure on the modulation of the hyper-radiosensitive response to low/intermediate dose gamma radiation, i.e. the proportionally increased cell death after low-/intermediate-dose exposure at high dose rate (0.1–0.5 Gy at 40 Gy/h) was addressed in a series of articles by Edin et al. [E3, E4, E5, E6, E7, E8]. Hyper-radiosensitive response of T47D human breast cancer cells or T98G human glioblastoma cells is abrogated by a first exposure to a low/moderate dose (adapting dose) (0.06–0.3 Gy) delivered at moderate dose rate (0.06–0.3 Gy/h) [E5, E6]. The effects of this adapting dose could be replicated by culturing non-irradiated cells in a conditioned medium harvested from adapted cells, or even in conditioned medium irradiated after cell removal. This effect was attributed to a soluble factor present in culture medium, most probably secreted by the cells even in absence of irradiation, which could then be activated by low-dose and low-dose-rate exposure. iNOS activity was required for the effects of the adapting irradiation. In subsequent work [E7], anti-TGF-β3 neutralizing antibodies were found to prevent inhibition of hyper-radiosensitive response by low-dose and/or low-dose-rate exposure, and conversely, the addition of purified TGF-β3 to un-adapted T47D or T98G cells could abrogate hyper-radiosensitive response. The effects of TGF-β3 required peroxinitrites, and "activation" of the adapted cells by IL-13 [E3]. These results were confirmed by in vivo experiments. The addition of serum collected from DBA/2 mice exposed to 0.3 or 0.03 Gy at a dose rate of 0.3 or 0.03 Gy/h, respectively, into the culture medium abrogates hyper-radiosensitive response in T47D and T98G cells. This effect is prevented by pre-treatment of the mouse-serum containing culture medium with a neutralizing anti-TGF-β3 antibody, or if the mice were injected with an iNOS inhibitor for six days before collecting their serum, starting one week after exposure [E8]. The pre-exposure of mice to 0.3 Gy over one-hour results in a better survival than for un-adapted mice after a subsequent exposure to 9.0 or 9.5 Gy. However, adapted mice do not survive longer than control animals if they are not further exposed to challenge doses [E4].

252. In a study by Qvarnström et al. [Q2], persistence of hyper-radiosensitivity was investigated in prostate cancer patients undergoing radiotherapy. Skin biopsies (452) from 42 prostate cancer patients who received 7-week fractionated radiotherapy treatment with daily dose fractions of 0.015–1.10 Gy to the skin were collected at regular time intervals before and after treatment (30 minutes, 2 to 72 hours post-irradiation). Epidermal skin keratinocytes were stained by immunofluorescence and immunohistochemical staining for γH2AX and 53BP1. There was a dose–response relationship for DSB foci (γH2AX and 53BP1 foci) observed in the skin keratinocytes throughout the treatment procedure. Presence of foci 72 hours after damage induction (irradiation) demonstrated the considerable persistence of DSBs produced. The authors conclude that this is the first evidence of preserved hyper-radiosensitivity as assessed by γH2AX and 53BP1 throughout the 7-week treatment.

253. Mechanistically, hyper-radiosensitivity is a response specific to G2-phase and is attributed to evasion of an ATM-dependent G2-phase cell-cycle checkpoint [M17], however, hyper-radiosensitivity is not caused by a failure to recognize DSBs. Exploiting three pairs of transformed isogenic cell lines with known and distinct differences in radiosensitivity and DNA repair (ATM, NHEJ), no relationship was evident between the initial numbers of DSBs monitored immediately after radiation exposure to doses less than 0.2 Gy or cell survival for any of the cell lines indicating that the prevalence of hyper-radiosensitivity is not related to recognition of DSBs. Instead, persistence of γH2AX foci (4 hours after exposure) was significantly correlated with cell survival [W17]. In agreement, primary AT fibroblasts displayed a similar hyper-radiosensitivity positive response to normal fibroblasts [E16]. Assessment of early G2-checkpoint activity after irradiation in 4 normal hyper-radiosensitivity positive and 4 hyper-radiosensitivity negative fibroblasts of cancer patients showed that in hyper-radiosensitivity positive fibroblasts the checkpoint was not triggered at doses lower than 0.2 Gy X-rays [S47]. In another study [S48], residual phospho-ATM and γH2AX foci were examined 24 h after 0.1–4 Gy and were found at higher frequency after exposure to low dose/intermediate dose (0.1–0.5 Gy) radiation in hyper-radiosensitivity positive compared with hyper-radiosensitivity negative fibroblasts. In doses exceeding 1 Gy, no difference appeared between positive and negative hyper-radiosensitivity fibroblasts.

254. A recent study addressed the response to low-dose radiation with 220 kV X-rays, 90 keV/μ C ions and broad beam neutrons with average energy of 5.8 MeV in rat and human stem cells and showed that murine and human glandular tissue stem cells exhibit a dose threshold (<1 Gy) in DNA damage response activation, resulting in intermediate-dose hyper-radiosensitivity with implication for salivary gland secretory function [N3]. Furthermore, inhibition of ATM led to impaired DNA repair in human salivary gland organoid cells after 1 Gy, but not after 0.25 Gy. Whereas (as expected) the initial number of γH2AX foci per cell increased with increasing doses indicating an efficient DNA signalling by DNA damage response, similar levels of residual damage were observed at 24 hours following both 0.25 and 1 Gy. These data might suggest an efficient DNA damage signalling, but poor repair at relative low doses of ionizing radiation in the organoid cells.

255. To investigate the mechanism of hyper-radiosensitivity and induced radioresistance after irradiation with medium- and high-LET particles, normal and two *ATM* deficient/mutant human skin fibroblast lines were irradiated by C ion beam and the involvement of ATR pathway in hyper-radiosensitivity/induced radioresistance response was detected with pre-treatment of specific inhibitors [X2]. The early G_2/M arrest was observed in *ATM* deficient/mutant cells with an effective ATR signalling, even as low as 0.2 Gy for C ion radiation. Inhibition of the ATR pathway in normal cells affected the hyper-radiosensitivity/induced radioresistance occurrence similarly as ATM inhibitor. These data demonstrate that ATR pathway may cooperate with *ATM* in the mechanism of intermediate dose hypersensitivity induced by C ion beams. Pre-treatment of AT fibroblasts with ATR inhibitor fully recovered mitotic entry after C ion beam radiation (0.2 Gy).

256. To summarize, some experiments suggest that in response to low- or intermediate-dose exposure delivered in one hour, cells and animals up-regulate their production of TGF-β3 to provide increased protection from hyper-radiosensitivity against a wide range of radiation doses delivered at high dose rate. The production of TGF-β3 seems to require IL-13 and iNOS activity. Thus, inflammatory factors may participate in radio-adaptive effects in relation to hyper-radiosensitivity. Hyper-radiosensitivity occurs in primary differentiated cells, stem cells and tumour cells after low- and high-LET radiation independent from ATM and ATR. Most evidence suggests that the efficiency of DSB repair based on γH2AX foci after intermediate dose (<0.2 Gy) is not affected early after treatment. Evidence indicates that persistence of DSB (i.e. γH2AX foci) correlates with cell survival.

G. Stem cells and target cell populations for radiation carcinogenesis

257. While not specifically mentioning stem cells, the UNSCEAR 1993 Report, annex E, "Mechanisms of radiation oncogenesis" [U3] recognized the likely single cell origin of cancers, and the accumulation of genetic and other alterations in stem and progenitor cell populations. Similar observations have been made in the UNSCEAR 2000 Report, annex G, "Biological effects at low radiation doses" [U5]. Previous work of the Committee has not undertaken a comprehensive evaluation of the effects of radiation on stem and progenitor cell populations.

258. Stem and early progenitor cells are widely held to be the main target cells from which cancers arise (e.g. [P24]). This is because of the inherent properties of such cell types, notably including long lifespan in the organism, thus allowing sufficient time for the accumulation of multiple alterations to the cellular phenotype, required to convert normal cells into malignant cells. Substantial evidence underpins the stem cell origin of cancers in general [L42], and experimental animal studies indicate stem and early progenitor cell origin for some radiation-induced cancers, notably acute myeloid leukaemias in the mouse [V8]. There are lines of evidence, however, that more mature differentiated cells can undergo a process of de-differentiation and thereby contribute to carcinogenesis [W1]. The relative contributions of the stem cell and de-differentiation routes is not known, but clearly any de-differentiation route would require the accumulation of larger numbers of cell phenotype alterations before cancers present clinically. Studies modelling cancer incidence over time indicate that for leukaemias one or two genetic alterations are required and while a number in the range of 5–7 is required for solid cancers. These estimates find support in more recent whole genome sequencing analyses of cancers [M19] where solid cancers were found to carry on average four driver mutations with a range of 1–10.

259. There remains a debate on the origin of mutations in cancers. Some recent studies suggest that in the majority of cancers errors in DNA replication in stem cells is the major source of mutations [T17, T18]. Therefore, the numbers of stem cells in a tissue and their frequency of division would be a major contributor to carcinogenesis. This view has been challenged as underestimating the role of mutations induced by environmental carcinogens [L37]. Mutation frequencies determined by whole genome sequencing in three adult stem cell populations with varying proliferation rates and cancer risk (colon, small intestine and liver) have been found to be similar [B42], a finding that also contrasts with the proposals from Tomasetti and colleagues. Further work on whole genome sequencing of radiotherapy-associated cancers that identifies putative genome sequence markers specific to radiation-induced cancers [B27] lends support to the view that a significant contribution of induced as opposed to replicative mutations is important for radiation carcinogenesis.

260. Clonal haematopoiesis refers to the presence of clones derived from single stem cells in the mature haematopoietic system of humans, identified by the presence of chromosome aberrations in haematopoietic cells and is a risk factor for haematological malignancy. It has been found recently by means of whole genome sequencing that clonal haematopoiesis is more common in elderly people. Ten per cent of over 65 year olds compared with 1% of under 50 year olds, carry clones with leukaemia-associated mutations [G3]. The fact that clonal haematopoiesis can be detected in the healthy before disease presentation suggests it may be a useful early marker of subsequent haematological malignancy. Similar age-related increase in clonal haematopoiesis with clones carrying leukaemia-associated translocations has been confirmed independently [J12]. These studies confirm the importance of early clonal expansion of leukaemia mutation carrying clones as a precursor to clinical presentation of haematological malignancy. Furthermore, the occurrence of clonal haematopoiesis has been found to be associated with prior exposure to radiation in the course of radiotherapy (but not chemotherapy) and tobacco usage [C26]. Recently, it has been shown in a mouse model of radiation acute myloid leukaemia that expanding clones with leukaemia-associated mutations can be detected prior to disease presentation and further indicate that the stem/progenitor cells of origin can differ between irradiated male and female mice [V8]. Such studies and approaches provide a link between experimental and modelling studies (see also chapter IV).

261. Notwithstanding the above discussions, a consideration of the effects of radiation at low doses in stem cells is of relevance to understanding mechanisms driving low-dose radiation carcinogenesis. It is important to establish if the responses of stem cells to radiation exposures are similar to those of the more widely studied differentiated cell types, and if responses vary between stem cell populations.

262. While there are not large numbers of relevant papers available (table 5), many different stem cell types have been examined, including embryonic, epithelial, neural and mesenchymal stem cells, some including low-dose and low-dose-rate studies. A number of key points can be derived from the studies reported to date. These are summarized in the following paragraphs, note that not all papers listed in table 5 are discussed in further detail below.

Table 5. Stem cell and radiation response literature

Species/cell type	*Irradiation conditions*	*Effect reported at low dose*	*Other effects/comments*	*Reference*
1. EMBRYONIC STEM CELLS				
Mouse Embryonic stem cell	Gamma radiation Adaptive dose: 25–50 mGy Challenge Dose: 1.5 Gy Dose rate: 130–136 mGy/min	25 mGy reduced effect of 1.5 Gy (5 hours later) on micronuclei and cytotoxicity		[K10]
Mouse Embryonic stem cells ex vivo irradiated 8-cell stage embryos	X-radiation Dose: 0.1–1 Gy Dose rate: 0.7 Gy/min	Increase in apoptotic cells in irradiated embryos and reduced normal full-term pregnancies	p21, Smad2, Foxo2 expression increased by irradiation but no effect of stem cell factors	[H14]
Human Embryonic stem cell/ induced pluripotent stem cell	Gamma radiation, X-radiation Dose: 0.1–4 Gy Dose rate: most at 0.4–~0.8 Gy/min	0.4 Gy increased targeted vector integration by homologous recombination (about 30-fold) with no effect on viability or HPRT mutation, or point mutations/indels in exome, some effects on gene expression 24 hours after 0.4 Gy. γH2AX foci repair observed and apoptosis at 0.4 Gy		[H12]
Human Chord blood-derived endothelial colony forming cells	X-radiation Dose: 60–380 mGy Dose rate: 7–35 mGy/min	Reduced proliferation, increased doubling times, cytotoxicity		[K26]
Human Embryonic stem cell	Gamma radiation Dose: 0.1–1 Gy Dose rate: not given, assumed to be acute	Gene expression at 2 and 16 hours after exposure by Taq-man assays – wide range of dose–responses observed and variation between embryonic stem cell lines	Considerable variation in results presented	[S54]
Human Embryonic stem cell	Gamma radiation Dose: 0.05–1 Gy Dose rate: not given, assumed to be acute	Agilent oligo microarrays – transient expression changes at 0.05, but fewer genes respond than at 1 Gy, significant cell line variation, especially at 0.05 Gy in responsive transcripts		[S55]
Human Embryonic stem cell/ induced pluripotent stem cell	Gamma radiation (LINAC) Dose: 0.25–15 Gy Dose rate: not given, assumed to be acute	Only very modest alterations in gene expression (*P53, RAD51, PRKDC, XRCC4, BRCA2)*	Most response observed at 2 Gy and above, probably quite crude PCR method employed	[S65]

Species/cell type	*Irradiation conditions*	*Effect reported at low dose*	*Other effects/comments*	*Reference*
Human Embryonic stem cell	Gamma radiation Dose: 0.4–2 Gy Dose rate: not given, assumed to be acute	No reported effects on core stem cell transcription factors and no effect on pluripotency; cytotoxicity observed but proliferation continues		[W13]
Human Embryonic stem cell	Gamma radiation Dose: 0–2 Gy Dose rate: 1.16 Gy/min	Chromosome aberrations, micronuclei and γH2AX foci induction at 0.5 Gy. Differentiated derivatives of human embryonic stem cells displayed higher induction of aberrations, micronuclei and foci than undifferentiated human embryonic stem cell	Patterns of gene expression changes induced by 1 Gy varied between differentiated and undifferentiated human embryonic stem cells, and peripheral blood lymphocytes	[V11]
2. HAEMATOPOIETIC STEM CELLS				
Human and mouse Various			Review paper	[F14]
Mouse Purified primitive haematopoietic stem cells and more committed lineages	Gamma radiation Dose: 20 mGy–2 Gy Dose rate: acute	Hyper-radiosensitivity of haematopoietic stem cells (only) seen in vitro, but not in vivo (four months after exposure), sensitivity driven by ROS	Concludes that low doses have long-term detrimental effects that could lead to leukaemias	[R14]
Mouse Irf5–/– and wild-type mice on C57BL/6 background	Gamma radiation (^{137}Cs) Dose: 4 weekly doses of 1.75 Gy from 5 weeks of age in vivo Dose rate: not given	Reduced lymphoma yields in –/– mice attributed to reduced apoptosis in haematopoietic stem cells leading to less compensatory proliferation	Proposes Irf5 as potential target for amelioration of bone marrow injury following radiation	[B33]
3. NEURAL STEM CELLS				
Mouse Embryonic brain ventricular zone/subventricular zone	X-radiation Dose: 10–20 mGy in vivo Dose rate: 4.9 mGy/min	Linear dose–response for 53BP1 foci, apoptosis especially at E13.5, linear induction. Delayed repair if doses below 50 mGy	No response to 50 Hz magnetic fields	[S3]
Mouse Adult and embryonic brain sub-ventricular zone, ventricular zone and sub-granular zone regions	X-radiation Dose: 50–200 mGy in vivo Dose rate: acute	Embryonic brain highly sensitive to DSBs and apoptosis induction; adult highly sensitive to apoptosis also	Summarizes work of Jeggo laboratory	[B15]
Human Neuronal stem cell in culture	Various charged particles Dose: 50 mGy–1 Gy Dose rate: 100–500 mGy/min	Elevated ROS and RNS (reactive nitrogen species) over one-week post-irradiation		[B25]

Species/cell type	*Irradiation conditions*	*Effect reported at low dose*	*Other effects/comments*	*Reference*
Human Neuronal stem cells, differentiated neurons and fibroblasts	X-radiation Dose: 0.46 or 2.3 Gy Dose rate: 0.01 or 0.05 Gy twice daily, 5 days per week	Elevated mitochondrial numbers and function in differentiated cells but not Neuronal stem cells		[S37]
Mouse Adult brain subventricular zone	X-radiation Dose: 50 mGy–2 Gy Dose rate: acute, 0.5 Gy/min	Apoptosis induced linearly with dose, 50–500 mGy, within this range Proliferation arrest has threshold around 200 mGy and induction of differentiation a threshold around 500 mGy		[B16]
4. MESENCHYMAL STEM CELLS				
Human Primary bone marrow derived mesenchymal stem cells	Gamma radiation Dose: 100 mGy Dose rate: 800 mGy/min	Some proliferation delay induced by 100 mGy in 3/5 lines. One line differentiation affected. Co-culture with BM haematopoietic stem cells increase differentiation of CD34/38 positive cells (more mature), again only in some	Authors conclude that 100 mGy can affect mesenchymal stem cell function, but variation is a concern. Accurate dose delivery problematic	[F23]
Human Myc/hTERT immortalized mesenchymal stem cell line	Gamma radiation Dose: 0.01–1 Gy Dose rate: 0.79 Gy/min	Global gene expression assessed at 1–48 hours post-exposure. Some low dose specific responsive genes identified and most with non-linear D-R. Most doses statistically very similar response for individual transcripts	Accurate dose delivery problematic, particularly for 10 mGy. Questionable if anything of significance in this study	[J21]
Rat Primary bone marrow derived mesenchymal stem cells	X-radiation Dose: 20 - 100mGy Dose rate: 100 mGy/min	Proliferation stimulated and increase S-phase at 50 and 75 mGy; stimulation of MEK, ERK, Raf phosphorylation also		[L27]
Human 4 primary oral mucosa derived mesenchymal stem cell lines	X-radiation Dose: 25 - 250 mGy Dose rate: 40 mGy/min	Linear accumulation of γH2AX and pATM foci, slower repair at 20–80 mGy		[O19]
Human Primary bone marrow derived mesenchymal stem cells (one donor)	X-radiation Dose: 80/1 000 mGy Dose rate: 40 mGy/min	Delayed H2AX foci repair at 80 as opposed to 1 000 mGy, residuals not with pATM and in proliferating cells. 1 000 mGy exposure leads to senescence at passage 11 post-irradiation and reduced proliferation	With one donor, no information on variability which is widely reported elsewhere	[P25]
Human Primary fat-tissue derived mesenchymal stem cells	X-radiation Dose: 100 mGy only Dose rate: 100 mGy/min	Transient apoptosis (2 hours) with 100 mGy and release of oxidized DNA. Propose release of oxidized DNA may be signalling molecule for adaptive response		[S25]
Miscellaneous			Review paper, including Alessio et al. results below [A11]	[S60]

Species/cell type	*Irradiation conditions*	*Effect reported at low dose*	*Other effects/comments*	*Reference*
Human Primary bone marrow derived mesenchymal stem cells	X-radiation Dose: 75 mGy Dose rate: not given	Slight and transient increase in mesenchymal stem cell proliferation observed, and increase in S-phase cells. Increased secretion of IL-11. Mild reduction in ability of mesenchymal stem cells to support T-cell proliferation. Claimed to be mediated by Rb, CDK1 and CDC25b	Only modest effects reported, and probably from a single donor	[Y5]
Human Primary bone marrow derived mesenchymal stem cells	X-radiation Dose: 40–2 000 mGy	40 mGy reduced proliferation and induced senescence and autophagy; impaired DSB repair at 40 mGy. Reduced stemness at 40 mGy, also at higher doses		[A11]
5. MISCELLANEOUS				
Mouse Spermatogonial stem cell in vivo	X-radiation Dose: 100 mGy Dose rate: 1 000 mGy/min	Increase in 53BP1 at 45 minutes but not caspase or γH2AX, no apoptosis observed	More mature cells show γH2AX and its repair. Conclude repair in spermatogonial stem cells may differ from that in more mature cells	[L10]
Mouse Muscle spindle (stem) cells	Gamma radiation Dose: 60–7 500 mGy	Reduced satellite cell numbers over time (50 mGy and above), no evidence of hormetic effects		[M21]
Mouse Skin, bulge and sebaceous gland stem cells	Gamma radiation Dose: 50 mGy/5 Gy Dose rate: 10 mGy/min or 2.34 Gy/min	50 mGy leads to apoptosis in sebaceous gland stem cells, but not Bulge stem cells. Survival of Bulge stem cells due to Hif1α expression and metabolic shift to glycolosis. Surviving bulge stem cells lead to Basal cell carcinoma development at 50 mGy in Ptch +/– mice		[R5]
Human Induced pluripotent stem cells	Gamma radiation Dose: 2–6 Gy Dose rate: not given	Find enhanced DNA damage response, especially HRR in stem as opposed to NO-induced differentiated cells	Concludes that pluripotent stem cells have enhanced DNA damage response by comparison with more differentiated cells, notable higher homologous recombination but variation in response with different tissue stem cell types	[M44]
Mouse Keratinocyte and melanocyte stem cells	Gamma radiation Dose: 0.5/1/2.5 Gy in vivo Dose rate: 0.3 mGy/min and 0.3 Gy/min;	Low-dose-rate exposure reduced hair follicle density and pigmentation, no significant reduction in melanocyte stem cell number, so assume functional change in skin stem cell compartments		[S66]
Human induced pluripotent stem cell line ND41658H differentiated to chondrocytes	Gamma radiation Dose: 1–5 Gy Dose rate: 2.5Gy/min	DNA damage response and repair observed, along with relatively low ROS levels but high induction of senescence	Concludes induced pluripotent stem cell derived chondrocytes have greater repair activity than standard chondrocyte cell lines	[S61]

Species/cell type	*Irradiation conditions*	*Effect reported at low dose*	*Other effects/comments*	*Reference*
Human dental pulp stem cells from deciduous teeth	X-radiation (Cone-beam CT) Dose: 5.4–107.7 mGy	Transient induction of phospho-H2AX and full repair even at 5.4 mGy. Increased release of cytokines IL-8, IL-1α and TNF-α over 24 hours	Concludes that cone-beam CT doses may carry risk	[V12]
Mouse Oesophageal epithelium progenitor cell	Gamma radiation (^{137}Cs) Dose: 1–5 fractions of 50 mGy Dose rate: 16 mGy/min	Single or multiple 50 mGy exposures prompted wild-type progenitors to reduce proliferation and increase differentiation. P53-mutation carrying progenitors do not differentiate but continued to expand into clones overgrowing the normal epithelium	The overgrowth of p53-mutation carrying cells was abrogated by treatments with antioxidants	[F10]
Mouse Lgr5$^+$ intestinal stem cells in organoid culture	X-radiation Dose: 1 000 mGy (Operated at 150 kV with 20 mA and a 0.5 mm Al plus 0.3 mm Cu filter) Dose rate: 600 mGy/min,		Reduced fitness of 1 Gy irradiated cells	[F22]
Mouse Lgr5$^+$ intestinal epithelial stem cells	Gamma radiation (^{137}Cs) Dose: 1 Gy Dose rate: 0.003 Gy/h X-radiation Dose: 1 Gy Dose rate: 30 Gy/h	High dose-rate reduced stem cell replenishment of epithelium, low-dose-rate exposure did not affect stem cell replenishment of the epithelium		[O20]
Mouse Hair follicle stem cells	X-radiation (LINAC) Dose: 10–50 fractions of 100 mGy	Exposures caused accumulation of 53BP1 foci in nuclear heterochromatin followed by ATM dependent senescence but not apoptosis. Highly damaged stem cells differentiate promoting stem cell proliferation		[S17]
Mouse Lgr5$^+$ intestinal stem cells	X-radiation (260 keV) Dose: 0.1–4 Gy Dose rate: 0.5 Gy/min	In the small intestine, 53BP1 foci detected immediately after irradiation, but rapidly disappeared thereafter, especially in Lgr5$^+$ stem cells. Cellular growth was temporarily arrested; however, cell numbers and mitotic cell numbers in the crypt did not change. The kinetics of DNA damage repair in Lgr5$^+$ stem cells were similar to those in the small intestines, while the colon was more susceptible to radiation-induced damage		[O21]

263. Stem cells are responsive to low-dose as well as moderate-/high-dose exposures to ionizing radiation, and such responses are observed under both in vitro and in vivo exposure conditions. DNA damage responses are broadly similar to those observed in differentiated cells but with some indications that responses in terms of DNA repair, notably homologous recombinational repair are greater in stem cells as opposed to their more differentiated counterparts, at least at high doses of 2–6 Gy gamma radiation [M44]. Moderate doses of 400 mGy gamma radiation can enhance DNA integration through homologous recombination in human induced pluripotent and embryonic stem cells [H12], supporting an enhanced HRR function in stem cells. This would suggest that repair of double-stranded DNA damage in stem cells being generally of a higher fidelity than in differentiated cell types. Support for this is found in a comparative study of chromosome aberration, micronucleus and γH2AX foci induction where undifferentiated human embryonic stem cells had reduced yields of these endpoints compared to differentiated counterparts [V11]. By contrast, mouse spermatogonial stem cells appear to have reduced HRR [L10, L11].

264. In common with findings in differentiated cells, the induction of DNA damage foci in stem cells (γH2AX, 53BP1 and others) is linear with dose, even in the low-dose area, down to 10 mGy [O19, P25, S3]. Furthermore, slower repair of DNA damage foci after low-dose exposures has been observed in neural [S3], and mesenchymal [A11, O19, P25, S3] stem cells. As with results from studies employing more differentiated cells, the long-term consequences of this delayed repair are not clear.

265. The induction of apoptosis by low doses in stem cells is variable according to stem cell type [R5] and developmental stage [B15] and this may be influencing the induction of cancers by low doses [R5]. The Revenco et al. [R5] study used a transgenic mouse model based on *Ptch1* [R5]. In this study, work was carried out on wild-type mice and effects followed in epidermal stem cell populations in the sebaceous gland and hair follicle bulge region. DNA damage induced by a dose of 50 mGy gamma-radiation exposure led to death of the sebaceous gland stem cells, while the bulge epidermal stem cells survived and underwent a metabolic switch with activation of Hif1α and overexpression of many cellular pathways (with endocytosis as the only pathway that was decreased). In *Ptch1* heterozygous mice, low-dose irradiation led to the development of skin cancers that look similar to human basal cell carcinoma. In this model system, moderate doses of radiation had the same but a more pronounced effect – more skin cancer foci per animal developed. Nevertheless, regardless of dose, 100% of these animals developed skin cancer.

266. In adult mouse neural stem cells irradiated in vivo with 50–500 mGy, a linear dose-dependence for the induction of apoptosis was observed, with no apparent threshold and with statistical significance achieved at 50 mGy [B16]. By contrast, the induction of reduced proliferation in the stem cell compartment required a dose of 200 mGy or above, and induction of stem cell differentiation required doses of 500 mGy or above. Barazzoul et al. [B16] argue that because quiescent stem cells accumulate fewer chromosomal translocations, the threshold of around 200 mGy for the induction of proliferation arrest may serve to protect stem cells from the consequences of irradiation, and that this may be relevant for low dose risk assessment [B16].

267. Fractionated exposure to X-rays (100 mGy fractions, 5 fractions per week at daily intervals, 2–10 weeks total) in mouse hair follicle stem cells is reported to lead to accumulation of DNA damage (53BP1) foci and the induction of senescence in highly damaged cells that promotes proliferation in the stem cell pool [S17].

268. Fernandez-Antoran et al. [F10] have demonstrated the sensitivity of oesophageal epithelial progenitor cells to 50 mGy exposure to gamma radiation in vivo that leads to a reduction in proliferation and increase in differentiation of wild-type progenitors. Progenitors carrying mutations in the *p53* gene respond differently to low-dose exposure and out-compete wild-type progenitors in the epithelium.

Treatment with antioxidants can reverse the competitive advantage afforded to pre-malignant *p53* mutation carrying cells/clones, allowing proliferation of wild-type cells and loss of mutation carrying cells through differentiation. This study suggests that after low-dose exposures the tissue can remove damaged cells through differentiation, but the exposure can promote the growth of pre-existing pre-malignant cells, depending on the oxidative environment. This effect can be seen as a pro-carcinogenic effect of low-dose exposure through promotion. By contrast, in the intestinal epithelium it has been reported that while high dose-rate exposure (1 Gy dose) reduced replenishment of the epithelium, the same dose delivered at low dose-rate (0.003 Gy/h) had no impact on replenishment rates [O20].

269. In the mouse haematopoietic system, primitive stem cells respond to in vitro low-dose exposure with a specific radiation hypersensitivity which is not observed in more differentiated haematopoietic cells [R14]. Such hypersensitivity is not seen in vivo indicating an important role of the stem cell microenvironment (or niche) in modulating responses. Low-dose responses were found to be driven by alterations in reactive oxygen metabolism, and the niche may play an important role in controlling the oxygen tension in vivo [R14]. Elevated ROS and reactive nitrogen species (RNS) have been observed in human neural stem cells at least one week post-exposure of haematopoietic stem cells [B25].

270. A number of analyses of gene expression at up to 48 hours after exposure to low and moderate doses have been reported. There are indications that the responsive genes may differ between dose levels but there is no clear consensus on the nature of low- as opposed to moderate-dose responsive genes; however, these studies are indicative of non-linear responses operating at the level of gene transcription. Adaptive response has been reported in embryonic stem cells [K10] but by contrast hormetic effects were not observed in mouse muscle stem cells (spindle cells) after gamma irradiation [M21].

271. A review by Manda et al. [M8] was unable to conclude whether low-dose radiation is likely to cause transformation of stem cells into cancer stem cells due to insufficient evidence being available. This review mentioned that exposure of neural stem cells to a moderate dose of 300 mGy low-LET radiation resulted in increased protein expression of Wnt1, Wnt3a, Wnt5a and β catenin leading to proliferation as well as differentiation, while exposure of diabetic rats to repeated doses of 75 mGy (total accumulated doses, 375–825 mGy) increased the number of bone marrow and circulating stem cells and improved wound healing. Similarly, regenerative neovascularization was also associated with moderate-dose exposures of four times 0.3 Gy doses [M32]. Thus, moderate to low doses radiation seem to increase proliferation followed by differentiation that can only indirectly support cancer development.

272. The International Commission on Radiological Protection (ICRP) published a report on "Stem cell biology with respect to carcinogenesis aspects of radiological protection" [I3], which presented arguments that low dose rate effects in vivo may differ from acute low dose effects in stem cell populations due to the ability of stem cells to repopulate tissues, based on studies with haematopoietic stem cell systems. The competition for occupancy/space in stem cell niches between undamaged and damaged stem cells is likely to be of relevance here in that damaged cells generally appear to be less "fit", i.e. less able to occupy a niche when undamaged cells are also present. Similar loss of fitness of 1 Gy X-irradiated cells has also been shown in an in vitro organoid culture system based on intestinal cells [F22].

Summary

273. Stem and early progenitor cells are viewed as main target cell populations for radiation cancer due to their long lifespan in the organism. Stem cell populations in several tissues have been shown to respond to low doses of radiation, and in terms of DNA damage response they appear to respond in a similar

fashion to other somatic cells, and this holds true for mutation frequencies that do not strictly correlate with replication cycles traversed.

274. However, some stem cells, most notably embryonic stem cells, but also induced pluripotent stem cells, depend more on homologous recombinational repair of DNA damage than NHEJ. This repair pathway preference likely serves to preserve genome integrity and avoid mutation in the presence of DNA damage given the higher fidelity of recombinational repair compared to NHEJ. This is likely therefore to act to protect stem cells from mutation, much as the retention of parental DNA strands through DNA replication and cell division that has been observed in some stem cell populations.

275. Some stem cell populations enter apoptosis readily following radiation exposure, and this likely serves to protect from cancer. Loss of cells through apoptosis though may lead to substantial proliferation of stem cell populations to restore tissue that brings risk of replication-associated mutation, at low doses this is expected to be a minor consideration.

276. A recent study has demonstrated how low (50 mGy) radiation exposures can lead to the preferential expansion of cells and clones carrying mutations in the *Trp53* gene [F10]. This supports a role of low doses in promoting the growth of pre-existing pre-neoplastic clones.

H. Effects at the whole organism level

277. In this section, the effects of radiation exposure at the level of the whole organism, and how these relate to the induction of cancer or the modulation of cancer risk are considered. The studies considered include those investigating the effects on the immune system that might modulate cancer risk, animal carcinogenesis studies and a range of studies that investigate specific aspects of carcinogenesis.

1. Immune system and cytokine responses

278. The Committee published a comprehensive evaluation of the effects of radiation on the immune system as its UNSCEAR 2006 Report, annex D, "Effects of ionizing radiation on the immune system" [U7]. This report noted the well-documented effects of high radiation exposures and concluded that it remained uncertain if low dose exposures stimulated or suppressed responses.

279. The impact of radiation over a range of doses on the immune system has been examined in a number of exposure contexts, including environmental, medical and accidental exposures. The UNSCEAR 2006 Report [U7] summarized general features of the immune system, the effects of radiation on immune functions observed in experimental systems, the possible mechanisms underlying these effects, and epidemiological studies on the effects of radiation on the immune system in humans. The review concluded that low-dose and low-dose-rate exposures to radiation could stimulate or suppress the immune system, while exposures to higher doses radiation was clearly immunosuppressive. In this annex, more recent studies on the effects of exposure to radiation on the immune system and function are considered. The impact of radiation over a range of doses has been examined in a number of exposure contexts, including human exposure (survivors of the atomic bombings, environmental, medical and accidental exposures) and experimental exposure of cell lines and animals.

(a) *Immune system and cancer*

280. Sigurdson et al. [S45] investigated the relationship between polymorphisms in a series of eight genes involved in apoptosis, inflammation and proliferation with breast cancer risk in a cohort of radiological technologists, considering both occupational and medical breast exposure. Occupational dose to the breast was categorized as <0.05 Gy (77%), <0.05–0.1 Gy (13%), >0.1–0.2 Gy (8%) and >0.2 Gy (2%). The distribution of personal medical diagnostic X-ray breast dose was similar. Their results showed that a minor allele of the gene coding for Casp-8, involved in apoptosis, had a significant protective effect, while a minor allele of the gene coding for IL-1α by contrast increased the risk of breast cancer development. Schonfeld et al. [S16] investigated the role of oxidative stress and chronic inflammation genes as modifiers of the risk of radiation-induced breast cancer in the same cohort. Their results suggest that a polymorphism in the *PTGS2* gene, coding for the COX-2 enzyme, is associated with an increased risk of breast cancer following occupational exposure. Together, these results show that genes coding for proteins involved in immune or immune-related processes can modify the risk of cancer development associated with low-dose radiation exposure.

281. Snijders et al. [S51] reported on the existence in mice of an oestrus cycle-dependent gene expression signature in mammary gland that includes 117 genes linked to interferon signalling. Human breast cancer patients with high expression of this signature had increased chances of metastasis-free survival, suggesting here again that the expression of factors associated with immune response can influence the outcome of cancer development. In breast-cancer- and radiation-sensitive BALB/c mice, but not in cancer- and radiation-resistant C57BL/6 mice, oestrus cycle-dependent variations of leukocytes and inflammatory cytokine levels in the serum were also observed. Although low-dose radiation (100 mGy X-rays) did not affect the expression of the interferon-dependent gene signature in any of the mouse strains, it resulted in low blood lymphocyte counts and circulatory inflammatory cytokine levels in BALB/c, but not C57BL/6 mice, suggesting that it may participate in the radiosensitivity of these animals.

282. In the study of Shin et al. [S40], AKR/J mice, prone to the development of thymic lymphomas, received 4.5 Gy of gamma rays, either at 0.8 Gy/min or at 0.7 mGy/h. The lower-dose-rate exposed mice survived longer and developed fewer thymomas. Gene expression analysis at 130 days post-exposure showed that in the thymus of lower-dose-rate exposed mice, most of the changes were the increase in apoptosis-related and B- and T-lymphocyte activation-related genes. In contrast, in high-dose-rate exposed mice most of these changes were down-regulation not only of genes involved in apoptosis and immune activation but also of genes involved in DNA repair and genome stability, DNA damage signalling, cell cycle, cancer and p53 signalling pathways. This difference might explain why, in contrast to high-dose-rate delivery, lower-dose-rate radiation better promotes a thymoma-free survival in response to high-dose radiation [S40]. More recently, Gao et al. [G2] analysed the modulation of long non-coding RNA (lncRNA) and their target genes in the thymus of ICR mice exposed to 0.075 or 4 Gy. Thymi were collected 12 hours post-exposure. Gene ontology classification of the mRNA potentially regulated by radiation-modulated lncRNA in mice exposed to 4 Gy identified cell death and cell-cycle regulation processes while those down-regulated are rather associated with nuclear organization. Thus, some of the effects observed in irradiated AKR mice 130 days post-exposure in [S40] may have already been initiated 12 hours post-exposure.

283. Radiation exposure is a recognized risk factor for the development of sarcomas. Injection of mice with the beta-emitting radionuclide ^{45}Ca (1 μCi/37 kBq per gram, intra-peritoneal) results in a high incidence of osteosarcomas. In this model, Kansara et al. [K13] showed that the onset of osteosarcoma development is accelerated in mice heterozygous for the tumour suppressor gene, *Rb1*. *Rb1* inactivation in primary human osteoblasts leads to a reduction of radiation-induced senescence in these cells, accompanied by a reduction in the expression of genes coding for inflammatory factors of the senescence-associated secretory pattern. In a cohort of patients, high *RB1* and high-senescence-associated secretory

pattern levels are associated with metastasis-free survival. IL-6 was shown to be required for tumour suppression in vivo. The number of NKT cells infiltrating tumours was found to be reduced in the absence of *IL-6* or *Rb1* expression, and the development of radiation-induced osteosarcoma was accelerated in NKT cell-deficient mice, without affecting senescence induction. A model was proposed in which *RB1*-dependent senescence and senescence-associated secretory pattern induction leads to the recruitment of NKT cells able to clear pre-malignant cells.

284. A series of articles addressed the role of NK cells and macrophages in the reduction of pulmonary engraftment of (intravenous) injected L1 sarcoma cells in low-dose exposed BALB/c mice. Whole-body exposure to 0.1 or 0.2 Gy X-rays at 2.2 Gy/h, reduces lung colony formation by half. Radiation exposure results in an increased cytotoxic activity of activated peritoneal macrophages and NK cells. The blockade of these activities abrogates the radiation-induced reduction in L1 cell engraftment [C13]. The production of NO by activated macrophages appears to be required for both in vitro cytotoxic activity against L1 cells and reduction of engraftment after low-dose exposure [N19]. Dose fractionation (10 times 0.01 Gy, 0.02 Gy and 0.1 Gy X-rays) appears inefficient at reducing the number of pulmonary L1 sarcoma cell colonies, but still induces an increase in the cytotoxic activity of "NK-enriched" splenocytes and activated macrophages purified from irradiated mice, albeit with different kinetics and amplitude than in mice acutely irradiated to the same total doses [N21]. An increase in cytokine secretion is observed in activated macrophages (IL-1β, TNF-α, IL-12), PHA-stimulated splenocytes (IL-2) and "NK-enriched" splenocytes (IFN-γ) purified from mice exposed to 0.1 and 0.2 Gy. The kinetics and/or amplitude of secretion in the nine days following exposure differed according to the dose delivery (acute compared with fractionation) [C14]. In a new series of experiments, using more mice, exposure to 0.1, 0.2 and 1 Gy in 10 fractions was this time found to induce a reduction of pulmonary L1 cell engraftment [N20]. All exposures induce a biphasic increase in the cytotoxicity and NO production of activated macrophages ex vivo. These effects of fractionated radiation exposure (increased cytotoxic activity of "NK-enriched" splenocytes and activated macrophages, production of NO by activated macrophages, reduction of pulmonary sarcoma cell engraftment) have been largely confirmed in a different mouse strain (C57BL/6) [N20].

(b) *Studies of the Japanese atomic bombing survivors*

285. Past studies, reviewed in the UNSCEAR 2006 Report, annex D, "Effects of ionizing radiation on the immune system" [U7] addressed the late effects of atomic radiation on immune cells in the atomic bombing survivors. A reduced frequency of blood T-lymphocytes with an imbalance in Th1/Th2 responses was found in survivors, with a reduced proportion of naïve T-cells, while on the contrary memory T-cells were increased. The proportion of B-lymphocytes was increased, as was the concentration of serum IgA in females and IgM in both sexes. No consistent changes were reported in circulating innate immune cells, although radiation-associated inflammatory responses were observed. Some of these qualitative and quantitative changes suggested that radiation exposure could accelerate the normal ageing of immune system, characterized by a functional decline with age, a process known as immunosenescence.

286. T-lymphocytes are generated in the thymus from circulating BM-derived progenitors. Thymic activity (its ability to support T-lymphocyte differentiation) can be evaluated by measuring in the peripheral blood the number and frequency of T-cells containing TRECs (T-cell receptor excision circles), circular DNA fragments generated during the somatic rearrangement process required for T-cell receptor gene expression. As TRECs do not replicate, they are more abundant in populations enriched in recently produced naïve T-cells. TRECs were quantified in the blood of 1,073 aging atomic bombing survivors who donated blood samples between 2003 and 2009 (median radiation dose 0.17 Gy, range 0–2.1 Gy, median age 77 years, range 62–90 years), in relation with lifestyle/obesity indicators [Y12]. TREC numbers showed a strong positive association with naïve T-cell frequency, were inversely

correlated with age at examination and more abundant in females, but no significant association with radiation dose was found, neither in CD4 nor in CD8 population. However, TRECs level was inversely correlated with obesity (fatty liver, type-2 diabetes) and inflammatory (serum C reactive protein concentration) markers.

287. The late effects of radiation exposure on the morphology and function of the thymus were analysed from thymic tissue samples collected from deceased atomic bombing radiation exposed individuals during autopsy (N=165, radiation dose <5 mGy (non-exposed), 5–200 mGy, >200 mGy, age 20–91 years) and stored at Radiation Effects Research Foundation [I14]. Immunohistochemistry and immunofluorescence staining of tissue slides allowed determination of the size and respective proportions of epithelial and lymphoid areas, and the presence of immature thymocytes. These data were analysed in correlation with the age at the time of death and the dose of radiation received and compared with results obtained from tissues (N=11) and slides (N=25) archived at Duke University. As expected, thymic atrophy increased and thymopoiesis decreased with age. This decline was evident earlier in males before the age of 60 years, but was similar in both sexes thereafter. Thymic measurements were also decreased in tissues from exposed individuals in a relation depending both on the dose of radiation and the age at the time of bombing. A decline in thymic measurement is more likely to be observed in individuals exposed at older age. The effects of radiation exposure were stronger than the effects of aging, suggesting that any radiation-dose can accelerate thymic aging, and these effects persist for years after exposure.

288. Radiation-induced T-cell immunosenescence was evaluated by fluorescence-activated cell sorting analysis of various T-cell populations in peripheral blood of aging atomic bombing survivors. Changes in the frequency of total conventional T-cells (Tconv), Tregs, and naïve, effector, central memory and effector memory $CD4^+$ T-cells were measured in relation to radiation dose (0.005–4.0 Gy), inflammation, sex and age at time of blood collection [K54]. The frequency of Tconv but not Tregs, declined with age. There was a radiation-dose dependent increase in Tregs and central memory $CD4^+$ T-lymphocytes. Radiation also induced a reduction in the frequency of naïve $CD4^+$ T-cells. The concentration of TNF-α was inversely associated with the modulation of naïve $CD4^+$ T-lymphocytes but not with changes in Tregs. Thus, radiation exposure seems to accelerate immunosenescence by a shift towards immunosuppressive Tregs and inflammation-related decrease in naïve T-cells.

289. The association between changes in T-lymphocytes homeostasis, obesity/metabolic status and telomere length was measured in a group of 620 atomic bombing survivors (median radiation dose 0.102 Gy, range 0–1.7 Gy) [Y13]. Telomere length decreases with age in T-lymphocytes and granulocytes. Surprisingly, when analysed in relation with dose, it was observed that there is first an increase in telomere length until 0.5–1 Gy, and then a decrease such that there was no difference in telomere length between the 0.005 and ≥1 Gy categories. Overall, radiation-associated telomere length shortening was observed in individuals exposed to ≥0.5 Gy in lymphocytes, but not granulocytes. This decrease was modulated (up or down) by different metabolic indicators, suggesting that metabolism may modify the long-term effects of radiation exposure.

290. In blood samples donated 11 years apart by a different group of 415 atomic bombing survivors categorized by the study authors into low (median radiation dose 0.23 Gy, range 0.005–0.69 Gy) and high (median radiation dose 1.3 Gy, range 0.70–3.8 Gy) dose exposure groups, telomere length was found to be significantly shorter in the high dose group than in control group (<0.005 Gy) [L52]. When all groups were classified according to the age at time of bombing, telomere length shortening in the high dose group was still significant in younger (age at time of bombing <12 years old) but no longer in older (age at time of bombing >12 years old) individuals. The rate of telomere length shortening with aging over 11 years was similar in all groups, irrespective of age at time of bombing. Telomere length was positively or negatively associated with different metabolic and/or cellular indicators in individuals exposed to <0.005 Gy, but most

of these associations disappeared in the exposed groups, suggesting that radiation exposure modifies the metabolic status and/or immune parameters, resulting in the loss of their correlation with telomere length.

291. The association of metabolic markers with the homeostasis of peripheral lymphocyte populations was also analysed in 3,113 atomic bombing survivors (median radiation dose 0.08 Gy, range 0–1.8 Gy) [Y14]. Several indicators of obesity and related diseases (fatty liver, diabetes) were associated with increases in total T, Th1, $CD8^+$ T- and B-cell populations, while the association was negative for naïve $CD4^+$ T and NK cells. In addition, age and radiation exposure were found to correlate with decreased total T, $CD4^+$ T and naïve $CD4^+$ T-cell populations, while proportion of Th1 and Th2 cells increased. However, the only significant interaction between radiation dose and metabolic/disease indicators was that of C reactive protein and radiation for B-lymphocytes.

292. The number of circulating white blood cells is an indicator of inflammation. The effects of radiation exposure on age-associated changes in total and differential (neutrophils, monocytes, lymphocytes) white blood cells were investigated in a longitudinal study involving 7,652 atomic bombing survivors who donated blood every two years between 1964 and 2004 [H39]. Both total and differential white blood cells declined with age in males and females, but with different patterns. Smoking increased the numbers of all cell types. Radiation exposure (>2 Gy) also results in increased white blood cell counts, but to a lower level than smoking. When age at time of bombing was considered, a significant radiation dose-dependent increase in white blood cells counts was identified in men exposed before the age of 20 years, but not in women. Thus, irradiation can result in persistent elevation of total and differential white blood cells for decades after exposure.

293. More recently, the effects of radiation exposure on white blood cells were re-assessed in a larger study (N=14,346) with a longer follow-up period (until 2010), different endpoints (frequency of white blood cells in addition to counts) and different statistical methods [Y15]. Baseline frequencies of lymphocytes, neutrophils and monocytes were similar at the study entry in never exposed not-in-city residents, individuals exposed to ≥1 Gy (median dose 1.5 Gy) and individuals exposed to <1 Gy (median dose 0.038 Gy). The different cell populations evolve differently, but for each of them the trend is different before and after 60 years of age for both men and women. The frequency and counts of monocytes were found to be dose-dependently increased by radiation exposure, and this increase is more pronounced after 60 years. This radiation-induced persistent elevation in monocytes could indicate ongoing inflammation and might be related to the acceleration of age-dependent clonal haematopoiesis.

294. The effects of radiation exposure and aging on inflammation have been compared between a group of 260 atomic bombing survivors exposed to more than 5 mGy and a group of 182 non-exposed (<0.005 Gy) individuals [H15]. The amount of several cytokines (IL-6, IL-4, IL-10, TNF-α), Igs, CRP, ROS and erythrocyte sedimentation rate were measured. All showed significant but weak association with radiation dose (except IL-4) and age. Multivariate analysis of selected cytokines/factors revealed that different combinations of factors, including or excluding ROS, could explain a large proportion of radiation and age dependency, suggesting the existence of different pathways associated with radiation- and age-associated inflammation. The effects of aging on inflammation appeared more pronounced than the effects of radiation. Radiation exposure may potentiate age-dependent inflammation.

295. In order to evaluate other age-related changes of the innate immune system, Kajimura et al. [K8] analysed the abundance and response to stimulation of circulating dendritic cells in 229 atomic bombing survivors. These individuals were categorized by the authors into non-exposed (median dose 0.001 Gy, range 0–0.002 Gy), and exposed to low (median dose 0.13 Gy, range 0.12–0.49 Gy) and high (median dose 0.98 Gy, range 0.50–3.80 Gy) radiation dose groups. The number of total dendritic cells, plasmacytoid and classical dendritic cells was not modulated by radiation exposure in men, while the number of plasmacytoid dendritic cells decreased with increased radiation dose in women. Of note, the

total number of dendritic cells, plasmacytoid and classical dendritic cells decreased with age also only in women. Ex vivo dendritic cells activation, evaluated by RT-qPCR and secretion of cytokines was influenced by the age of donors, but not by radiation dose or sex.

296. To determine the effects of radiation exposure early in life on the functionality of the immune system, the efficiency of vaccination against influenza virus was measured in a group of 292 aged atomic bombing survivors (non-exposed, low-moderate exposure, high exposure) who donated blood before and after subcutaneous vaccination with commercial trivalent inactivated influenza vaccine during the 2011 or 2012 flu season [H16]. The response to vaccination was evaluated by quantification of hemagglutination inhibition antibody titres in serum, and the production of cytokines by peripheral blood mononuclear cells stimulated in vitro with the relevant influenza vaccine. Although the response to vaccination was modest, as expected in this aged populations, it was similar in control and exposed groups, indicating that radiation exposure early in life does not prevent nor impair vaccine response decades later. Thus, the functional decline of the immune system does not seem to be aggravated by past radiation exposure.

(c) *Environmental, occupational, medical and accidental exposures*

297. Attar et al. [A25] compared immune parameters in residents of a HBRA in Ramsar, Islamic Republic of Iran, and of a control area. They reported modulation of lymphocyte numbers, cytokine and antioxidant levels in the serum, and neutrophil activity, suggestive of a shift of T-lymphocyte populations from Th1 to Th2, and activation of innate immunity, including antioxidant defences in HBRA residents. In a different study Borzoueisileh et al. [B52] addressed the influence of the duration of residence in the same region on immune parameters in a group of 50 healthy adults who lived in this area for 10 to 35 years (mean exposure duration 22±8.7 years). They observed a significant increase in the frequency of blood $CD4^+$ T-cell and a decrease of blood $CD107a^+$ cells (cytotoxic $CD8^+$ T-cells and NK cells) with time of residence, i.e. cumulative exposure. This last result was partially confirmed in an independent study comparing the abundance of $CD107a^+$ cells in the blood of a group of 30 HBRA residents and a group of 30 residents from a control region [B52]. HBRA group included individuals living in this area for at least 10 years, and the mean annual effective radiation dose was 9.77 mSv (4.5–57 mSv). For the control group, the mean annual effective radiation dose was 1.2 mSv (0.57–3.0 mSv). The frequency and number of white blood cells and lymphocytes was similar in both groups, but the concentration (cells/µL) of $CD107a^+$ cells was lower in donors from the HBRA group.

298. Li et al. [L22] studied immune parameters in two comparable groups of 100 healthy women living in China, one living in the Yangjiang district HBRA (mean age 65±10 years) and the control one in the Enping district (median age 61±8.1 years). Cumulative dose exposure in HBRA subjects was 160±38 mSv (range 59–249 mSv) and 44±7.3 mSv (range 31–66 mSv) in the control group. There was no statistical difference in the frequency of total or $CD4^+$ blood T-lymphocyte. However, the frequency of $CD8^+$ T-cells increased with cumulative dose. Furthermore, the number of $CD8^+$ T-lymphocytes was increased in function of body mass index after adjustment for age and dose. The analysis of serum cytokine levels by antibody array identified a group of 10 factors elevated by 1.5 to 2.6 times in HBRA residents. The levels of 6, including sIL-6R, MCP-1, IFN-γ, EGFR and CRP were confirmed by ELISA to increase with increasing cumulative dose, in a linear relation for CRP, MCP-1 and sIL-6R.

299. In a different experimental approach, Jain and Das [J10] performed an in-depth transcriptional profiling (Affymetrix Gene Chip) of peripheral blood mononuclear cells isolated from residents of the Kerala coast region in India. They analysed cells from four groups of nine healthy donors exposed to <1.5 mGy/y (group I), 1.51–5.0 mGy/y (group II), 5.01–15 mGy/y (group III), and >15 mGy/y (group IV). The number of differentially expressed genes (up- or down-regulated with fold changes of 1.3, 1.5

and 2.0) increased with exposure in groups II, III and IV. In each group, the number of genes regulated decreases as the fold change increases. Gene ontology analysis showed that immune response pathways are among the over-represented biological processes defined by differentially expressed genes in groups III and IV, with 24 and 28 pathways, respectively. In group IV, some pathways related to T-cell receptor signalling, B-cell receptor signalling, and cytokine-cytokine receptor signalling are up-regulated, whereas some other related to T-cell receptor signalling, Toll-like receptor signalling, and cytokine-cytokine receptor signalling are down-regulated. Expression of certain genes including *NFKB2* (up-regulated) and *CXCR1*, *TNFS10*, *CCR2* (down-regulated) is dependent of the radiation dose in groups II–IV. The regulation of expression of 30 genes was validated by RT-qPCR. This study illustrates the complexity of the regulation of the immune system/immune processes in response to chronic low-dose exposure.

300. To evaluate the effects of chronic exposure/internal contamination on the immune system, Sajjadieh et al. [S7] measured the levels of IL-4 and INF-γ together with the $CD4^+$ T-cell population in Ukrainian children living in a post-Chernobyl contaminated area, with or without irritable bowel disease without inflammation. Compared to children from the same area without irritable bowel disease, children with irritable bowel disease had less $CD4^+$ T-cells, a higher level of IL-4 and a lower level of IFN-γ, suggesting a stronger polarization toward a Th2 phenotype. There was no difference with age, suggesting that there was no radiation-dose effect.

301. Apoptosis of lymphocytes and mutations in the T-cell receptor genes in circulating lymphocytes were analysed in Techa river basin residents chronically exposed for years to a combination of external and internal radiation [B41]. The study group consisted of 161 people (mean age 69 years, range 58–83, 69% women) with mean accumulated red bone marrow dose of 0.96 Gy (range 0.01–4.5 Gy), and a maximum annual dose to red bone marrow of 0.26 Gy and included 38 people who survived chronic radiation syndrome. Chronic radiation syndrome is a clinical syndrome which develops in man after whole body annual radiation exposures exceeding 0.7–1.0 Gy and cumulative doses >2–3 Gy over 2–3 years, characterized by inhibition of haemopoiesis and immune reactions, structural and functional disorders of the central nervous, cardiovascular and other organ systems [I2]. The control group consisted in 72 unexposed individuals with similar characteristics. The frequency of apoptotic cells was found increased in the study group (0.35%) compared with the control group (0.25%). The frequency of T-cells with mutated T-cell receptor genes was increased three times (0.33%) in the study group and even reached 0.41% in the chronic radiation syndrome survivors. The induction of apoptosis may limit the accumulation of mutated T-lymphocytes at higher accumulated doses.

302. A comprehensive survey of immune parameters was conducted in a different group of residents of the Techa river basin in relation to their level of mutation in T-cell receptor genes [A8]. The study group (N=66) was divided into a main group (N=29), consisting of individuals with higher levels of T-cell receptor gene mutations, and a comparison group (N=37). The mean age, sex ratio and socio-economic status of both groups were similar. The absorbed dose to red bone marrow was 0.89±0.09 Gy (range 0.09–2.0 Gy) and 1.03±0.07 Gy (range 0.30–2.3 Gy) for the main and comparison groups, respectively. The absorbed dose to soft tissue was 0.07±0.01 Gy (range 0.01–0.33 Gy) and 0.07±0.02 Gy (range 0.01–0.49 Gy) for the main and comparison groups, respectively. Adaptive (number of B- and T-lymphocyte subsets, serum Ig concentration) and innate (number of NK and NKT cells, myeloid cells, their phagocytic and lysosomal activities, cytokine levels) immune parameters were quantified in blood. The lysosomal activity of neutrophils, the number and frequency of NKT cells and the amount of IL-1α in serum were found to be increased in the main group together with a decrease in IL-2 and GM-CSF levels. lysosomal activity of neutrophils increase was correlated to the dose rate and absorbed dose to red bone marrow, whereas the amount of NKT cells was associated to the soft tissue dose rate. The authors view these changes as the reaction of the immune system to the increased frequency of mutated T-cells in exposed individuals.

303. Analysis of immunological changes was also performed in workers from the Mayak production association workers cohort [R23] occupationally exposed to cumulative doses of external gamma rays ranging from 0.5 to 3.0 Gy (N=14, mean dose 1.8±0.76 Gy) or to a combination of gamma rays (N=77, range 0.7–5.1 Gy, mean dose 1.8±0.76 Gy) and internal alpha radiation from plutonium incorporation (mean body burden 1.8±0.23 kBq, range 0.3–16.4 kBq). The control group included 43 matched subjects with no history of exposure. The amount of serum IgM, IgG, IgA, IgE, MMP-9, TNF-α, IFN-γ, IL-10 and TGF-β1 and the proportion and number of selected circulating T-lymphocyte and NK cell populations were measured. The level of IgM, IgA, MMP-9, and the frequency of NK cells were increased while the NK cell frequency of T-lymphocytes was decreased in both groups of exposed workers compared to the control group. The level of IgG and the frequency of total and T-helper lymphocytes were reduced while the amount of TGF-β1, IFN-γ, the frequency of NK and NKT cells and the number of NKT cells were increased in externally exposed workers. Finally, the frequency of helper T-cells was significantly lower and the amount of serum TNF-α and TGF-β1 significantly higher in workers with mixed exposure compared to workers exposed to external radiation only. In the external radiation group, the proportion of $CD4^+$ helper T-cells was inversely correlated with the level of gamma-radiation exposure. Thus, chronic occupational exposure leads to long-term modulation of immune parameters, different according to the exposure scenario.

304. Howell et al. [H37] described the response of rodents captured in the Chernobyl exclusion zone to chemotherapeutic agents and lipopolysaccharide stimulation. After intra-peritoneal injection of lipopolysaccharide (0.5 mg per kg body weight), the induction of the glutathione peroxidase 1 and superoxide dismutase 2 genes in the liver was significantly lower in animals from contaminated areas than in control animals. The results indicate a modulation of the response to lipopolysaccharide in animals living in contaminated environments.

305. Occupational exposure of medical staff is an important consideration. Karimi et al. [K15] compared haematological parameters, lymphocyte response to mitogenic stimulation and cytokine levels in a group of 30 radiology staff who received less than 50 mSv ("the maximum permissible annual dose") and 20 unexposed control laboratory staff. Detailed exposure information is not available. It was found that lymphocytes from radiology workers proliferate more intensely in response to phytohaemagglutinin than those of unexposed workers. Radiology workers had higher levels of IFN-γ and lower levels of IL-4 than unexposed donors, suggesting a shift toward a Th1 status.

306. Zakeri et al. [Z2] analysed chromosomal damage and selected markers of cellular and humoral immune responses in serum of interventional cardiologists compared to unexposed clinical physicians. The group of 37 cardiologists was exposed to an annual mean dose of 8.1±7.8 mSv, with a total dose of 30.5±24.3 mSv over the last five years. The frequency of aberrant cells, chromosomal breaks and dicentrics plus centric rings increased 2, 4 and 5 times, respectively in cardiologists when compared to a control group of clinical physicians. Interventional cardiologists had a more than 10-fold higher serum IgG level, but without any correlation with duration of employment and exposure. The levels of IL-2 and IL-10 were respectively significantly up- and down-regulated in cardiologists. No variation was found for IL-4, IL-8, IFN-γ and IL-6. There was no variation in the number and frequency of the main lymphocyte subsets in cardiologists at the steady state level. However, upon phytohaemagglutinin stimulation, $CD4^+CD69^+$ T-lymphocytes proliferate more.

307. Gyuleva et al. [G21, G23] analysed several immune parameters in a cohort of 438 workers (421 men and 17 women) of the Kozloduy nuclear power plant in Bulgaria and a control group of 65 persons (49 men and 16 women) without any work-related exposure. Nuclear power plant workers were grouped according to employment duration (mean dose of 9.5±5.8 mSv in group 1 to 350±120 mSv in group 4). The frequency of blood $CD4^+$ T-cells was decreased in group 1 (dose range of 0.1–20 mSv) and group 3 (dose range of 100–200 mSv) when compared to the control group; the frequency of $CD8^+$

T-lymphocytes was decreased in group 1, and the frequency of NKT cells was found to be decreased in groups 1 and 3, and that of T-lymphocytes with NK activity in all groups. However, all these fluctuations were found to be within the range of normal reference values. No changes were observed in the percentages of B-lymphocytes, total T-lymphocytes and NK cells in the different groups of workers. In a follow-up study, the frequency and absolute number of $CD4^+CD62L^+$ memory T-lymphocytes in groups 1 and 2, and of $CD4^+CD25^+$ Treg cells in group 1 were found decreased compared to control group. The frequency of $CD8^+CD28^+$ cytotoxic T-lymphocytes was decreased only in group 4 (>200 mSv). Here again, all these parameters were within the normal reference values.

308. These analyses were repeated and extended in a second cohort of Kozloduy nuclear power plant workers (105 workers stratified in four groups with mean doses of 11.8±9, 53.2±21, 130±30 and 324.6±89 mSv, respectively). The frequency and absolute number of B-lymphocytes, total T-lymphocytes, $CD4^+$ and $CD8^+$ T-lymphocytes, cytotoxic $CD8^+CD28^+$ T-lymphocytes, NK cells and NKT cells were found to be unaffected by radiation exposure. Within the $CD4^+$ T-lymphocyte population, naïve and terminal effector T-cells were found to be unchanged, but the absolute number of central memory $CD4^+$ T-lymphocytes was found to be decreased in group 1 and increased in group 4 when compared to a control group of 32 unexposed workers. No changes were observed in the levels of circulating Igs (IgG, IgA and IgM) or cytokines (IL-2, IL-4 and IFN-γ) in exposed workers [G22]. Collectively, these studies indicate that work-related exposure with cumulative doses up to 350 mSv does not induce any pathological changes in the frequency and absolute number of the main blood lymphocyte and NK-cell populations in these occupationally-exposed workers.

309. Chi et al. [C22] reported on the follow-up of three subjects accidentally irradiated while sterilizing food products. Subjects A, B and C received an estimated physical dose of 11.2, 1.82 and 1.0 Gy of ^{60}Co, respectively in about 20 minutes after entering a defective radiation-sterilization room. Subjects A and B received bone marrow transplantation on days 14 and 21 after the accident, while subject C received GM-CSF 48 hours after exposure. Three years later, they all showed a reduced percentage of $CD4^+$ T-lymphocytes, and a reduced $CD4^+/CD8^+$ ratio. B-lymphocytes and NK cells were still decreased only in subject A. Analysis of gene expression in leukocytes showed persistent modulation of the numerous genes involved in inflammation, including *PTGS2*, coding for the COX-2 enzyme, and oxidative stress management, showing that radiation exposure, at least at high doses, has long-lasting molecular and cellular consequences.

310. Li et al. [L21] report on the 10-year follow-up of the accidental "persistent" exposure of a group of 54 people (29 males and 25 females, median age 31.5, range 20–42 years) exposed to ^{192}Ir between May 9 and July19, 2002. Neither the exact exposure scenario nor the method used for dosimetry are provided. Subjects are said to have been exposed to doses estimated to be between 0.05 and 0.65 Gy by biological dosimetry (lymphocyte chromosome aberration). Ninety per cent of the subjects exhibited physical symptoms one month post-exposure. About half of them had abnormal white blood cell counts, and most (90%) exhibited abnormal morphology of bone marrow granulocytes, erythrocytes and megakaryocytes at that time. These abnormalities decreased with time (analysis at 1, 3, 5 and 10 years), but two of the subjects still had abnormal white blood cell counts, and abnormal bone marrow cell morphology 10 years after the accident, respectively. Chromosomal aberrations (dicentrics and rings assessed using conventional staining) sharply decrease with time post-accident. The number of $CD4^+$ and of $CD8^+$ T-lymphocyte and the number of NK cells in blood were decreased for up to 1 year post-accident, but returned to normal levels thereafter. Despite the lack of information on the scenario and dosimetry of exposure, this study provides valuable information about long-term follow-up of the consequences of accidental exposure.

(d) *Low-dose exposure for treatment of cancer, inflammatory, infectious and chronic diseases*

311. Kojima et al. [K34] reported on the use of various regimens of radiation exposure as therapy for cancer and non-cancer patients. Patient A had surgery for prostate cancer, but his prostate-specific antigen level rose again and remained high. Exposure to 30-weekly dose fractions of 150 mGy from X-rays (total or half body irradiation) resulted in normalization of his prostate-specific antigen level. Patient B was affected by an inoperable prostate cancer with bone metastasis. A combination of X-rays (10 fractions of 150 mGy, three fractions each week) and radon exposure (use of a 44 × 93 cm radon sheet, surface activity 37 μGy/h), six hours each day for ten months resulted in the disappearance of the bone metastasis and normalization of his prostate-specific antigen, which stayed stable for at least six months after the treatment. Finally, a patient afflicted with ulcerative colitis for 16 years was treated with a combination of daily intakes of radon-containing water (200 mL of radon-containing water (330 Bq/L) with every meal), exposure to radon in a room mimicking the conditions found in radon spas (9.8 kBq/m^3) twice a week for 40 minutes and daily exposure to radon at bedtime. The clinical symptoms disappeared, and the patient's bowel movements were restored to normal levels. This patient has now been healthy for more than eight years.

312. Exposure to low doses of radon (effective dose estimated to be around 0.3 mSv) in radon spa therapies is reported to relieve pain in patients suffering from musculoskeletal disorders associated with chronic inflammation. A lasting reduction in the serum concentration of collagen degradation products, observed in patients from 12 weeks after the beginning of the treatment, suggests an amelioration of bone resorption, which might result, at least in part, from a reduction in the concentration of the adipokine vifsatin, which follows the same kinetics [C31]. This study also reported a decrease in Treg cells as another sign of attenuated inflammation. Rühle et al. [R21] performed a detailed longitudinal monitoring of immune cell populations in the blood of a cohort of 100 patients undergoing radon spa therapy. Immune cells were compared before the beginning and up to 30 weeks post-therapy. T-lymphocytes and monocytes were slightly increased from 6 to 30 weeks. Within T-lymphocytes, the frequency of Treg cells, effector and effector memory cytotoxic T-cells were up-regulated, whereas cytotoxic T-cells, naïve T-cells and central memory cytotoxic T-cells were down-regulated at 6, 12 and/or 30 weeks. Importantly, no change was observed in the frequency of $CD4^+$, Th1 and Th2 cells. Myeloid and plasmacytoid dendritic cells were only transiently increased at week 6, whereas neutrophils and eosinophils were slightly decreased at that time. No changes were observed for B-cells. The frequency of total NK cells remained constant during the 30 weeks, but different NK cell subsets were up- (NK1) and down-regulated (NK2 and NK3) after six weeks after therapy. The activation level of T-, B- and NK cells, as defined by CD69 expression, is lastingly reduced after therapy, whereas a small subset of T-cells shows increased expression of HLA-DR, indicating that they are activated. Thus, exposure to a very low dose of radon can induce discrete but long-lasting changes in immune cell homeostasis and/or activation in patients, which appears to impact on inflammatory status and the course of musculoskeletal diseases.

313. Kojima et al. [K35] describe a case report where low-dose radon exposure therapy using conditions encountered in radon-spa therapy (40 minutes each day, 5 days per week in a room with radon activity 200 kBq/m^3 plus inhalation of vapours from radon-containing water) resulted in a long-term reduction of rheumatoid arthritis physical symptoms (swelling, pain, mobility) and biological markers (reduction to normal levels of the circulatory concentrations of C-reactive protein and MMP9).

314. Low- to moderate-dose low-LET radiotherapy is also used to treat a variety of human chronic inflammatory disorders, but the underlying mechanisms are still elusive. Arenas et al. [A15, A16] analysed the effects of low-dose radiotherapy on the interactions between leukocytes and endothelial cells in mice, i.e. the early stages of inflammation. Intravital microscopy showed that a single abdominal

exposure to low and moderate doses of 0.1–0.6 Gy inhibits lipopolysaccharide-induced adhesion of leukocytes to endothelial cells in intestinal venules. Leukocyte rolling is inhibited by doses of 0.1 and 0.3, but not by a dose of 0.6 Gy. A dose of 0.3 Gy was found to be the most effective, with effects visible as early as five hours post-exposure and lasting for two (adhesion) or three (rolling) days. The inhibition of leukocyte adhesion/rolling was accompanied by a concomitant increase in plasmatic TGF-β1. The administration of anti-TGF-β1 neutralizing antibody allowed to partially rescue leukocyte adhesion, but not rolling inhibition. The increase in circulatory TGF-β1 is mirrored by a local increased production in intestinal tissues.

315. The mechanistic basis of the anti-inflammatory effects of radiotherapy was also investigated in vitro by analysing the response to a range of X-ray doses (0.1–3 Gy) of EA.hy.926 endothelial cells [R11, R12]. Several aspects of the response were found to be discontinuous or biphasic. For example, the activation of the transcription factor AP-1 is biphasic with maxima at doses of 0.3 and 3 Gy. Adhesion of blood polymorphonuclear cells to irradiated EA.hy.926 cells were found to be more decreased and polymorphonuclear cells produced less CCL20 chemokine after a dose of 0.5 Gy than after doses of 0.3, 1 or 3 Gy. This effect is partially controlled by TGF-β1, and totally abrogated by inhibition of the expression of the X-linked inhibitor of apoptosis protein (XIAP), which is maximally induced after a dose of 0.5 Gy. Inhibition of XIAP expression also resulted in decreased NF-κB activation and increased apoptosis in 0.5 Gy exposed EA.hy.926 cells [R13].

316. A discontinuous response was also observed when the secretion of IL-1β by irradiated (doses of 0.01–1 Gy) murine activated peritoneal macrophages was investigated. A decrease in the production of IL-1β after exposure of macrophages to doses of 0.5 and 0.7 Gy occurred only when the macrophages were purified from the radiosensitive BALB/c mouse strain, but not from the radioresistant C57BL/6 strain, even if they express a human TNF-α transgene. Thus, it appears that the anti-inflammatory effects of moderate-dose radiotherapy might be linked to the genetic background rather than to the inflammatory status [F20].

317. Joo et al. [J24, J25] and other authors [C1] addressed the effects of low-dose gamma irradiation on IgE-mediated allergic response. Acute doses (0.01 to 0.1 Gy) and protracted doses (0.01, 0.05 Gy) inhibited the release of inflammatory mediators by IgE crosslinking in a rat mast cell line after in vitro sensitization. This inhibition is also reflected at the level of inflammatory cytokine production and intracellular calcium mobilization. Low-dose exposure had no effects on the activation of mast cells by lipopolysaccharide or a calcium ionophore. This inhibition results from a radiation-dependent decreased activation of FcεR1-mediated intracellular signalling pathways. These results were confirmed in human mast cells lines, although not with the same dose-effect relation. Low-dose radiation exposure was also shown to affect the development of immediate-type allergic reactions and late-phase reaction in mice, with a concomitant in situ decrease of FcεR1 expression in tissues.

318. Chronic or long-term inflammation is one of the mechanisms contributing to carcinogenesis. Inflammation is often associated with chronic disease. In the following paragraphs, this annex presents studies addressing the effects of low-dose/low-dose-rate radiation on inflammation, in several models of chronic disease, as it may influence the risk of cancer.

319. The effects of exposure to 0.05 Gy of X-rays (delivered at 0.5 Gy/min) and 0.05 Gy of ^{12}C ion (90 MeV, LET 28.3 keV/μm) on human peripheral blood lymphocytes in vitro were compared 24 hours post-irradiation. The proportion of the $CD4^+$ and $CD8^+$ T-lymphocytes, and of NK and NKT cells were not affected by exposure. The expression of the genes coding for IL-2, TNF-α and IFN-γ was increased and the secretion of these cytokines was accordingly up-regulated. The cytotoxic activity of peripheral blood lymphocytes was also up-regulated after ^{12}C ion and X-ray exposure. For all these parameters, the effects of ^{12}C ions appeared always stronger than those elicited by X-rays [C17]. A similar study was

performed on peripheral blood lymphocytes obtained from patients suffering from alimentary tract cancer (oesophagus, colon, gastric cancer). Here, the proportion of total T-lymphocytes as well as of $CD4^+$ =and $CD8^+$ T-lymphocytes was found to increase after exposure to 0.05 Gy of X-rays or ^{12}C ions (90 MeV, LET 28.3 keV/μm). The frequency of the different NK cell populations did not change. The expression of IL-2 and IFN-γ was again found to be increased both at the mRNA and protein level, but the expression of TNF-α was unchanged. Thus, the outcome of the radiation response may be influenced by the health status at the time of exposure [C18].

320. The effects of repeated low-dose radiation exposure (25 mGy X-rays at 12.5 mGy/min every other day for 2, 4, 8, 12 or 16 weeks) on the development and pathophysiological consequences of type I diabetes were investigated in a murine model of experimentally induced disease [Z8, Z9]. Induction of type 1 diabetes in C57BL/6 mice results in systemic and local (kidney, heart) inflammation (elevated serum levels of TNF-α, MCP-1, IL-18, ICAM-1), and kidney and heart damage (oxidative stress, fibrosis). Exposure of diabetic mice to 25 mGy every other day for 2 to 16 weeks reduces both systemic and local inflammation and diabetes indicators, including oxidative kidney damage as early as 2 to 4 weeks after diabetes induction [Z8]. Repeated low-dose exposures also reduced cardiac inflammation and heart damage [Z9]. Thus, repeated low-dose exposure can significantly suppress several aspects of diabetes-induced inflammation and associated renal and heart dysfunction. It must, however, be noted that repeated low-dose exposure also induces a low level of systemic and local (kidney, heart) inflammation in healthy control C57BL/6 mice.

321. Beneficial effects of multiple low-dose exposures to X-rays were also found in a mouse model of diabetes after exposure of a different regimen of multiple low-dose radiation exposures. Diabetic nephropathy develops in kidneys in diabetic mice and this was prevented by low-dose-rate radiation exposures in two studies from the same group [C20, C21]. Diabetes was induced in 10-week-old C57BL/6J mice by repeated exposure to streptozotocin, and then animals were exposed to 12.5, 25 or 50 mGy every other day for 4 or 8 weeks. Mice exposed to all of these doses of radiation showed less fibrosis and some reduction in creatinine and proteins increased in diabetic animals such as connective tissue growth factor.

322. Moderate-dose exposure protection in diabetic animals was found in rats which otherwise suffer from diabetic testicular atrophy [Z17]. Type 2 diabetes was induced in rats over two months by high fat diet coupled with exposure to streptozotocin. Development of this disease triggered testicular atrophy, unless exposure to moderate-dose radiation was carried out. Exposure of rats to 25 mGy of X-rays every second day for four weeks until the accumulated dose reached 350 mGy protected animals from testicular atrophy. These rats had close to normal expression of anti-oxidative proteins NQO1, SOD, and catalase as well nearly normal expression of glucose metabolism relevant proteins Akt and GSK-3b.

323. The effects of repeated low-dose radiation exposure on the development of experimentally induced type 2 diabetes was also analysed. Mice were exposed to 25, 50 or 75 mGy of X-rays at 12.5 mGy/min every other day for 4 or 8 weeks after disease induction [S27]. Radiation exposure was found to attenuate dyslipidaemia, renal inflammation and kidney dysfunction in diabetic mice. Repeated exposures of 25 mGy had no impact on hyperglycemia but repeated exposures of 50 and 75 mGy transiently reduce insulin resistance at 4 weeks. Differences in the response to different radiation doses were observed: the effects of repeated 50 and 75 mGy exposures are often transiently visible at 4 but not 8 weeks, while the effects of repeated 25 mGy exposures are often visible at 8 weeks only (for example for levels of urinary albumin, plasma HDL, renal tri-glycerides, TNF-α, PAI-1, malondialdehyde, Nrf2 expression). Expression of Nrf2 target genes (SOD1, HO-1 and NQO-1) was increased at 4 and 8 weeks for all radiation doses. These results were partially confirmed in a follow-up study comparing the effects of repeated low-dose exposures (50 mGy every other day for 4 weeks) and fibroblast growth factor 21 (FGF-21) alone or in combination on the development of type 2 diabetes induced nephropathy [S28]. FGF-21

is known to regulate carbohydrate and lipid metabolism and have beneficial effects in diabetes. The attenuation of diabetes consequences/indicators was better after the combination than any treatment alone. Of note, repeated low-dose exposures and FGF-21 have opposing effects on the expression of Nrf2 and its target genes, but the combination resulted in a normalization of their expression. Low dose rate and FGF-21 act independently to protect against type 2 diabetes pathophysiological consequences.

324. In a study using diabetic mice, Tsuruga et al. [T21] exposed 11 weeks old female db/db mice (BSK.Cg-Leprdb +/+ Leprddbb/Jcl) and heterozygous littermates, db/m mice (BKS.Cg-m +/+ Leprdb/Jcl) to 940 μGy/h of gamma rays for 24 days. Under these conditions, diabetic animals had improved tolerance for glucose while no changes were found in healthy heterozygous littermates. This finding was ascribed to improved MnSOD production in irradiated db/db animals.

325. Alpha-radiation exposure was also found to protect mice from renal damage induced by CCl_4 injection, which induces a strong oxidative stress in kidney. Hence, 24 hours post-injection, mice exhibit an increase in blood creatinine, and kidney lipid peroxidation, and a decrease in renal SOD and catalase activity and morphological changes such as dilatation of Bowman's space and glomerular atrophy. Inhalation of radon (1,000 or 2,000 Bq/m^3 for 24 hours) before CCl_4 administration reduces creatinine and lipid peroxidation increase and limits or reverses the effects of CCl_4 on SOD and catalase activity, respectively, but does not prevent tissue damage [K18]. Some of these effects are similar to those induced by α-tocopherol, suggesting that radon inhalation can augment anti-oxidative defences.

326. The effects of radiation exposure on the development of thyroid autoimmune diseases were analysed in two mouse models [N2]. The exposure to a single dose of 0.5 Gy gamma rays 1 week before beginning the supplementation of drinking water with NaI for 8 weeks exacerbates the development of autoimmune thyroid disease (Hashimoto's thyroiditis) in susceptible NOD-H2h4 mice, revealed by an increase in the level of circulating anti-thyroglobulin. Total-body irradiation before (0.05 or 3 Gy) or after (0.05, 0.5 or 3 Gy) the beginning of NaI supplementation or exposure of the thyroid only (0.05 Gy) did not increase the severity of the disease. The pre-exposure of animals to 0.05 Gy either 1 or 2 weeks before exposure to 0.5 Gy does not significantly modulate thyroiditis scores. No effects of radiation exposure (single or repeated 0.05 Gy) were observed in a different experimental model of thyroid autoimmunity (induction of Grave's hyperthyroidism in Balb/c mice). Thus, radiation may, under a specific exposure regimen, exacerbate the development of certain autoimmune thyroid disease in susceptible animals with a discontinuous dose response pattern.

327. The effects of chronic low-dose-rate exposure on the development of asthma were investigated in a murine model of sensitization to ovalbumin (OVA) [K25]. Female C57BL/6 mice received on days 0 and 14 two intraperitoeal injections of OVA emulsified in aluminium hydroxide and were challenged at days 21, 22 and 23 by inhalation of nebulized OVA (1% weight/volume). Mice were chronically exposed to gamma radiation (^{137}Cs) at 0.554 mGy/h or 1.818 mGy/h from the first injection to the last challenge, i.e. 24 days for total doses of 0.3 and 1 Gy, respectively. They were sacrificed 48 hours later for histopathology analysis of lungs, and analysis of inflammatory markers in bronchoalveolar lavage and serum. Radiation doses of 0.3 and/or 1 Gy were found to significantly attenuate histopathological signs (inflammatory cell invasion of lungs) and systemic markers (IL-4, IL-5, anti-OVA IgE) of asthma. These changes were associated with a marked reduction in Mucin-5, p-ERK and p-JNK in lungs. The exposure to intermediate doses delivered at low dose rate can therefore attenuate airway and systemic inflammation during asthma [K25].

328. Decrease of arthritis in TNF-α transgenic mice was achieved by a single partial-body exposure to 500 mGy of X-rays [D15]. Endpoints such as mineralization properties of osteoblasts and neutrophil infiltration were explored in vivo. The authors ascribed this success to the notion that 500 mGy exposure induces apoptosis of fibroblast-like synoviocytes that reduced expression of the inflammatory phenotype.

329. Behçet's disease is a multi-systemic disorder of unclear aetiology characterized by blood vessel inflammation throughout the body and, among others, mouth, skin, eyes sores, painful and swollen joints that may result from a dysfunction of Th1 $CD4^+$ T-lymphocytes. Kang et al. [K12] used a virally induced murine model of Behçet's disease to investigate whether low-dose radiation exposure could modulate the production of cytokines. Induced mice were exposed (whole-body or half-body only) to 0.1, 2 or 10 Gy of X-rays. The level of intracellular and serum IFN-γ (Th1) and IL-4 and IL-10 (Th2) were measured by fluorescence-activated cell sorting and ELISA, respectively. Whole-body exposure to 0.1 Gy results in increased levels of all cytokines in cells and serum four to six days post-radiation. The IL-4/IFN-γ ratio was clearly in favour of IL-4, suggesting an increased Th2 immunity. Half-body exposure to 0.1 Gy results in an increased serum concentration of these cytokines compared to whole-body irradiation. Both exposures to 0.1 Gy lead to a reduction of the severity score two weeks post-exposure.

330. The effects of low and high doses of ^{137}Cs gamma ray (1 mGy–2 Gy at 1 mGy/min) exposure on *Escherishia coli*-induced sepsis was investigated in C57BL/6 mice [S58]. It was found that 0.1 Gy total-body irradiation 48 hours prior to infection significantly rescued mice from death (75% survival at 72 hours compared with 25% in non-irradiated animals). One day post-infection, the bacterial burden was significantly decreased in the blood, spleen and kidney of pre-exposed mice, and the cytokine storm (large increase in serum IL-1β, IL-6 and IL-10) was prevented. The number of peritoneal macrophages was lower in pre-irradiated infected mice than in non-irradiated mice. The frequency of $CD8^+$ T-cells and macrophages was increased in the spleen of pre-exposed mice, but exposure to radiation did not modulate the expression of activation markers on splenocytes. In contrast, radiation exposure reduced the induction of iNOS and increased the expression of Nfr2 and HO-1 in splenocytes. In vitro, pre-exposure of murine RAW264.7 macrophage cells before *Escherishia coli* infection mostly recapitulates the same effects. Thus, part of the effects observed in vivo could result from the stimulatory effects of low-dose exposure on anti-microbial macrophage activity.

331. With the similar aim to evaluate the effects of radiation exposure on immune response in vivo, Misra et al. [M35] evaluated the effects of internal contamination at a young age on the ability of adult mice to respond to influenza A infection. Fourteen-days old pups received an intra-peritoneal injection of 50 μCi/185 kBq of ^{137}Cs, resulting in a mean accumulated dose of 2.5–2.7 Gy over the next 50 days. They were infected 26 weeks later with 100 HAU (haemagglutinating units) of influenza A/HKx31 (H3N2) virus. Contaminated mice recovered over a 2-week period and had serum antibody titres and levels of MCP-1 and club cell secretory protein mRNA in the lung similar to control mice. Thus, ^{137}Cs contamination early in life resulting in an accumulated dose of 2.5–2.7 Gy in the following several weeks does not impair efficient anti-viral responses at later times.

(e) *Low-dose/low-dose-rate exposure effects in healthy animals*

332. The analysis of C57BL/6 mice exposed to ^{137}Cs gamma radiation at low dose rate (0.7 mGy/h and 3.95 mGy/h for 12 and 21 days for total dose of 0.2 and 2 Gy, respectively) seven days post-exposure did not show any changes in body weight, in haematological parameters nor in the distribution of B- and T-lymphocytes in the spleen. The analysis of the level of circulating cytokines in the serum (analysed by antibody arrays) showed, however that a number of cytokines were significantly up- or down-regulated, with changes suggestive of the recruitment, differentiation and activation of myeloid cells and a shift toward Th2 type immunity [S39]. Thus, even if no overt changes in the homeostasis of immune cells were visible, a modulation of inflammatory mediators involved in intercellular signalling occurs at the systemic level and may shift the balance between Th1 and Th2 immunity.

333. However, a different study addressed the response of C57BL/6 mice to 0.01, 0.05, 0.1, 0.5 and 2 Gy of gamma radiation, from 4 hours to 7 days post-exposure. Apoptosis was already detected as early as

4 hours post-radiation in $CD4^+$ T-cells after 0.01 and 0.05 Gy. In NK cells and dendritic cells a dose of 0.05 and 0.1 Gy has no effects, whereas higher doses induce apoptosis in all populations analysed, including $CD8^+$, Treg and B-cells. The number of $CD4^+$ T- and Treg-cells was transiently increased at day 1 after 0.01 Gy. All populations decreased, more or less dose dependently by day 3, and recovery was already evident by day 7. These changes may be related to changes in the expression profile of cytokines. Several of the cytokines analysed show a dual profile, with a reduction or no change at 0.01 and 0.05 Gy and an increased expression after 2 Gy. The response to 0.1 Gy seems intermediate between these two patterns. It is not possible to discern a Th1 or Th2 trend. The expression of cytokines with haematopoietic activity (GM-CSF and IL-5) is dose dependently increased from 4 hours to day 3. These data illustrate the different response in time of splenic immune cells to low- and moderate-dose radiation in vivo [B46].

334. The effects of acute or fractionated low-dose radiation on immune cells was analysed in the spleen of C57BL/6 mice exposed to 0.001, 0.01 or 0.1 Gy of ^{137}Cs gamma rays delivered either acutely or fractionated in three equivalent doses delivered on three consecutive days. Spleen cell number is stable, except for a sporadic increase at day 7 in the acutely exposed mice exposed to 0.001 Gy. In acutely exposed mice, the proportion of splenic $CD4^+$ T-cells increases transiently at day 2, while dendritic cells and macrophages decrease. These factors are normalized in later days, but the frequency of $CD8^+$ T-cells decreases for higher doses at day 7 and 14. The expression of activation markers (CD69, CD28, CD86) is unchanged or decreased for all doses at all timepoints. Fractionation only slightly changes this pattern. However, the pattern of cytokine production by splenocytes differs after acute and fractionated dose delivery. Some of these changes can be explained by changes in the expression of protein involved in signal transduction (Nrf2, IκBα), differently regulated according to dose and fractionation. These results suggest that acute exposure elicits a mixed response that evolves to a Th1 type profile, whereas fractionated exposure evokes first a Th1 response that evolves towards a Th2 phenotype [S57].

335. The effects of acute low- or intermediate-dose exposure and aging on the murine T-cell receptor (TCR) repertoire expressed by peripheral blood T-lymphocytes were compared in CBA/Ca mice after a single irradiation of 0.1 or 1 Gy 250 kV X-rays with 1.2 mm HVL Cu [C3]. High throughput profiling of rearranged TCR-β chain genes in the blood of irradiated mice 1, 3 and 6 months post-exposure showed that radiation affected the homeostasis of the T-cell pool. The effects of 0.1 Gy were more pronounced and lasted longer than those elicited by 1 Gy, which were partially reversed over time. These effects can be assimilated to accelerated aging. They result, at least in part, from effects of radiation on haematopoietic stem cells.

336. To investigate how extracellular vesicles participate in systemic intercellular signalling, including bystander cell-to-cell communication, extracellular vesicles purified from C57BL/6 mice exposed to 0.1 or 2 Gy of X-rays were injected into non-irradiated syngeneic mice (bystander mice). Changes in the level of cytokines in the plasma were compared between bystander mice and directly irradiated mice 24 h after injection or irradiation. M-CSF and pentraxin-3 levels were up-regulated in both low- and high-dose irradiated mice and bystander mice injected with extracellular vesicles purified from 0.1 and 2.0 Gy irradiated mice. In addition, CXCL16 and Lipocalin-2 were increased in mice exposed to 2 Gy and in bystander mice injected with extracellular vesicles from mice exposed to 2 Gy, and C-C motif chemokine ligand (CCL) 5 and CCL11 were similarly down-regulated in low and high-dose bystander mice. Extracellular vesicles purified from the blood of 0.1 and 2 Gy exposed mice were found to contain miRNAs targeting similar pathways (acute myeloid leukaemia, T-cell receptor signalling, MAPK, TGF-β), but no common miRNA was identified. Thus, extracellular vesicles can recapitulate some of the effects of direct radiation exposure in recipient mice, and some of these effects may result from the miRNA cargo of these extracellular vesicles [S75].

337. The potential protective effects of nicaraven, a hydroxyl-radical scavenger radioprotectant, on haematopoietic stem cells was tested in mice exposed for 30 consecutive days to 50 mGy of gamma radiation (^{137}Cs). Nicaraven was injected 30 minutes before each irradiation. There were two sets of controls: One were mice that were irradiated with the same protocol and received no nicaraven (non-treated irradiated mice) and the second were mice that were not irradiated (non-irradiated controls). Mice were sacrificed 24 hours after the last irradiation. Cytopenia was clearly reduced in nicaraven treated mice, and there was no loss of circulating c-kit$^+$ haematopoietic stem cells compared with the non-irradiated control mice, in contrast with non-treated irradiated mice. The number of c-kit$^+$ bone marrow cells was also higher in irradiated mice which received nicaraven compared with non-treated irradiated mice but was only about half of non-irradiated controls. Notwithstanding, the number of colony-forming unit in the blood and bone marrow of nicaraven-treated mice was similar to non-irradiated controls, while it was clearly reduced in non-treated irradiated mice. These effects could not be attributed to changes in intracellular ROS levels. Analysis of gene expression on dedicated RT-PCR arrays (370 genes representing 13 pathways) showed that the majority of the genes representing the inflammatory/immunity pathway (pro- and anti-inflammatory cytokines, co-activation molecules) were significantly down-regulated. Thus, modulation of immune responses but not of the redox status participates in the protection of haematopoietic stem cell by nicaraven in vivo after repeated exposure to low-dose radiation [A12].

338. The effects of exposure to radiation with different LET on the homeostasis and function of immune cells in vivo and ex vivo was also addressed, including eventual induction of an adaptive response by low-dose and low-dose-rate X-irradiation prior to subsequent exposure to high-LET radiation. In mice exposed to 0.01, 0.05 or 0.1 Gy of protons (210 MeV) delivered over several days at an average dose rate of 1 mGy/h for 12 hours per day, the number of white blood cells, lymphocytes, monocytes/macrophages and granulocytes in the blood and spleen were unchanged in the three weeks following exposure. However, a more detailed analysis revealed a modulation of the splenic T-lymphocytes in mice exposed to 0.01 Gy, due to a decrease in CD4$^+$ T-lymphocytes at the end of the exposure period (day 0) and at day 21, but not at day 4. Splenic CD8$^+$ T-lymphocytes were not affected. B-lymphocytes were reduced at day 21. The expression of genes related to T-cell subsets differentiation was analysed by RT-qPCR using a dedicated PCR array in splenic CD4$^+$ T-cells purified from control mice and mice exposed to 0.01 and 0.1 Gy at day 0. Five and nine genes were up-regulated in CD4$^+$ T-cells exposed to 0.01 and 0.1 Gy, respectively, while three genes were commonly up-regulated (*CD40*, *CEBPB* and *TNFSF4*). Only three genes were down-regulated after 0.01 Gy, and none after 0.1 Gy. The down-regulated genes code for the p105 subunit of NF-κB, JNK1 kinase and IL-4 receptor. Thus, protracted low-dose proton exposure induces early dose-dependent modulation of signalling in CD4$^+$ splenic T-cells after whole-body irradiation [G12].

339. In a different series of experiments, low-dose and low-dose-rate (0.01 Gy delivered at 0.18 mGy/h) exposure to gamma radiation was found to result in a transient reduction in B- and T-lymphocytes (CD4$^+$ T-lymphocytes but not CD4$^+$CD25$^+$ and CD4$^+$CD25$^+$Foxp3$^+$ Tregs) in the spleen of irradiated animals, visible at day 4 but no longer at day 21. This low-dose and low-dose-rate exposure does not apparently modulate the response of mice and CD4$^+$ T-cells to a subsequent exposure to proton radiation representing a simulated solar particle event (sSPE, 1.7 Gy, 30 to 210 MeV) [G13]. However, it was later found that low-dose and low-dose-rate exposure induces changes in the level of several key proteins (NF-κB p65, JNK1, p38 MAPK, Lck, ZAP70, up- or down-regulated at 4 and 21 days post-exposure) involved in signal transduction in CD4$^+$ T-lymphocytes. CD4$^+$ T-lymphocytes purified from mice at day 21 and activated in vitro secreted three times more IL-4 and slightly less TGF-β1 than control mice, suggesting a possible polarization towards a Th2 type response [R9]. Pre-exposure to low-dose and low-dose-rate exposure could prevent or reduce most of the changes induced in CD4+ T cells by a 1.7 Gy sSPE exposure. Thus, low-dose and low-dose-rate exposure prior to a sSPE results in radio-adaptation and can modify intracellular signalling elicited by high-dose proton exposure [R9]. Despite these findings, protracted low-dose and low-dose-rate exposure was found to have only a marginal influence on the

effects of proton exposures (2 or 3 Gy, 250 MeV, 0.4 KeV/µm) on the dynamics of leukocytes populations, platelets and erythrocytes in mice 4 and 17 days post-proton exposure [L51].

340. To determine whether protracted low-dose and low-dose-rate exposure affected only a subsequent high-LET radiation response, a direct comparison was performed. Mice exposed or not to 0.01 Gy gamma radiation delivered at 0.3 mGy/h were subsequently exposed to 2 Gy gamma radiation (0.9 Gy/min) or 2 Gy protons (250 MeV, LET 0.4 KeV/µm, 1 Gy/min). The different leukocyte populations as well as T-cell populations, their secretion of cytokines and their content in active signalling protein kinase were analysed 21 and 56 days later. Low-dose and low-dose-rate exposure alone had limited impact on cell homeostasis and cytokine secretion, indicating long-lasting changes suggestive of a shift towards Th1 type immunity. Prior low-dose and low-dose-rate exposure did not overtly modulate the response of mice and $CD4^+$ T-cells to 2 Gy gamma or proton radiation. Only a limited number of subtle changes could be observed on Tregs homeostasis or secretion of VEGF. Thus, the radio-adaptive ability of low-dose and low-dose-rate exposure to a challenge dose of high-dose gamma or protons radiation is quite limited [G14]. The liver of mice exposed to similar radiation exposure regimen (proton energy was only 230 MeV) showed no overt signs of inflammation at day 56 and only a small increase in oxidative burst capacity by stimulation with *S. cerevisiae* Zymosan was observed after low dose and low dose rate + proton exposures. The expression of apoptosis-related genes, analysed by dedicated RT-qPCR array (84 genes), did not change in the liver of mice exposed to low-dose and low-dose-rate exposure only, but pre-exposure to low-dose and low-dose-rate exposure modulates the gene expression in mice exposed to 2 Gy gamma rays or to 2 Gy of protons. Thus, only two genes are down-regulated in 2 Gy gamma-irradiated mice, but 11 are modulated (up- and down-regulated) in mice previously exposed to low dose and low dose rate. Similarly, 6 and 12 genes are modulated (up or down) in proton compared with low dose and low dose rate + protons exposed mice. Thus, low-dose and low-dose-rate exposure can modify the apoptotic response of liver cells to high-dose gamma or high-dose proton radiation long after exposure. However, this study does not allow a conclusion to be drawn as to whether this radio-adaptation is beneficial (increased apoptosis of exposed cells) or potentially detrimental (increased survival of possibly exposed damaged cells) [G14].

(f) *Effects of low-dose radiation on natural killer cells, dendritic cells and macrophages*

341. Yang et al. [Y3] analysed the effects of a range of low (25–75 mGy) and moderate (150 – 500 mGy) dose X-ray exposure in vitro on human NK cells generated from peripheral blood mononuclear cells after culture with IL-2 and OK432 (anti-neoplastic compound). A dose of 75 mGy was found to induce the expansion of in vitro generated NK cells and stimulate their production of TNF-α and IFN-γ and their specific cytotoxicity. These effects were abrogated by p38-MAPK inhibitors, which also inhibited the mRNA expression of FasL and perforin, two molecules involved in NK cytotoxic activities.

342. Human NK cells are however extremely radiosensitive. They were found to be the most radiosensitive of the human peripheral blood mononuclear cells after in vitro exposure of freshly purified cells. The fraction of viable NK cells is reduced 48 hours after exposure to 50 mGy X-rays, and clear signs of apoptosis and secondary necrosis are detected 48 hours after exposure to 0.1 Gy [F6]. Furthermore, a transcriptomic analysis of human whole blood exposed in vitro showed that numerous genes related to NK cell activity are dose-dependently down-regulated 48 hours after exposure to 0.5–8 Gy of gamma radiation. Importantly, the magnitude of this decrease is much more important than the loss of NK cells in culture, implying that these genes are specifically down-regulated in response to radiation [P7].

343. In contrast, Sonn et al. [S59] reported that in vitro exposure to a moderate radiation dose (0.2 Gy gamma rays) of purified murine NK cells increased their cytotoxicity towards tumour target cells. Luzhna and Kovalchuk [L53] reported that low-dose exposure (0.1 Gy X-rays) induced up-regulation of numerous genes belonging to the Kyoto Encyclopedia of Genes and Genomes NK cell mediated cytotoxicity pathway in the mammary gland of rats, four days post-exposure.

344. However, chronic exposure of SJL mice for eight months to very low dose rate (annual dose of 100 mGy) induced only a transient increase in splenic NK cell frequency at weeks 28 and 32, without increasing their cytotoxic activity. The only other change observed among immune cell populations was a transient increase in $CD8^+$ T-lymphocytes in lymph nodes at week 32 [L1]. At the interface of innate and adaptive immunity, dendritic cells play a key role in the development of immune responses. The effects of low-dose gamma irradiation on dendritic cells were analysed from a functional point of view, both in human and mice. Different results were obtained in each experimental system.

345. Human dendritic cells were produced in vitro from purified $CD14^+$ monocytes from peripheral blood mononuclear cells. Sorted cells were allowed to differentiate into dendritic cells for four days in the presence of IL-4 and GM-CSF, with or without fractionated (one dose per day) X-ray exposure (0.162 Gy/min), to a total of 0.05, 0.5, 1.0 or 2 Gy delivered in 4 fractions of 0.0125, 0.125, 0.25 or 0.5 Gy, respectively. Radiation did not affect dendritic cell differentiation, phenotype and activation, maturation or cytokine secretion following lipopolysaccharide stimulation. Thus, exposure did not affect the ability of dendritic cells to stimulate the proliferation and activation of allogeneic T-cells in co-culture. Though in vitro, the study showed that radiation exposure has no effects on the differentiation, maturation and functionality of human dendritic cells generated from purified monocytes [J8].

346. The effects of low- to moderate-dose radiation exposure in vivo were analysed in C57BL/6 mice exposed to 0.1, 0.25 and 2 Gy X-rays. Changes in the phenotype of splenic dendritic cells were found only in 2 Gy irradiated mice. Exposure to 0.1 Gy does not stimulate antigen uptake and, by contrast, decreases antigen presentation to T-cells by irradiated dendritic cells. Cytokine secretion is only mildly affected by low-dose exposure (small increase in IL-10, no changes in IL-1α, IL-1β, IL-6, IL-12, TNF-α), whereas the secretion of all these factors is increased by 0.25 and 2 Gy. Thus, in vivo low-dose radiation exposure does not activate or even reduces murine dendritic cell functions, in contrast to moderate- and high-dose radiation exposure [P14]. However, when mature $CD11c^+$ dendritic cells purified from the spleen of BALB/c mice were exposed in vitro to 0.02, 0.05, 0.1, 0.5 and 1.0 Gy before being used to stimulate allogeneic purified T-cells in a mixed lymphocyte reaction, dendritic cells isolated from 0.05 Gy exposed mice could stimulate T-lymphocyte proliferation. Although their phenotype remains unchanged (no induction of activation markers), these cells produce more IL-12, IL-2 and IFN-γ, but similar amounts of IL-10 when compared with control cells or dendritic cells from 1 Gy exposed animals. Hence, ex vivo low-dose exposure can specifically activate dendritic cells to promote Th1 $CD4^+$ T-lymphocyte differentiation [S31]. The differences observed in these two murine studies could result from in vivo compared with ex vivo exposure, the different mouse strains or differences in dendritic cell identification and sorting purity.

347. Inter-cellular communication is paramount in immune responses. Wunderlich et al. analysed if and how radiation exposure interferes with these events using an in vitro system [W16]. Murine peritoneal macrophages were activated or not activated in vitro with lipopolysaccharide and then exposed to a single dose of X-rays ranging from 0.01 to 2 Gy. Irradiation (>0.1 Gy) of lipopolysaccharide-activated macrophages reduces their expression of MHC II molecules. The ability of these macrophages to induce allogenic $CD4^+$ T-cell proliferation is reduced only after a dose of 2 Gy. Conditioned medium collected from irradiated activated macrophages cultures did not modulate the maturation of bone-marrow derived dendritic cells, nor their capacity to induce T-cell proliferation. Thus, radiation exposure appears to differentially modulate paracrine signalling from activated macrophages (see also section III.F.3).

348. Radiation exposure can also elicit paracrine signalling from non-immune cells. The effects of low and moderate dose radiation on the expression of inflammatory factors by skin cells for example, in EpiDermFT skin explants exposed to 0.03, 0.1 or 2 Gy X-rays, were analysed ex vivo one and two days post-irradiation [V5]. The modulation of the secretion of 23 cytokines was monitored. A small but significant induction (1.4-fold) and reduction (0.9-fold) of the secretion of IFN-γ and CCL3, respectively, was observed 48 hours after an exposure of 0.03 Gy. Exposure to 0.1 Gy results only in an induction (3.3 times) of IL-2 two days after irradiation, while 2 Gy exposure induces the up-regulation of several cytokines (IL-2, IL-10, IL-13, IFN-γ, CCL3, TNF-α) one and/or two days post-exposure. The secretion of a limited number of cytokines (IL-1α, IL-6, IL-8, IL-10, TGF-β) was also compared in EpiDermFT skin explants exposed to intermediate and high doses of X-rays or ^{12}C ion [S46]. X-ray exposure (2 Gy) increased moderately the production of IL-6 and IL-8 1 and 2 days after irradiation, but exposure to 0.5 or 10 Gy had no effects. ^{12}C ions irradiation induced a small increase in IL-10 and IL-8 secretion 2 days after 0.5 and 2 Gy, respectively. Thus, low- and moderate-dose exposure induces only subtle changes in inflammatory cytokine secretion in reconstituted skin explants. The profile of secretion evolves with time, dose and the quality of radiation.

(g) *Summary and conclusions on immune system*

349. Radiation was found to affect several aspects of the immune system. It is, however, difficult to draw any general conclusion regarding the effects of low-dose/low-dose-rate exposure, as different experimental systems were used, and different endpoints were analysed among the various reported studies. The eventual interrelationship between low-dose radiation and the immune system in cancer development is illustrated by the observed association of variant alleles of immune genes with breast cancer in a cohort of radiological technologists.

350. For human exposure studies, some of the late effects of radiation exposure in atomic bombing survivors suggest that radiation can accelerate discrete aspects of aging of the immune system sometimes in a dose-dependent manner. However, immune functions (e.g. vaccination response) appear normal in aging survivors. Other human exposure studies (occupational, environmental, accidental) failed to produce any consistent results, probably because of differences in exposure scenarios.

351. Studies analysing the effects of low-dose/low-dose-rate exposure in vivo and on purified cells illustrate the diversity of responses (increase, decrease, no response) obtained in different experimental systems. The effects of low-dose radiation exposure are different according to the initial inflammatory status. One study reported that internal contamination in early life did not impair the response to influenza 24 weeks later.

352. In conclusion, low-dose/low-dose-rate radiation exposure may affect the homeostasis and/or modulate the activation of the immune cells, but it remains unclear whether these effects are activating or inhibiting the immune system; additionally, the precise mechanisms responsible and the short- and long-term consequences remain elusive, especially in relation to cancer development. The resilience of the immune system may, however, prevail in the long-term.

2. In vivo effects on gene inversion and other genetic/cellular endpoints

353. In order to evaluate the potential of low-dose radiation to cause DNA modifications, this area of research often employs transgenic animals in which mutation induction can be studied in a simplified way. For example, when a bacterial gene is incorporated into mouse genome – mutations in this foreign

DNA are irrelevant for animal health while they permit easy means for monitoring mutations. The *Escherishia coli* gene *lacZ* has been used for this purpose in rodents. In *pKZ1* mice *lacZ* is incorporated in inverse orientation adjacent to the chicken beta actin promoter; provided that an inversion occurs *lacZ* expression leads to production of blue beta galactosidase in the presence of X-gal substrate. Work with *pKZ1* mice in Japan has been extensive, not the least because this strain was originally developed in Japan [M24]. This type of transgenic mouse model was used, among others, for studies at the Institute for Environmental Sciences [B53].

354. A colony of *pKZ1*$^{Tg/+}$ heterozygotes maintained by backcrosses with C57BL/6J mice was used in extensive work published by the Sykes laboratory [D6, D7, D8, D9, O17, S71, S72, S73, Z5]; interestingly, different tissues were found to have similar degrees of chromosomal inversions. In prostates of mice exposed to a range of doses (0.001, 0.005, 0.01, 0.02, 1, 10 and 1,000 mGy; delivered at dose rates 0.0014, 0.0017, 0.34, 13.9 or 180 mGy/min) inversion frequencies scored in tissues obtained three days post-exposure increased (at 0.005 and 0.01 mGy), decreased (at 1 and 10 mGy) and then increased again (at 1,000 mGy). These differences were found to be statistically significant [Z5]. The same male animals were also explored in a different study by the same group [H35]. Dose-associated changes in frequencies of *lacZ* inversions in spleen documented in that study were similar (once again "bimodal") with findings documented in prostate. Authors of this work have proposed the following explanation for the bimodal data. They propose that a low fidelity, error-prone DNA repair preventing apoptosis functions in the ultra-low-dose region below 0.01 mGy (the NEOTRANS model); on the other hand, maximal protection from mutation fixation was provided by p53-independent, high fidelity DNA repair coupled with apoptosis (proposed "PAM" or p53-independent protective apoptosis-mediated process) in the low-dose region around 1–10 mGy.

355. The same group of researchers again used *pKZ1*$^{Tg/+}$ heterozygotes to investigate *lacZ* inversions three days post-exposure in prostates of mice exposed to an adapting dose of 0.001, 0.01 mGy (dose rates 0.0014 mGy/min), 1 mGy (dose rate 0.34 mGy/min) or 10 mGy (dose rate 13.9 mGy/min) followed four hours later by a 1,000 mGy (dose rate 180 mGy/min) challenge dose of X-rays. All low-dose exposures protected equally well from 1 Gy challenge dose suggesting that there is no threshold in the range of priming doses used in this study, for protective X-ray exposure [D6]. When challenge doses were altered to include 4,000 mGy, no increase in *lacZ* inversions was found unless two 4,000 mGy doses were given in sequence. At the same time, two 1,000 mGy doses in sequence led to fewer inversions than a single 1,000 mGy dose [D8]. Additional studies with very low doses of radiation and their potential protective effects were also extended to exploration of whether low doses can be effective if delivered after challenge doses. When 0.01 or 1 mGy doses were delivered four hours after 1,000 mGy dose, total mutations levels were lower than in controls, while 1 mGy dose on its own (or followed by 0.01 mGy dose) decreased numbers of inversions compared to controls [D8]. It is important to note, however, that post-irradiation period variation modulated registration of radiation-induced inversions [O17]; inversion frequencies differences were the most pronounced when analyses are carried out at one or three days but not seven days after exposure.

356. Among recent reviews that compared different strains and genotypes of animals, especially valuable is work by Shimura and Kojima [S34]. The focus of this review is to pinpoint "lowest doses" with recorded effects. This review paper considering research over past 20 years covering exposure of humans and animals, focusing on functional endpoints (e.g. fetal abnormalities) as well as cellular events, from micronuclei formation to nucleoplasmic bridges and, using chromosomal inversions in *pKZ1* mice. No universal conclusion could be drawn because different studies used different strains of animals and endpoints; again, animals with genetic changes were the ones to show the most intense effects (e.g. chromosomal aberrations in SCID mice after 50 mGy). Otherwise, the lowest doses with recorded effects in wild-type animals were exposures to 100 mGy of gamma rays or 9 mGy of fast neutrons from a ^{241}Am-Be neutron source. Another well researched review focused on animal research was published by Tang

et al. [T9]. In this work the authors emphasize both beneficial and harmful effects found in numerous in vivo studies.

357. Eight-week-old male and female *pKZ1*$^{Tg/+}$ heterozygote mice were given either regular or tritium containing water at activity levels of 10 kBq/L, 1 MBq/L or 20 MBq/L HTO over a period of 28 or 224 days; these three exposures correspond to total doses in the range 0.01–180 mGy [B12]. External beam radiation with dose rates matching two highest HTO exposures was also used in this study. No changes in either spleen weight, cell numbers or recombination of beta galactosidase were found in any of the treatment groups.

358. Wild-type mice with differences in radiosensitivity were used in a study focused on bone marrow cells [Z24]. C57BL/6 and CBA/Ca mice, susceptible or resistant, respectively, to myeloid leukaemia and radiation-induced chromosomal instability were exposed to X-rays at 8 to 12 weeks of age. A dose rate of 1 mGy/s was used to deliver total doses up to 100 mGy, for higher total doses (500, 1,000 or 3,000 mGy), a dose rate of 7.5 mGy/s was used. Bone marrow cells were inspected for expression of p21, p53, Casp-3 or TUNEL assay staining. Positive p53 and p21 cells were noted at doses of 50 or 100 mGy in C57BL/6 or CBA/Ca mice, respectively, while caspase and TUNEL became detectable only at doses 500 mGy or greater, regardless of genetic background.

359. Use of cells isolated from living organisms for temporary maintenance in cell culture is frequent in radiation research. Quite often these cells are peripheral blood mononuclear cells and leukocytes. While most often such studies irradiate cells after they have been isolated, other approaches are sometimes used too. An interesting example of such work is the study by Nafee et al. [N1] who isolated peripheral blood mononuclear cells and leukocytes from three-month-old female Wistar rats after they have been exposed to 9 mGy of fast neutrons (^{241}Am-Be neutron source, dose rate 0.2 mGy/h). Comet assays were used to evaluate DNA damage and this study contends that nearly complete DNA repair occurred within three hours post-irradiation.

360. Less frequently used for preparation of single-cell isolates are muscle cells. Nevertheless, an example relevant for low-dose work comes from Masuda et al. [M21]. In this work, 12 week-old C57BL/6 male mice were gamma irradiated with dose rates of 0, 2, 10, 50 and 250 mGy/d over a period of 30 days, resulting in cumulative doses of 60, 300, 1,500 and 7,500 mGy. Animals were sacrificed immediately or three months after being irradiated, and satellite cells (muscle stem cells) were isolated from muscles, stained by anti-Pax7 antibody and 4′,6-diamidino-2-phenylindole and counted. Exposures to 60 and 300 mGy did not reduce cell numbers immediately after radiation, but there was a small though statistically significant decrease in cell numbers for all doses used when animals were sacrificed at three months after irradiation.

361. Spleen cells isolated from irradiated animals were used in a study published by Koturbash et al. [K40] designed to explore DNA damage caused by fractionated low doses of radiation [K40]. Wild-type C57BL/6 male mice were whole body irradiated on day 60 of age with 100 mGy of X-rays at dose rate of 50 mGy/s. At 6 or 24 hours post-exposure the first group of mice was sacrificed and spleens extracted. For additional groups of mice, irradiation was repeated each 24 hours, as well as the pattern of animal harvest until experimental day 6. At the end of experiment, spleens of mice exposed to 100, 200, 300, 400 or 500 mGy and sacrificed 6 or 24 hours post-exposure were collected. DNA strand breaks were generally greater at 6 compared to 24 hours, and total DNA damage was increased in 400 and 500 mGy exposed mice compared to the lower dose counterparts, except in 100 mGy exposed mice at 6 hours. Similarly, apoptosis was greatest in 400 and 500 mGy exposed animals, both at 6 and 24 hours; for other doses, 24-hour group animals had more apoptotic cells. A dose-dependent increase in p38 protein was found, while spleens from 200 mGy exposed mice had the most BCL2^{+} cells. Expression of Ki67 and PCNA followed BCL2 as well. Phospho-ATM was increased only in 100 mGy exposed mice (both

timepoints) while p53 was decreased compared to control. Authors concluded that the repair occurring in these animals was p53- and ATM-independent, which is in keeping with the concept of "PAM" (p53-independent protective apoptosis-mediated process) for the low-dose region, postulated by the Sykes laboratory, albeit for *pKZ1* mice and doses around 1–10 mGy.

362. Healthy wild-type mice were also used in a study that looked at the fate of bone marrow cells ex vivo after moderate-dose radiation exposure of live mice [L30]. Four-month-old female mice of the strain BALB/cBYJ were used for this study. X-ray irradiations were carried out with doses of 170, 500 or 1,000 mGy at a dose rate of 180 mGy/min. Animals were sacrificed at days 3 or 21 post-exposure. Bone microarchitecture was affected by exposure to radiation differently when different endpoints were considered. The fate of isolated bone marrow cells was explored, and the following conclusions were made: differentiation into adipocytes and growth of adipocyte colonies was increased only by exposure to 1,000 mGy in cells isolated after 21 days. On the other hand, osteoblastogenesis was increased on day 3 in cells from mice exposed to 170 mGy, and in cells on day 7 after exposure to 1,000 mGy.

363. Partial body irradiation studies were focus of several interesting recent studies. For example, Yamada et al. did thoracic irradiations of female Wistar rats irradiated at 1, 5 or 15 weeks of age. Dose rates about 500 mGy/min were used and total doses were 1, 3 or 5 Gy [Y1]. No life shortening was found for any of the doses when irradiated animals were 1-week old. In addition, total thoracic dose of 1 Gy did not cause life shortening in any of the rats. However, cancer types found at necropsy were different in irradiated mice compared with controls. Pituitary cancer was the most frequent cancer type in non-exposed rats, but not in exposed rats. The frequency of lung cancer was at least doubled with statistical significance in all irradiated animal groups compared to controls, while mammary cancers increased only in 3 and 5 Gy groups. While it is difficult to compare moderate-dose partial body exposures with low-dose exposures, this type of research (i.e. in vivo studies with animals allowed to live out their lifespan and exposed to radiation at different ages) informs us that it may be necessary to broaden the spectrum of questions that need to be considered when discussing radiation-induced cancer.

364. Exposure to radioactive particles by inhalation can be considered as special case of partial body irradiation. Extensive work of this type was carried out with beagle dogs in the United States between 1950s and 1990s; in many cases, these studies were concluded decades after final exposures. An example of such a study is work by Park et al. [P4]. In this study, groups of 8 to 24 dogs inhaled 0.14, 0.63, 3.2, 13, 44 or 210 kBq of weapons-grade $^{239}PuO_2$ aerosol (particle sizes of 2.3 μm) and were kept and monitored throughout their lifespan. Among the dogs (20 per dose, 10 per sex) exposed to the two lowest doses (0.012 or 0.059 kBq/kg), median life expectancy was not reduced compared to controls while lung cancer incidence was lower than in controls. At the same time, only incidences of bone cancer were found in animals exposed to 0.012 or 0.059 kBq/kg and the same two groups of animals included some that died of bacterial pneumonia. Nevertheless, it should also be mentioned that in depth consideration of these and some additional data led another group of researchers to conclude that genetic inbreeding is an important factor in proper analyses of dog data [W11]. Work by Wilson et al. suggested that use of Wright's coefficient of inbreeding as an explanatory variable led to improved model fitting with lung fibrosis data in beagle dogs that had inhaled ^{238}Pu and ^{239}Pu.

3. Effects on gene and protein expression in experimental animals

365. A recent study investigating proteomics changes in liver compared acutely exposed C57BL/6J male mice given 4 Gy of X-rays at 10 weeks of age and animals exposed to 4 or 8 Gy at 24 weeks of age, with chronically exposed mice [N6]. Chronic exposures were carried out with C57BL/6J male mice exposed to dose rate of 1 mGy/d, total dose of 400 mGy, or a dose rate of 20 mGy per day, total dose of 8,000 mGy.

These mice were sacrificed immediately or three months after chronic exposure, while acutely exposed mice (8 Gy) were sacrificed six days or three months later. Protein expression changes in irradiated mice were generally sustained over three months post-exposure. Laminin-2, DRAK1, BID, Bim, Bmf had persistently altered expression in chronically exposed mice, while MyD88 and Bcl-xL had altered expression immediately after acute exposure to 8 Gy and three months after acute exposure to 4 Gy.

366. In a similar study, liver proteins were measured in C57BL/6J chronically exposed to low-dose radiation [N5]. Twenty-two hours a day irradiation for 485 days was carried out with ^{137}Cs gamma irradiator at dose rates of 0.032, 0.65 and 13 μGy/min. This led to total accumulated doses of 21,420 and 8,000 mGy. After 2D-electrophoresis and protein identification, protein rhodanese expressed in mitochondria was found to be significantly overexpressed in 420 mGy animals.

367. Liver mRNA expression in seven weeks old brown rats (*Ratus norvegicus*) was also investigated after exposure to low-dose radiation [N14]. Irradiations with gamma rays were carried out with 140 mGy/d for two weeks and a total dose of 2,000 mGy. Unfortunately, the time between irradiation and animal sacrifice was not specified in this work. Up-regulated genes confirmed by RT-PCR included *Btg2*, *Gadd45*, *Jun*, *Plk2*, *Agpat3* and *9*, and *Mir-27b*.

368. The liver proteome has been a primary focus of the Ding laboratory [Y7, Y8, Y9]. In order to evaluate different potential environmental exposures, a "local" source of gamma rays was used in the form of "uranium tailings". The material was accumulated in quantities needed to deliver dose rates of 30, 100 and 250 μGy/h for 22 hours each day for 500 days [Y9]. In this study, glycine N-methyltransferase and nucleophosmin were both up-regulated at 250 μGy/h, while glutathione S-transferase was down-regulated in all irradiated groups. A shorter-term experiment was carried out with, once again, male six to seven-week-old C57BL/6J mice. Gamma rays were delivered by a ^{137}Cs source at dose rates of <50, 50–500 and 500–1,000 μGy/h for 22 hours a day for 90 days and then sacrificed. Liver tissue was used for 2D-electrophoresis followed by mass spectrometry and Western blots for several selected proteins, as well as real-time PCR. Genes responding to radiation in this work include catalase (mRNA increase beginning with <50 μGy/h group), glycine N-methyltransferase (increased from 50–500 μGy/h group) and glutathione S-transferase P1 (increased in 500–1,000 μGy/h group). The same group focused especially on calreticulin [Y8] in a study using six-week-old C57BL/6J male mice. Radiation doses were doubled, but dose rates kept the same as in previous experiment; the most interesting proteins from this screen were catalase, glutathione S-transferase P1 and calreticulin. Other than catalase and glutathione S-transferase P1 and a few other not emphasized proteins such as, e.g. serum albumin, relatively few proteins overlapped between livers of mice irradiated over 90 or 180 days.

369. Recently, Bakshi et al. [B9] investigated effects of in utero irradiation on heart proteome in mice. Inbred C57Bl/6J animals, mated during a two-hour time period to ensure synchronous embryonic development, were whole body irradiated while pregnant at embryonic day 11. Total doses of 0.2, 0.5, 1 or 10 Gy were delivered as X-rays at a dose rate of 350 mGy/min. Male offspring were sacrificed at six months and female offspring at two years of age; two dose groups shared 13 or 14 proteins in common as deregulated (this included both up- and down-regulated proteins). Only a few proteins were deregulated in common when six-month-old male and two-year-old female animals were compared; e.g. Peroxiredoxin 5 and MACRO domain-containing 1 ribose deacetylase are such examples. It would be interesting to explore the causes for such low overlap, e.g. age or sex of animals. In previous work, male outbred NMRI mice were exposed to radiation on postnatal day 10 using 20, 100, 500 or 1,000 mGy doses of gamma rays produced by a ^{137}Cs source (dose rate 0.023 mGy/min). Heart proteome changes were analysed seven months after irradiation [B7]. Nine proteins were affected by all used doses of radiation; several of them were commonly regulated by PPAR alpha. Amounts of phospho-PPAR alpha were decreased in all irradiated samples. Subsequent exploration of liver proteome by the same group gave similar results [B8]. Again – 10-day-old neonate male NMRI mice were exposed to 20, 50, 100 or

1,000 mGy and proteomic liver analyses carried out with samples collected at day one or seven months after exposure. About 40 proteins were found deregulated by 20 or 50 mGy exposures after either one day or seven months. In comparison, 60 or 70 proteins at day one or seven months were deregulated in mice exposed to 500 or 1,000 mGy. Four or five proteins were common to all exposures at different timepoints. Most importantly, PPAR alpha could again be found as regulator of commonly affected proteins. In liver, activity of this protein dropped at 100 mGy exposures.

370. Partial body (heart "only") irradiation of C57BL/6 mice with 200 mGy did not lead to significant changes in mitochondrial proteome at 40 weeks post-exposure [B19]. Only five proteins responsible for cytoskeleton desmin, actin, and three forms of myosin were found to be up-regulated.

371. Brain proteome and miRNA changes were also studied by the same group [K21]. Mice at post-natal day 10 were exposed to 100 or 500 mGy and their brains harvested 24 hours later, separated into cortex and hippocampus and analysed for expression of mitochondrial proteins. Findings include twofold changes for mitochondrial complex I proteins which were decreased at both doses in both parts of the brain, and transcription factor phospho-CREB (binding to cyclic AMP response elements) increased in cortex at both doses. Additional proteins relevant for synapses were modulated as well as several different miRNAs. In most cases, miRNAs were similarly regulated in response to radiation both in cortex and hippocampus. Most miRNA changes were found in cortex after exposure to 100 mGy.

372. In addition, the same groups collaborated to produce brain samples from female C57Bl/6 *ApoE*$^{-/-}$ mice [K22]. Eight-week-old mice were chronically irradiated with 1 or 20 mGy/d with ^{137}Cs gamma rays over a period of 300 days. Irradiations were carried out for 22 hours a day and cumulative doses were 300 or 6,000 mGy. Hippocampi from each hemisphere were processed separately as well as the two brain hemispheres. Almost none of the proteins had altered expression but their phosphorylation patterns were vastly different. The phosphorylation profile for neurofilament and eukaryotic translation initiation factor (Eif4b) showed up-regulation, but down-regulation in phosphorylation of G-protein coupled receptor 158 at both doses. Results of pathway analysis for these data were detailed in the study; for example, in mice chronically irradiated with 300 mGy pathways related to synaptic plasticity and altered in comparison to control were glutamate receptor signalling, Wnt/β catenin signalling, and tight junction signalling. Based on immunocytochemistry, no cell number changes were seen either for Ki67 or GFAP positive cells (proliferating and neural stem cells, or for mature neurons – NeuN^{+} cells, and Casp-3 pattern of expression was the same as in controls). Only immunocytochemical change detected was in increased density of post-synaptic density protein 95 and the microtubule-associated protein 2 (MAP2), but not phospho-MAP2, in dentate Gyrus. Finally, at 300 but not 6,000 mGy there was a decrease in Iba1^{+} microglia, which is a marker of neuroinflammation, and a reduction in TNF-α and lipid peroxidation quantified as total protein content that was modified with malondialdehyde.

373. Using the data and materials from study by Uehara et al. [U1] (dose rates of 20.0, 1.00 or 0.050 mGy/d for 401 days accumulating to total doses of 8, 0.4 and 0.02 Gy ^{137}Cs gamma ray, respectively), the same group of researchers explored the possibility to use complex data obtained from low-dose irradiation of mice to look for possible transcription factors [V4]. Sequences from 68 genes that were shown to be modulated by low-dose exposures and 93 genes that were not affected by the low-dose exposures were used for this study. The sequence span of 3 kb upstream and 1 kb downstream from the coding sequence were chosen and searched for transcription factor binding sites using TFSEARCH database. Potential binding sites were found for 154 transcription factors and their frequencies were calculated both in genes modulated or not affected by low-dose exposures. Binding sequences for glucocorticoid receptor, fork head domain factor *HFH-1*, *AP-1*, sterol regulatory element-binding protein 1 and olfactory neuron-specific factor, several early growth response genes, *NF-κB p65*, heat shock factor 1, POU factor *Brn-2*, ectopic viral integration site 1 encoded factor, among others, were found to be

associated with low-dose responding genes in liver of low-dose irradiated mice. Of these, only *AP-1* and NF-κB were known to be affected by low-dose radiation prior to this study.

374. Neuronal effects of moderate to high irradiation were the focus of work by Wei et al. [W8]. One-year-old Kunming mice (weighing 30–35 g) received 300 or 3,000 mGy of X-rays and were sacrificed for mRNA analysis 7 days later or immunohistochemistry 14 days later. Exposure to 300 mGy led to higher numbers of Wnt1, Wnt3a, Wnt5a, and β catenin expressing cells by immunohistochemistry as well as expression measured by Western blot and RT-PCR. The ratio of nestin/β catenin and BrdU/β catenin double positive cells was also the highest in 300 mGy exposed mice. These data support increased neural stem cell proliferation and neurogenesis at 300 mGy compared to controls, while opposite is true for 3,000 mGy exposures: Neurogenesis drops compared to control mice; also, these mice perform less well in behavioural studies.

375. Mice of NMRI (Naval Medical Research Institute) were exposed to whole body radiation from a ^{60}Co irradiator 10 days after birth. Doses included 350 or 500 mGy delivered at a dose rate of 20 mGy/min [B59]. The animals were sacrificed a day or six months after irradiation and changes in protein expression noted, especially in 500 mGy exposed animals. For example, tau protein was increased in the cerebral cortex and decreased in hippocampus one day after exposure, and while it increased to usual level in hippocampus by six months of age, tau expression remained increased in central cortex of six-month-old mice. Proteins CaMKII, GAP-43 and synaptophysin showed milder changes.

4. Modulation of different genetic effects by low-dose exposures

376. Mice heterozygous for $APC^{1638N/+}$ and wild-type female C57BL/6J mice were used in a study by Suman et al. [S68]. Animals irradiated with 250 mGy X-rays were either monitored for the development of intestinal cancer themselves or mated two days after exposure with other exposed or unexposed animals. Heterozygous mice from both-parents- and male-parent-irradiated groups had significantly more tumours than mice from a no-parents-irradiated cross. With regard to sex, male F1 heterozygotes had significantly higher tumour frequency in all experimental groups including animals that were irradiated.

377. Female mice of three different genotypes: SCID (C.B-17/Icr-SCID), C.B-17/Icr wild-type and F1 heterozygotes ($Prkdc^{+/-}$) were used in the experiment conducted by Ishii-Ohba et al. [I11]. Irradiations were carried out with gamma rays produced by ^{137}Cs; 1, 2, 3, 5 or 6.5 Gy doses were delivered at dose rate of 500 mGy/min; doses of 50, 100, 250 or 500 mGy were delivered at dose rate of 80 mGy/min. Finally, in order to induce thymic lymphoma in wild-type mice a dose of 1.6 Gy was delivered once a week for four weeks. In SCID mice the background incidence of lymphoma was about 38% and this number did not change with a 50 mGy exposure; for 100 mGy exposure, however, the incidence increased to 53% and the percentage continued to increase until it plateaued at a dose of 2 Gy. In wild-type mice or heterozygotes, the incidence of lymphoma did not increase compared to the incidence in non-irradiated mice except at 5 or 6.5 Gy doses. Solid tumours were far more frequent in these mice, but their frequency was not modulated dependent on the total dose received. In SCID mice non-lymphoma tumour frequencies also did not change with a change in dose. While lymphoma-free survival in SCID mice decreased for all doses above 50 mGy, it increased in wild-type mice exposed to 250 mGy.

378. Low and moderate doses of radiation were found to be protective on their own but in some cases, they also have strongly synergistic harmful effects in combination with chemical mutagens in an animal model of induced carcinogenesis. Bruce et al. used a mouse model for lung cancer induction – A/J strain in which lung tumours are chemically induced by benzo[a]pyrene and progression from hyperplastic foci to adenoma and carcinoma is easily quantified [B57]. Ten-week-old females were exposed to six fractions

of whole-body irradiation (gamma rays, ^{137}Cs irradiator) at dose rates of 1.07 or 1.33 mGy/s over three weeks for total doses of 60 and 600 mGy, respectively. Benzo[a]pyrene injection was carried out four weeks before first irradiation and all animals were sacrificed for lung evaluation at 46 weeks after chemical exposure. Low-dose irradiation alone protected these mice from the development of hyperplastic foci (about 33% incidence compared to 65% incidence in controls), however, while about 92% of mice exposed to benzo[a]pyrene had hyperplastic foci, combination of chemical exposure and 60 mGy exposure led to development of foci in all mice. Moreover, this dose of radiation and benzo[a]pyrene was associated with increased adenoma and carcinoma. Exposure to 600 mGy showed intermediate effects.

379. Exploration of experimental schemes where low-dose exposure is followed by high-dose has been mentioned already several times, sometimes low-dose pre-exposure to adapting doses was found to be protective. However, that is not the case when central nervous system is considered [A5]. Low-dose exposures of 100 mGy followed 24 hours later by high dose of 2,000 mGy (both delivered as gamma rays from a ^{137}Cs irradiator) were found to have synergistic effect on central nervous system of two months old C57BL/6 wild-type mice with respect to formation and maturation of neurons. Transgenic male mice with knockdown of *CCR2* were not affected in the same way.

380. Mota et al. [M42] used X-ray fluorescence microscopy to study elementalomics of peripheral blood and cardiac formalin fixed paraffin embedded tissue in low- and high-dose irradiated animals. Male Wistar rats were exposed to 0.1 or 2.5 Gy total body irradiation with X-rays (150 kV, 10mA and 17 mGy/s), and samples collected at 24 hours after exposure. Elemental decreases for K and Ca (indicating cell death but not very reliable with formalin fixed paraffin embedded) and S, Fe and Zn were noted at both doses.

5. Effects on vascularization, cell migration/invasion and epithelial-mesenchymal transformation

381. Several examples support the notion that moderate doses and possibly low doses of radiation support growth of new vasculature. That is one way in which low or moderate doses may contribute to carcinogenesis, facilitating the vascularization and therefore growth of tumours.

382. A study focused on angiogenesis as a factor critical for support of tumour growth used X-rays to deliver a dose of 300 mGy to NOD-SCID mice [S53]. Two in vivo endpoints were investigated with rodents – outgrowth of vasculature into Matrigel plugs implanted into animals 24 hours after exposure (each mouse serving as its own control, which was possible because of partial body irradiation), and the success rate for implantation and volume of tumour cells (MOLT-4 cells) injected into immunocompromised mice, again 24 hours post-exposure. Both the growth of new blood vessels on their own and tumour burden were increased in mice that were exposed to 300 mGy.

383. Diabetes was induced in male Wistar rats by a single intraperitoneal injection of streptozotocin and 60 days; after induction of diabetes, a skin wound was created in diabetic and control animals [G20]. Half of the animals were exposed to X-rays in fractions of 75 mGy five days a week for 1, 2 or 3 weeks, accumulating to 375, 600 or 825 mGy. Wound healing was most rapid in control animals, but in moderate dose exposed diabetic animals wound healing progression was better than in unexposed animals induced into a diabetic state by the injection of streptozotocin, with more PCNA (cell proliferation) and vascular endothelial growth factor receptor (VEGFR) (blood vessel growth stimulation) found by immunohistochemistry, more tissue remodelling enzyme activity (e.g. MMP-2 and MMP-9). Finally,

more $CD34^+$ cells and blood vessels and more $CD31^+CD34^+$ cells were found in circulation of moderate dose exposed diabetic rats compared to non-exposed diabetic animals.

384. Protection from reperfusion ischaemia injury by moderate doses of radiation was the primary question explored in work by Ministro et al. [M32]. In this study, 22-week-old female C57BL/6 mice were used as recipients of cells isolated from nine-week-old female C57BL/6-Tg(CAG-EGFP)10sb/J mice. Critical limb ischaemia was induced in mice by surgery and 12 hours later the mice were exposed to 300 mGy of X-rays each day for one to seven consecutive days – total doses of 300, 600, 900, 1,200 or 2,100 mGy. No benefits were found for doses below or above 1,200 mGy; however, this dose lead to significantly improved capillary density ($CD31^+$) especially between 15 and 45 days post-injury. This work also demonstrated that the radiation regimen increased numbers of circulating endothelial progenitor cells and subsequent collateral vessel development. RT-PCR for these cells showed increased expression of *Vegfr2*, *Vegfr1*, *Fgf2*, *Angpt2*, *Pdgfc*, *Tgfb2*, *Hgf*, and *Met* in mice exposed to radiation. When the study included bone marrow transplantation from GFP-expressing animals prior to injury and irradiation GFP^+CD31^+ cells were found to be increased (while only $CD31^+$ cells were increased in wild-type mice).

385. Male Sprague–Dawley rats were used to test effects of moderate to high-dose radiation on bone healing [C15]. After a bone fracture was introduced, animals were irradiated with 500 or 5,000 mGy X-rays or left as controls. Cell viability and proliferation were greatest in rats exposed to 500 mGy; moreover, higher gene expression associated with osteoblast differentiation was also documented in this group at later timepoints. Regenerative capacity may also rely on better growth of new vasculature.

386. Recent work by Marques et al. [M18] used human endothelial cells (HUVEC) in the in vitro wound healing assay and blood vessel growth assay in chicken embryo in the presence of conditioned media from irradiated and control pre-adipocyte or adipocyte cell cultures. Conditioned media from mouse 3T3-L1 pre-adipocytes exposed to 0.1 or 0.3 Gy X-rays (Clinac, 300 MU/min dose rate) produced the most effects in support of faster wound healing or more extensive vasculature growth. This effect was less pronounced when irradiated mature adipocytes were used. Thus, this work may provide mechanistic explanation that connects low and moderate dose-induced obesity in mice (e.g. [B53, T4]) with improved neoangiogenesis described in this section. Production of VEGF-A and MMP1 and 2 by preadipocytes could at the same time aid wound healing and yet lead to cancer progression.

387. The later stages of tumour progression are characterized by features such as increased cell migration and invasiveness, and transformation of epithelial cell morphology to a more fibroblastic form, known as the EMT. Each of these features relate to the increased metastatic potential of solid cancers. Unlike the studies described above, much work on invasion, migration and EMT utilizes in vitro cell models. This work is included in this chapter as it relates to cancer progression in vivo. Relevant literature is summarized in table 6.

388. Relatively few studies consider the effects of low or moderate doses of low-LET radiations. Zhang et al. [Z15] report that X-irradiation at moderate-high doses promotes EMT, cell migration and cell invasiveness. Ghosh et al. [G5] found that 100–200 mGy ^{60}Co gamma irradiation enhanced the migration and invasiveness of four cancer cell lines but not primary cells, 50 mGy exposures are reported to have no effect. The previously cited work of Sofia Vala et al. [S53] indicated that moderate dose (0.5–0.8 Gy) photon irradiation enhanced wound healing. By contrast, 100 mGy or 10 fractions of 10 mGy ^{137}Cs irradiation are reported to reduce migration and invasion of human breast cancer cell lines [K19]. One study suggests that 0.5 Gy X-irradiation increases cell migration and invasion [O9], interpreted to suggest that conventional radiotherapy may enhance metastatic spread of remaining tumour cells. The impact of low-dose irradiation on these endpoints relating to metastatic spread are therefore inconclusive at present.

389. Several studies relating to the use of protons and heavy ions for radiotherapy have reported effects of particle irradiation on cell migration and invasion. The effects of the irradiation are generally reported to reduce cell migration and invasion [F24, H3, O9, O10]. In these studies, the effects are interpreted to indicate that proton and heavy ion therapies provide a therapeutic advantage by not only direct killing of tumour cells but also through reducing metastatic spread. The pro-migratory effects of high dose low-LET radiations may be mediated by the release of exosomes with cargos rich in pro-migratory factors [A20, M47]. By contrast, the effects of heavy ion and proton irradiation on EMT are reported to be positive [W4, W5]. That is the irradiation causes or enhances EMT, which is a feature that is considered to enhance metastatic spread of solid cancers.

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Table 6. Endpoints relating to cell migration, invasiveness and epithelial-to-mesenchymal transition

Species/cell type	*Irradiation conditions*	*Effect reported at low doses/dose-rate*	*Other reported effects*	*Reference*
Pancreatic carcinoma cell line, PANC-1	6 MeV photons Dose: 2–6 Gy Dose rate: 4.18 Gy/min		2 and 4 Gy reduced migration and invasion, especially in presence of plant extract	[H3]
Human fibrosarcoma cell line HT1080, mouse osteosarcoma cell line LM8	190 MeV/nucleon protons Dose: 4–8 Gy 290 MeV/nucleon C ions Dose: 4–8 Gy 4 MeV LINAC (X-radiation) Dose: 0.5 Gy Dose rate: 1.8 Gy/min		All exposures increased adhesion to extracellular matrix proteins; migration and invasion decreased by protons and C ions, increased by X-rays; C ions reduced number of metastases in transplant model	[O9]
Human lung adenoma cell line A549	190 MeV/nucleon protons Dose: 4–8 Gy 290 MeV/nucleon C ions Dose: 4–8 Gy 4 MeV LINAC (X-radiation) Dose: 0.5 Gy Dose rate: 1.8 Gy/min		Protons reported to reduce invasion and migration	[O10]
Human pancreatic cancer cell lines	290 MeV/nucleon C ions, 80 keV/mm Dose: 0.5–4 Gy		C ions reduce migration and invasion in all but one cell line	[F24]
Aleutian mink lung cell line, Mv/Lu; hTERT immortalized oesophageal cells	170 MeV/nucleon Si ions, 99 keV/mm; 600 MeV/nucleon Fe ions, 180 keV/mm. Dose: 0.1–2 Gy Dose rate: 0.25–1 Gy/min	Si and Fe ions cause mild EMT, even at 0.1 Gy		[W4]
Aleutian mink lung cell line, Mv/Lu; hTERT immortalized oesophageal cells	100 MeV protons, mean energy 5 MeV and 10 MeV/mm. Dose: 0.1–4 Gy Dose rate: 0.25–1 Gy/min	0.1 Gy and higher doses enhance TGFβ induced EMT; mild induction of EMT in absence of TGFβ		[W5]
Human tumour cell lines U87, BMG-1, A549, HeLa	Gamma radiation (^{60}Co) Dose: 50–200 mGy Dose rate: 1.034 Gy/min	100–200 mGy increased connexin 43 expression and increased glioma cell line migration. No effect of 50 mGy. No observed effects in primary cancer cell lines	Connexin expression effect reported to be mediated by sustained activation of p38 and up-regulation of p21	[G5]

Species/cell type	*Irradiation conditions*	*Effect reported at low doses/dose-rate*	*Other reported effects*	*Reference*
Human breast carcinoma cell line MCF-7	X-radiation Dose rate: 0.4 Gy/min or 20 Gy/day (13.9 mGy/min)		EMT-like changes and increased migration and invasion	[Z15]
Human lung endothelial cells (non-transformed)	LINAC (Varian Clinac 2100 CD) photons Dose rate : 300 MU/min		0.5–0.8 Gy dose increased wound healing – a surrogate for migration	[S53]
Human breast cancer cell lines MCF-7, MDA-MB231	Gamma radiation (^{137}Cs) Dose: 10 fractions of 0.01 Gy Dose rate: 0.1 Gy/min	100 mGy or 10×0.01 mGy reduced migration and invasion, no evidence of EMT	Reported effect mediated by inhibition of JAK1/STAT3 signalling	[K19]
Human glioblastoma cell lines and primary astrocytes	X-radiation Dose: 2–8 Gy Dose rate: 2.3 Gy/min		Increased release of exosomes carrying cell migration related factors that enhance migration in recipient cells	[A20]
Human head and neck squamous cell carcinoma cell lines	Gamma radiation (^{137}Cs) Dose: 3–9 Gy Dose rate: 0.45 Gy/min		Exosomes derived from irradiated head and neck squamous cell carcinoma cells conferred a migratory phenotype to recipient cancer cells, linked to radiation-regulated exosomal proteins that increase AKT-signalling	[M47]

6. Carcinogenesis

390. An extensive study using Sprague–Dawley rats included irradiations of 4,016 animals of both sexes in order to evaluate the health of their offspring. Irradiations were both acute and fractionated (10 doses once every 4 weeks) at three dose levels (0.1, 1 and 3 Gy). Pregnant dams irradiated with 0.1, 0.5 and 1 Gy at day 12 of pregnancy produced 2,799 offspring, while male breeders exposed acutely to 0.1, 1 and 3 Gy before mating produced 2,557 offspring. Finally, dams fed on day 12 of pregnancy with standard feed irradiated by 1,000 or 4,000 Gy produced 1,139 offspring that were fed the same feed until spontaneous death. Animals exposed to external beam were irradiated at six weeks of age with ^{60}Co gamma rays to total doses of 100, 1,000 or 3,000 mGy. While both lethal tumour and any tumour incidence increased in rats exposed to 1,000 or 3,000 mGy, no significant increase was found in the group exposed to 100 mGy [S52]. Life shortening occurred in both high dose groups and was the greatest in females exposed to 3,000 mGy. The only biological endpoint in this study modulated by 100 mGy exposure was development of atypical hyperplasia in mammary glands.

391. An interesting approach to study carcinogenesis came from work by Sasaki and Fukuda [S10]. In this work, B3C3F1 mice (cross between C57BL/6JNrs and C3H/HeNrs) were exposed to 100, 480 or 950 mGy of ^{137}Cs gamma rays at seven days of age. In addition to usual comparisons such as life shortening (excess relative risk increased by 0.102, 0.697 and 1.206 for the three doses), other evaluations were carried out: numbers of tumours per mouse were counted as well as numbers of tumours in groups of mice dying within a certain age window. This evaluation provided new insights into health status of animals exposed to low doses of radiation. For example, even though no significant change in life expectancy was seen in mice exposed to 100 mGy compared to controls, their causes of death were different. In animals that reached age of 950 days, mice in the control group were dying from non-tumour causes, while tumours accounted for most of the deaths of younger animals. On the other hand, 950 days old mice that received 100 mGy continued to die predominantly of cancer (similar to younger animals). Moreover, four distinct tumour types could be found in this group of mice but not in the controls. In terms of specific tumour types, ovarian and lung cancers had greater frequency in mice exposed to 100 mGy compared to controls (probably at the expense of non-tumour diseases).

392. An extensive review of years of work carried out at the low-dose-rate irradiation facility in Japan's Institute for Environmental Sciences was published recently [B53]. This review paper succinctly informs about studies in mice of several different genotypes (B6C3F1, C3H, C57BL/6 and transgenic (gpt delta×SWR) F1), irradiated 22 hours a day by a ^{137}Cs source over a range of dose rates from 0.04–0.06 mGy/d, 0.8–1.1 mGy/d and 16–21 mGy/d. Total irradiation doses after exposure periods of 400 to 485 days were either 20–21, 400–420 or 8,000–8,050 mGy. Ten different endpoints – lifespan, neoplasm incidence, body weight, tumour cell transplantability, changes in chromosome structure, gene mutations, changes in mRNA and protein levels, and transgenerational effects on lifespan and neoplasm incidence were all studied in these mice, in both sexes. Statistical analyses considered $P<0.05$ as significant for non-cancer endpoints and $P<0.01$ for neoplasia. While most findings from this review are covered in specific references throughout this annex, this review is very valuable as a resource with the most succinct information provided by the researchers themselves. It should also be noted that a not yet published study is mentioned, where work was carried out with C57BL/6J mice (groups of 215–278 mice per sex per dose). Importantly, only male mice exposed to 20 mGy/d with total dose of 8,000 mGy demonstrated life shortening. Males exposed to the total accumulated dose of 20 or 400 mGy did not show life shortening. By comparison (based on [T3]), in B6C3F1 strain animals both sexes had shorter lifespans in groups exposed to 20 mGy/d with total dose of 8,000 mGy, while females showed life shortening at the dose rate of 1 mGy/d with total dose of 400 mGy.

393. Yamauchi et al. [Y2] used 60 animals per group of 6-week-old male B6C3F1/Jcl mice, put onto a calorie-restriction diet and exposed to chronic ^{137}Cs gamma ray radiation for 400 days at 20 mGy/d,

resulting in a total dose of 8 Gy. Life expectancy of low calorie animals was greatest, followed by low calorie irradiated mice which survived longer than non-irradiated, standard calorie diet mice. Life shortening by radiation in regular diet mice was not significant.

394. Lawrence et al. [L7] investigated effects of radiation in a mouse model prone to development of prostate cancer – transgenic adenocarcinoma of the mouse prostate (*TRAMP*). Six weeks old *TRAMP* FVB F1 mice were exposed to a single high dose of 2 Gy (1 Gy/min X-irradiation), a single low dose of 50 mGy (13.9 mGy/min X-rays) or five weekly exposures of 50 mGy X-rays. There was no significant difference in the time to palpable tumour between sham-irradiated animals and mice exposed to 2 Gy radiation. With regard to low to moderate doses of radiation (either 50 or 5×50 mGy) no changes with respect to control were found in either apoptosis frequency or any other endpoint examined in this study (e.g. Ki67 staining, histopathological status of the prostate).

395. Development of lymphoma in mice with thymocyte-specific Bax overexpression was focus of the study published by Jacobus et al. [J6]. Three different genetically modified mice were used in this study: *(a)* C57BL/6 *Lck-Bax* transgenic mice have an insertion of additional copies of the mitochondrial-localized BAX gene under the control of the lymphocyte-specific protein tyrosine kinase promoter (Lck) and 50% of mice develop lymphomas in the absence of radiation by 40 weeks of age if male and by 70 weeks of age if female; *(b) Lck-Bax38&1* mice additionally have increased levels of superoxide in thymocytes which still hastens development of lymphoma: 50% of non-irradiated mice develop it by 25–30 weeks of age; in this genotype, no sex differences in development of lymphoma were noted; *(c) Lck-Bax38&1* mice were also crossed with *SIRT3* knockout mice to produce double mutants for the purpose of this study – oxidative damage in these mice is still more pronounced and MnSOD activity decreased; as a consequence mice that are a cross of *Lck-Bax38&1* and *SIRT3* knockout die even earlier than the *Lck-Bax38&1* mice and develop lymphoma at a rapid rate of increase. Irradiation with 100 or 1,000 mGy gamma rays was provided by ^{137}Cs irradiator at a dose rate of 350 mGy/min; in addition, some mice were exposed to 100 or 1,000 mGy of Si- or Fe ions, administered over a period of approximately one minute. In *Lck-Bax1* mice, exposure to either dose significantly shortened latency of lymphoma in female mice, while in male mice no significant differences between irradiated and sham-irradiated animals were found. In *Lck-Bax38&1* mice, no significant changes in latency were caused by exposure to either dose; with regard to sex, exposure to 100 mGy lead to a nearly statistically significant separation where females had shorter latency than males. Finally, in *Lck-Bax38&1* mice no significant change in latency were caused by exposure to either dose of Si ions and no sex differences were detected. Exposure to Fe ions, however, led to a dose-dependent shortening of disease latency; sexes were separated in this study with female mice developing lymphoma sooner than males when exposed to 1,000 mGy of Fe ions. It should be noted that in this study animal ages varied between four and nine weeks and this may be part of the reason for some of the data variability; in other words, that data could perhaps have been more meaningful were mice more exactly age-matched.

396. In addition, a recent study using p53 heterozygotes found that a single X-ray exposure of 10 mGy significantly delayed onset of cancer in irradiated compared to non-irradiated $p53^{+/-}$ animals [L18]. Moreover, multiple exposure to similar doses to those received from a CT scan (i.e. 10 exposures of 10 mGy, one per week) after a single high exposure (4 Gy) increased the overall lifespan of the 4 Gy exposed mice, by approximately 8% (P=0.017) [L19].

397. Considering dependence of humans on other living organisms, and the fact that, for example, intestinal flora plays a significant role in resilience to cancer (e.g. [K55]) it may be postulated that some of the complex effects of radiation of human surroundings may have indirect, yet real effects on cancer induction. Several studies exploring high doses of radiation show this point. Goudarzi et al. [G8] investigated response of C57BL/6J 8-week-old male mice to high doses of radiation (5 and 12 Gy X-rays) through alterations of mouse metabolome dependent on intestinal microbiome. Collective gut

microbiome showed pronounced responses to radiation exposure, persisting in some cases for many days. In a study focused on high-LET radiation exposures and gut microbiome [C6] C57BL/6J mice were fed a consistent diet for five months, and then exposed at six months of age to ^{16}O (600 MeV/n) as a heavy ion. Delivery of doses of 100 and 250 mGy was found to alter the mouse gut microbiome extensively, leading to functional metabolic changes (for example, benzaldehydes were increased in mice exposed to 100 and 250 mGy both at 10 and at 30 days post-exposure). Equivalent studies in animals exposed to low doses of low-LET radiation are still not available. It is considered that any changes in microbiome would also affect changes in animals' cancer risk because of the metabolome changes that can be triggered.

(a) *Low dose effects on transplanted tumours*

398. A study focused on tumour cell transplantability [I13] used eight- to nine-week-old female BALB/c and C3H/HeN mice irradiated with 50, 100, 200, 500 or 1,500 mGy X-rays at a dose rate of 40 mGy/min. Tumour cells used for transplantation were mammary sarcoma cell line EMT6 isogenic for BALB/c mice and SCCVII squamous cell carcinoma isogenic for C3H/HeN mice; both hind limbs were injected either with 1,000 or 100 cells six hours after radiation exposure and tumours were allowed to grow for 50 days. In both cases, only 1,500 mGy dose increased transplantability. In C3H mice, there was no difference for sham-irradiated mice and those exposed to doses of 500 mGy and below. In BALB/c mice animals exposed to 200 mGy had the least successful implantations, however the differences were not significant.

399. A different approach to evaluation of tumour transplantability was tested when tumour cells used came from a mouse that developed cancer in response to exposure to high-dose-rate radiation – the ovarian granulosa cell tumour cell line: OV3121, was derived from B6C3F1 mice and transplanted into B6C3F1 and BALB/c mice [T2]. Recipient mice were exposed to chronic radiation at eight weeks of age for 200 or 400 days, gamma rays were produced by a ^{137}Cs source and total accumulated doses were 4,000 or 8,000 mGy. Cytologically, numbers of Th1 cells were increased in spleens of 8,000 mGy exposed mice and proliferation induction by allo-Mixed Lymphocyte Reaction was decreased; all other comparisons (for Th2, Th17, Treg etc.) failed to show significance. Tumour cells were injected into hind limbs of recipient mice immediately after radiation was concluded and tumour growth was monitored for 60 days. Tumours grew in 8,000 mGy irradiated mice more than in controls. The difference was not statistically significant when 4,000 mGy irradiated mice compared with controls, possibly because of the difference in age of the mice.

400. Low-dose radiation hormesis was also studied with "radiation-emitting sheets" producing a range of low-dose alpha, beta and gamma emissions at dose rates between 35 or 130 μSv/h (the gamma ray contribution was about 10% in each case). When three-week-old female BALB/c mice were bred and maintained on radiation emitting sheets with dose rate of 100 μSv/h, no changes in weight were observed [N9]. In addition, mice were maintained for seven weeks on radiation emitting sheets with dose rates of 40 or 100 μSv/h and then their hind limb was injected with syngeneic breast cancer tumour cell line EMT6. In all cases (control, 40 μSv/h, 100 μSv/h, each 25–30 animals per group) tumours grew in three out of four animals, but rates of tumour growth differed – the slowest rate of growth was recorded in animals maintained on 40 μSv/h sheets.

(b) *Low dose rate and liver, adrenal glands and ovaries*

401. Many studies focused on animals continuously exposed to low-dose-rate gamma rays until accumulation of medium to high total doses and explored cancer types and cancer incidence at the time of sacrifice or at the end of lifespan. For example, eight-week-old female B6C3F1/Jcl mice were exposed daily to 21 mGy/d of ^{137}Cs gamma rays [T4] and the mice were sacrificed after 100, 200, 300 and

400 days after beginning of irradiation (corresponding to total accumulated doses of 2,000, 4,000, 6,000 or 8,000 mGy). At that time exposures were stopped, and additional animals were sacrificed each hundred days up to 400 days after conclusion of irradiation or allowed to live out the entire lifespan. Irradiated animal groups consisted of 60 to 90 mice, each group was accompanied with equal number of age matched non-irradiated controls. This study found increased body weight in irradiated mice from sacrifice days 200 to 500 accompanied with many non-neoplastic lesions (adrenal subcapsular cell hyperplasia, fatty degeneration of the liver, atrophy and tubulostromal hyperplasia of the ovaries). Cancers in all animals included malignant lymphomas, which caused most of the life shortening in irradiated groups; hepatocellular adenomas/carcinomas; bronchoalveolar adenomas and Harderian gland adenoma/adenocarcinoma; adrenal subcapsular cell adenomas and ovarian neoplasms (tubulostromal adenoma, granulosa cell tumours) especially had earlier incidence in irradiated mice. Finally, animals from sacrifice days 400 to 700 and in the lifespan group had increased incidence of multiple neoplasia [T4].

402. In an earlier study, Tanaka et al. [T3] exposed eight-week-old B6C3F1 mice (2,000 of each sex) at dose rates of 21, 1.1 and 0.05 mGy/d ^{137}Cs gamma radiation, again for 400 days total, leading to accumulated total doses of 8,000, 400 and 20 mGy. In this study, animals were kept until natural death. Among the most frequent cancers in all cases were myeloid leukaemias in males, soft tissue neoplasms and malignant granulosa cell tumours in females, and haemangiosarcoma in both sexes. In mice receiving the highest doses, the frequency of these diseases compared to controls were significantly increased, age of onset for leukaemias was earlier, and frequency of multiple primary neoplasms per mouse was increased. While some life shortening was found in mice exposed to total doses of 400 and 20 mGy [T8] and more than 85% of all mice died of cancer, no specific fatal neoplasms could be associated with low-dose-rate, low-dose and medium-dose exposures. It should be noted that in this study too, increased body weight was noted in mice exposed to 21 mGy per day. Importantly, Nakamura et al. [N8] found that feed consumption was not increased in irradiated female mice compared to non-irradiated littermates. Overall increase in adiposity (body weight, adipose tissue weight, serum levels of leptin, and lipid contents of the liver and serum) must therefore be connected with metabolic alterations triggered by radiation exposures. Considering that the same mice had highest cancer incidences as well, it is once again necessary to state that the relationship between metabolic derangements and cancer needs further exploration. Importance of metabolic profile as a modifier of long-term radiation responses has been found in humans, e.g. among atomic bombing survivors [Y14].

(c) *Low dose and lung cancer*

403. Floyd et al. [F16] used the bi-transgenic *CCSP-rtTA/Ki-ras* mice (FVB/N background with doxycycline inducible expression of the *Ki-rasG12C* gene in lungs) to develop a general model to study the promotion of lung cancer, with multiple cancer foci in lungs developing after doxycycline exposure. Munley et al. [M46] exposed these animals to low to moderate doses of X-radiation (80–160 mGy). This further increased the frequency of lung cancer incidence. There was a statistically significant sex difference, with higher numbers of cancer foci in female than in male mice. The response to radiation exposure of other organs in these animals remains to be investigated.

404. Puukila et al. [P26] examined data from several studies where beagles were exposed to aluminosilicate beads containing ^{90}Sr, ^{144}Ce, ^{91}Y, ^{90}Y with effective lung half-lives of 2.5 days to 600 days; the beagles were separated into three groups not on the basis of dose first but based on the life shortening effect noted. Animals were grouped into those dying shortly after exposure (acute lung injury), those that die early (mostly lung cancer) and those whose death is non-significantly expediated. Evaluation of total doses delivered to animals that died later in life due to lung cancer induction showed that the total doses were not the same for all radionuclides used. The radionuclide with a long half-life,

^{90}Sr (0.1–0.6 Gy/d), induced cancer with greater efficiency than the radionuclide with a short half-life, ^{90}Y (8–10 Gy/d), indicating the importance of cell death and stress-cell recovery in the radiation response.

7. Role of sex in radiation responses

405. In Li et al. [L23], sex differences in ex vivo irradiated (moderate-high dose) human cells peripheral blood treated with 0, 0.5, 1, 2, 3, 4, 6 and 8 Gy with ^{60}Co gamma rays at a dose rate of 1 Gy/min were explored; expression patterns of candidate genes using quantitative real-time polymerase chain reaction (qRT-PCR) at 6, 12, 24 and 48 hours post-irradiation were identified. Sex-dependent regression models could explain 0.85 of variance at 24 hours and estimate absorbed dose up to 0.5–5 Gy.

406. Male and female C57BL/6 mice were exposed to X-radiation at eight weeks of age, either from 0.3 Gy to 3 Gy or 0.3 Gy followed by 3 Gy four days later. Ten males and ten females each were sacrificed 6 hours, 96 hours or 2 weeks after irradiation [W3] and miRNAs harvested and compared between different treatments and animal of different sex. Sex differences were detected, confirming previous findings by the same group [K38, K39]. Liver samples from female mice overexpressed miR-29c only after 0.3 Gy irradiation but not after 3 Gy or 0.3 Gy followed by 3 Gy exposure. This finding is additionally of interest because miR-29c expression is decreased in liver cancer mouse Hepa 1–6 (and human HepG2 and C3A) cell lines, but it is induced in normal mouse liver epithelial cell line NmuLi. This finding can perhaps be combined with work of Tanaka et al. [T4] that finds signs of non-neoplastic changes, e.g. fatty degeneration in livers of female B6C3F1 mice exposed to 21 mGy/d at 300 days after beginning of irradiation while development of nodules occurs only at 200 days after the completion of 400 days of radiation.

407. Sex differences in responses to radiation exposure were noted in transgenic reporter mice expressing luciferase from a promoter for secretory clusterin [K28]. This protein is an extracellular molecular chaperone that clears cell debris, it is stress-induced and exposures to ionizing radiation at doses greater than 20 mGy lead to its peak expression at 96 hours. Recombinant reporter gene *hCLUp-Luc* was grafted onto an FVB/N genetic background. Mice of equal weight were used which is often a practice in experiments with large numbers of mice, however, in this case all mice were also used at exactly 10 weeks of age. It is important to emphasize experimental rigour of this approach because it suggests that low-dose radiation research field could produce more accurate data if more attention to experimental procedures was given. Irradiations with 100 mGy was carried out at dose rates of 3,880 mGy/min with a ^{137}Cs source. At 24 hours after irradiation animals were injected with luciferin and female mice demonstrated greater reporter gene expression. This was explained by the fact that clusterin is under testosterone repression. Isolated internal organs were also imaged ex vivo after 10 mGy radiation; luciferase expression in bone marrow, spleen, colon, thymus and mammary glands was increased at 24 hours unless the animals were given 5 mg anti-TGF-β1 antibody per kg body weight. This suggests that expression of secretory clusterin in female mice exposed to 10 mGy depends on TGF-β1.

408. Takabatake et al. [T1] reported that B6C3F1 mice chronically exposed to a low dose rate ^{137}Cs gamma rays (21 mGy/d, total dose 8 Gy over 400 days) had shorter lifespans than controls. Irradiated and non-irradiated females had shorter lifespans than males. Genetic evaluation of lymphomas (the predominant cause of death in all cases, more frequent in females) showed that in irradiated mice there was a gain of chromosomes 11 and 15 and partial gains of chromosomes 12, 13, 14, 16 and X were predominant in lymphomas from non-irradiated mice. No sex differences were noted with respect to chromosomal changes.

409. Whoolery et al. [W10] demonstrated that ^{56}Fe and ^{28}Si ions interfered with hippocampal neurogenesis. Neuronal proliferation decreased with dose when ^{28}Si ions are used for whole body irradiation of 9-week-old C57BL/6J and Nestin-GFP mice at doses of 0.2 Gy or 1 Gy (300 MeV/nucleon, LET 67 keV/µm, dose rate 1 Gy/min). Sex differences were noted for controls with females having fewer Ki67 positive cells. After radiation there were no significant differences for female mice but in long-term male groups after radiation new neuron survival was reduced.

8. Summary and conclusions on whole animal studies

410. Cancer induction in animals is one of the late effects of radiation, especially at low doses (see table 7). Consequently, many research studies on mammals focus on mutated animals with increased cancer frequency. In other animal systems, investigation is often focused on non-cancer events that would be cancer initiating or promoting (or opposite) in mammalian model systems. In all cases, induction of cancer by radiation varies with the tissue or cell type whether the exposure is external or internal, the LET of radiation, dose rate, the sex and age of the animal at the time of exposure etc. While some studies suggest that exposures to low doses of radiation can reduce cancer incidence in experimental animals, others show that similar low doses can increase cancer incidence. In all cases, variation in response and group size/statistical power can be limiting, thus reaching a consensus on the impact of low radiation exposures on animals is not currently possible. It is clear that very careful control of experiments in terms of animal ages and weights is of great importance.

Table 7. Summary of animal studies

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
WILD-TYPE ANIMALS						
Fruit fly Canton-S		^{226}Ra radiation source Dose : 50, 100, 200, 400 mGy Dose rate: 36 mGy/h[d]	Lifespan, RNA (PCR)	Hormesis with respect to lifespan in 100 mGy males, 400 mGy females	Genes for circadian rhythm, DNA repair, Heat shock	[Z18]
Fruit fly Canton-S, 24–48 hours		X-radiation Dose : 0.1, 1, 10, 50, 100, 200, 400 Gy[d]		Hormesis with respect to lifespan for 100 mGy	Unique gene set for low dose	[A13]
Silkworm Eggs		Alpha, beta and gamma radiation via substrate Dose rate: 35–130 µSv/h[e]	Body weight	No adverse health effects at age studied		[N9, S30]
Zebrafish Strain brass, 6 hours embryo, sibling groups	Menadione	Gamma radiation (^{137}Cs) Dose : 50–1 500 mGy X-radiation Dose : 1–50 mGy[d]	DNA double-strand break (TUNEL)	Linear increase in DNA double-strand breaks at ≥100 mGy		[B37, B38, B39]
Zebrafish Embryo, 3 hours post-fertilization		Gamma radiation Dose rate: 0.8, 570 mGy/d for 96 hours[e]	DNA damage, health status	Hatching time is shorter with ionizing radiation; DNA damage and apoptosis at 570 mGy/d		[G1]
Zebrafish 2-cell embryo		3.37 MeV protons Dose : 10–2 000 protons[d]	Health	Triphasic response: increased apoptosis below 30 mGy and above 90 mGy, hormetic effect and reduced apoptosis between 30 and 60 mGy		[C25]
Zebrafish		X-radiation Dose: 5–100 mGy Different hardness[d]	Apoptosis	Triphasic response but shifted depending on X-ray hardness (the harder the X-rays the lower the dose with hormetic effect)		[K36]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Zebrafish AB strain		Gamma radiation (^{60}Co) Dose: 5.2, 31 Gy Dose rate: 8.7, 53 mGy/h[d]	Mating, health etc. month or year later, health of F1		Few genes in F1 from different doses overlapping, more overlapping between higher dose 1-month F1 and lower dose 1-year F1	[H42]
Dog Beagle		Gamma radiation Dose rate: 3, 7.5, 18.8 mGy/d[d]	Myeloproliferative diseases, life shortening	No lymphoproliferative diseases below 3 mGy/d		[F14] review
Dog Beagle		Gamma radiation (^{239}PuO) Activity inhaled: 0.14, 0.63, 3.2, 13, 44, 210 kBq[f]	Tumour incidence, life shortening	Lung cancer reduced in low dose group		[P4]
Mouse B6C3F1, 8 week-old		Gamma radiation Dose: 8 000, 400 and 20 mGy Dose rate: 21, 1.1, 0.05 mGy/d for 400 days[d]	Tumour incidence	Body weight increase for 1.1 and 21 mGy/d and higher tumour incidence for 21 mGy/d (total dose 8 Gy)		[B53, T3]
Rat Sprague–Dawley, 6 weeks		Gamma radiation (^{60}Co) Dose: 0.1, 1, 3 Gy[d]	Tumour incidence	No tumour incidence increase at 100 mGy, only some mammary hyperplasia		[S52]
Rat Sprague–Dawley	Bone injury	X-radiation Dose : 0.5, 5 Gy[d]	Pathology, bone recovery	Bone healing with low doses; at 500 mGy high early osteoblast viability and proliferation and high late differentiation	Proteins increased at 7 days in 500 mGy COL1A, ALP, Osterix and OCN, PCNA, etc.	[C15]
Rat	Streptozotocin – diabetes	X-radiation Dose: 350 mGy Dose rate: 25 mGy every second day[g]	Diabetes testis atrophy	Protection from atrophy		[Z17]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Rat Wistar	Streptozotocin – diabetes	X-radiation Dose: 375, 600, 825 mGy Dose rate: 75 mGy fractions, 5 days per week for 1, 2, 3 weeks[g]	Diabetes impairment of skin wound healing	Neovasculature growth ($CD34^+$), increased $CD31^+/CD34^+$ in circulation	PCNA and VEGFR increased by IHC, MMP-2 and MMP-9 increased	[G20]
Rat Wistar, 3 month-old	Female	Fast neutrons (^{241}Am-Be) Dose: 9 mGy[d]	DNA repair	Repair nearly complete within 3 hours		[N1]
Rats Wistar, 1, 5, 15 week-old	Female	Thoracic X-radiation Dose: 1, 3, 5 Gy[d]	Neoplasia, life shortening	No life shortening for any age/dose, cancer types different than controls		[Y1]
Mouse DBA/2		Gamma radiation (^{60}Co) Dose rate: 300 mGy/h, 6 weeks before challenge[d]	Challenge dose 9 or 9.5 Gy survival	Better survival with pre-exposure		[E4] females had higher LD_{50} in all groups
Mouse C57BL/6J	Streptozotocin at 10 weeks – diabetes	X-radiation Dose rate: 12.5, 25, 50 mGy fractions every other day for 4–8 weeks[g]	Diabetic nephropathy evaluation	Less kidney fibrosis, protection by 12.5 or 25 mGy for 8 weeks best	Lower creatinine and connective tissue growth factor	[C20, C21]
Mouse C57BL/6, prion disease	Infection with scrapie strain ME-7	Gamma radiation (^{60}Co) Dose: four fractions of 5–750 mGy per fraction[g]	Prion disease management	Four fractions at 500 mGy around day 50 post-infection effectively reduce onset time and final decline		[P20]
Mouse B6C3F1		Gamma radiation Dose rate: 1, 20 mGy/d for 400 days[d]	Pathology	Tumour incidence and life shortening significant in 8 000 mGy group		[B53, T4]
Mouse B3C3F1 (C57BL/6JNrs × C3H/HeNrs), 7 day-old		Gamma radiation (^{137}Cs) Dose : 100, 480, 950 mGy[d]	Tumour incidence	Old mice from 100 mGy cohort die more of tumours than from non-tumour toxicities		[S10]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse C3H/HeN		Chronic total body gamma irradiation Dose: 200, 20 mGy daily over 22 hours for 5–400 days from 8 weeks old[d]	Chromosomal abnormalities	Linear increase in number of dicentrics in spleen cells over time for both dose rates. Unstable aberrations per cell show a spike at 1 Gy cumulative dose for 20 mGy/d group, data points not significant however		[B53, N5]
Mouse C57BL/6, 12 week-old	Male	Gamma radiation Dose: 60, 300, 1 500, 7 500 mGy Dose rate: 2, 10, 50, 250 mGy/d for 30 days[d]	Muscle effects, immediately after ionizing radiation or 3 months after	Satellite cells isolated from muscles had lower numbers when isolated from animals after 3 months		[M21]
Mouse C57BL/6, CBA/Ca		X-radiation Doses: up to 100 mGy Dose rate: 1 mGy/s Doses: 500, 1 000, 3 000 mGy Dose rate: 7.5 mGy/s[d]	Bone marrow radiation-induced chromosomal instability	TUNEL assay not positive before 200 mGy	p53 and p21 cells were noted at 50 or 100 mGy in C57BL/6 or CBA/Ca mice, respectively	[Z24]
Mouse C57BL/6		Total body X-irradiation Dose: 200 mGy Harvest 40 weeks later[g]	Heart mitochondrial proteome		desmin, actin, and three forms of myosin.	[B19]
Mouse C57Bl/6 J	None; Male	^{16}O high-LET 600 MeV/n Dose: 0.1, 0.25, 1.0 Gy Dose rate: 0.21–0.28 Gy/min[f]	Microbiome and metabolome	Bacterial 16S rRNA amplicons; Fecal metabolomics		[C6]
Mouse C57Bl/6 5 week-old	Female	X-radiation Dose: 75 mGy fractions before challenge Dose rate: 1.2 mGy/h over 450 days[g]	Lymphoma incidence, 7.2 Gy (in 4 fractions) challenge	Chronic irradiation – better health overall, more $CD4^+$, $CD40^+$, more efficient protection from lymphoma but 75 mGy also protects		[I7]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse C57Bl/6, 4–8 week-old	Both	X-radiation Dose : 50, 200, 400 mGy[d]	Low dose protection from LD_{50} 5.9 Gy	50 mGy best protection at 6 hours and 2 weeks before the challenge		[I12]
Mouse C57Bl/6	Female	Gamma radiation Dose: 0.3, 6.0 Gy Dose rate: 1, 20 mGy/d for 300 days[d]	Gene expression analysis		Differentially expressed gene lists	[K22]
Mouse C57BL/6J and BALB/cJ, 5-month-old	Female	Gamma radiation (^{60}Co) Dose: 20, 100 or 500 mGy Dose rate: 0.5 or 10 mGy/min[d]	Challenge dose 1 or 2 Gy, spleen micronuclei	No protection from any of low doses (BALB/cJ always had more micronuclei)		[B13]
Mouse C57BL/6J, 5–6 week-old	Female	Gamma radiation (^{60}Co) Dose: 20, 100 mGy[d]	Response to 2 Gy challenge	Pre-exposure did not protect splenocytes or thymocytes from 2 Gy		[B40]
Mouse C57BL/6J, 7–8 week-old	Both or male for tritiated water	Gamma radiation (^{60}Co) Dose: 20, 100 mGy Tritiated water Dose: 10 kBq/L, or 1, 20 MBq/L for 1 month[f]	Response of splenocytes to 2 Gy challenge, 24 hours post-challenge	Pre-exposure by gamma rays protected from apoptosis, tritiated water (in males) did not		[F13]
Mouse C57BL/6J, BALB/cJ, 2–5 month-old	Female	Tritiated water Dose: 10 kBq/L–20 MBq/L[f]	Spleen cell damage, micronuclei	No effects on splenocytes		[B14]
Mouse C57BL/6, 9–10 week-old	Female	Gamma radiation Dose : 50 mGy[d]	Health status after 2 or 3 Gy proton as challenge, after 4 or 17 days	Organ weights, RBC and leucocyte counts – none affected much but if so – pre-exposure to gamma rays modulated it		[L51]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse C57BL/6J, B6C3F1		Gamma radiation (^{137}Cs) Dose: 400 mGy Dose rate: 1 mGy/d Dose: 8 000 mGy Dose rate: 20 mGy/d[e]	Life shortening, neoplasias etc.	B6C3F1 females show life shortening at 400 mGy and 8 000 mGy, males at 8 000 mGy; in C57BL/6J only males live shorter at 8 000 mGy		[B53, T3] 2018 paper is a review – little unpublished data
Mouse C57Bl/6J		X-radiation Dose rate: 0.35 Gy/min[d]	Proteomics		Differentially expressed protein lists	[B9]
Mouse C57BL/6J, 6 month-old		600 MeV/n high-LET (^{16}O) Dose: 100, 250 mGy[f]	Metabolomics at 10 and 30 days	Metabolomics can be carried out	Benzaldehydes and more	[C6]
Mouse BALB/cJ, C57BL/6J	None; Male	Gamma radiation (^{137}Cs) Dose : 50, 100, 1 000 mGy[d]	DNA damage (chromosomal aberrations), 1 and 4 hours, 1 and 6 months	No long-term genomic instability except 1 Gy, protection by 50 mGy		[R6]
Mouse A/J	4 weeks before ionizing radiation	Gamma radiation (^{137}Cs) Dose: 60, 600 mGy Dose rate: 1.07, 1.33 mGy/s over 3 weeks[e]	Lung cancer development at 46 weeks	60 mGy alone is protective, increased adenoma and carcinoma for combined 60 mGy–benzo[a]pyrene, 600 mGy combined less cancer		[B57]
Mouse C57BL/6, EGFR-expressing C57BL, 22 week-old	Critical limb ischaemia; female	X-radiation Dose: 1–7 300 mGy fractions, from 12 hours post-CLI[g]	Neovasculature; ischaemia recovery	$CD31^+$ cells increased, neoangiogenesis only improved by 4×300 mGy	ionizing radiation increased Vegfr2, Vegfr1, Fgf2, Angpt2, Pdgfc, Tgfb2, Hgf, and Met	[M32]
Mouse C57BL/6JJcl females mated with C3H/HeNJcl males, 6 week-old		Gamma radiation Dose: 360, 3 600, 7 200 mGy Dose rate: 20, 200, 400 mGy/d[e]	Pregnant mice exposure form day 0 to day 18 of gestation	Fetus size, fetal organ sizes, decreased in all groups		[G19]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse BALB/c and SPRET/EiJ mice		Total body X-irradiation Dose: 100 mGy[d]	Gene expression		Differentially expressed gene lists	[T10]
Mouse Backcross of (BALB/c × SPRET/EiJ) × BALB/c	Syngeneic $p53^{-/-}$ breast tumours, female	X-radiation Dose : 100 mGy[d]	Pathology of orthotopic breast cancer	Decreased latency when recipient mice receive 100 mGy		[Z12]
Mouse BALB/c, C3H/HeN	Female, syngeneic tumours transplantation	X-radiation Dose: 50, 100, 200, 500, 1 500 mGy 6 hours before tumour injection[d]	Protection from growth of transplanted tumours	Only a trend of protection in BALB/c mice animals exposed to 200 mGy		[I13]
Mouse BALB/cA, 8 week-old	Syngeneic tumours transplantation	Gamma radiation (^{137}Cs) Dose: 4 000, 8 000 mGy over 200, 400 days[e]	Tumour cells injected immediately after ionizing radiation	Tumours grew faster in 8 000 mGy recipients		[T2]
Mouse BALB/c	Syngeneic tumours transplantation	Alpha, beta and gamma radiation via substrate Dose rate: 40–100 µSv/h[e]	Tumour cells injected after 7 week-long exposure	The slowest rate of growth of hind limb tumours was recorded in animals maintained on 40 µSv/h sheets		[N9]
Mouse BALB/cBYJ, 4 month-old		X-radiation Dose : 170, 500, 1 000 mGy[d]	Bone architecture 3 or 21 days later	Bone marrow cells growth of adipocyte colonies only 1 Gy; osteoblastogenesis increased on day 3 in 170 mGy		[L30]
Mouse BALB/c, 4–6 week-old at DOX administration	Doxorubicin 7.5 mg/kg, female	X-radiation Dose: 75 mGy, 72 hours before DOX[d]	Oxidative stress in heart	Cardiomyocytes health increased, less apoptosis	Reduced BAX, increased Bcl2	[J18]
Mouse CF1 and outbred ICR, 2-hour zygote in utero exposure		X-radiation Dose : 200, 400 mGy[d]	Number and health of the embryos at days 19 or 18	No significant effects		[J7]
Mouse *hCLUp-Luc* on FVB/N		Gamma radiation (^{137}Cs) Dose : 100, 1 000 mGy[d]	Reporter mice – luciferase increase to ionizing radiation		TGF-β1 controls expression from clusterin promoter	[K28]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse Kunming, 12 month-old		X-radiation Dose : 300, 3 000 mGy[d]	mRNA, IHC one or two weeks after	Increased neural stem cell proliferation and neurogenesis at 300 mGy	Increased number of Wnt1, Wnt3a, Wnt 5a, and β-catenin	[W8]
REPAIR DEFICIENT ANIMALS						
Zebrafish Strain brass, same clutch 6 hour-old	Ku80 antisense	Gamma radiation Dose : 50–1 500 mGy, X-radiation Dose : 1–50 mGy[d]	DNA double-strand break (TUNEL)	Linear increase in DNA double-strand breaks at ≥10 mGy		[B37]
Mouse SCID, *ATM*$^{-/-}$, *ATM*$^{+/-}$	SCID (*Prkdc*$^{-/-}$), *ATM*$^{-/-}$	X-radiation Dose: 100 mGy single or multiple fractions, or 6 Gy[g]	DNA damage evaluated based on foci, 0.5, 24, or 72 hours after exposure	Cortical neurons, skin, intestine: ICC for DNA break foci		[S12]
Mouse SCID, *ATM*$^{-/-}$, *ATM*$^{+/-}$	SCID(*Prkdc*$^{-/-}$), *ATM*$^{-/-}$	Lung gamma radiation Dose: 100 mGy Dose rate: 2 Gy/min, 5 days a week for 2, 4, 6, 8, 10 weeks[g]	DNA damage in lung cells, 0.5, 24 or 48 hours after exposure	DNA damage decreased with time in wild-type mice (until 50 exposures), in SCID not so much, in *ATM* knockout not at all		[F15]
Mouse DNA ligase IV$^{-/-}$, *ATM*$^{-/-}$, double mutants, E13.5	*LIG4*$^{-/-}$, *ATM*$^{-/-}$, *LIG4*$^{-/-}$, *ATM*$^{-/-}$	X-radiation Dose : 50–200 mGy[d]	DNA damage and apoptosis (DNA double-strand break foci and TUNEL) 1 hour after exposure	Less repair and more apoptosis in mutant embryos, spontaneously and after ionizing radiation, spontaneous damage decreases with age		[B15]
Mouse NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*$^{tm1Wjl/SzJ}$ (NSG); 6–8 week-old at grafting	SCID(*Prkdc*$^{-/-}$), human CD34^{+} cells from cord blood grafted	High-LET 350 MeV/n (^{28}Si) Dose: 400 mGy X-radiation Dose: 1 Gy[f]	DNA damage in circulating human cells (1.2 ions traverse each cell)	Chromosomal aberrations: 2x more damage with X-rays		

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse SCID, 10–12 week-old	SCID(*Prkdc*$^{-/-}$)	Gamma radiation (^{137}Cs) Dose : 50, 100, 1 000 mGy[d]	DNA damage (FISH) in bone marrow cells, 1 and 4 hours, 1 and 6 months	No long-term genomic instability up to 100 mGy based on chromosomal aberrations		[R8]
Mouse SCID, C.B-17/Icr wild-type and F1 heterozygotes for *Prkdc*$^{+/-}$	SCID(*Prkdc*$^{-/-}$), *Prkdc*$^{+/-}$, Female	Gamma radiation (^{137}Cs) Dose: 50, 100, 250, 500 mGy[d]	Lymphoma incidence	Thymic lymphoma incidence unchanged in SCID only at 50 mGy; in wild type the latency extended at 250 mGy		[I11]
Mouse SCID	SCID(*Prkdc*$^{-/-}$)	X-radiation Dose : 300 mGy[d]	Success of MOLT-4 tumour growth, neovasculature	Tumour growth and neovascularization improved by ionizing radiation		[S53]
Mouse F1 for *ERCC2* mutants in CH3 crossed with C57BL/6J; 10 week-old	*Ercc2*$^{+/-}$	Gamma radiation (^{60}Co) Dose: 63, 125, 500 mGy[d]	Bone marrow isolation at 4 and 24 hours, 2 or 18 months	No chromosomal or telomere changes. Life expectancy increased after 63 mGy, decreased at higher doses		[D1] study was geared to study cataracts
TUMOUR SUPPRESSOR MALFUNCTIONING ANIMALS						
Mouse heterozygotes *p53*$^{+/-}$, 7–8 week-old	*p53*$^{+/-}$	X-radiation "CT scan quality" Dose one or more 10 mGy fractions[g]	Tumour incidence, apoptosis	Low dose protection from sarcomas and lymphomas, extending lifespan in *p53*$^{+/-}$; less apoptosis with more fractions in wild type		[L18, L19, P17]
Mouse *APC*$^{1638N/+}$ male or both parents or grandparents irradiated 2 days before conceiving	*APC*$^{1638N/+}$ both sexes	X-radiation Dose: 250 mGy to single or both parents or grandparents[d]	Transgenerational tumour incidence	For males or both grandparents irradiated, F2 mice had more tumours, in females both grandparents irradiated had more tumours		[S68] male F1 heterozygotes had higher tumour incidence than female

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse Ptch on background of C57BL/6JCrl and C3H/He, 1 day-old	*Ptch* $^{+/-}$	X-radiation Dose: 50, 100 mGy Dose rate: 100 mGy/min Dose: 200–3 000 mGy Dose rate: 650 mGy/min[d]	Life shortening, LOH, microarrays, animals exposed at day 1 after birth	Radiation induced LOH pattern unique, at 50 mGy ratio of what looks like "spontaneous": irradiated LOH is 13:7, at 100 mGy 1:1		[I10]
Mouse *Ptc1*$^{+/-}$ in CD1 background crossed with *Rad54*$^{-/-}$ and *DNA-PKcs*$^{-/-}$ mice on C57BL/6 background; in utero exposure	*Ptc1*$^{+/-}$, *Rad54*$^{-/-}$, *DNA-PKcs*$^{-/-}$	X-radiation in utero Dose: 250 mGy, 3 Gy at E13.5 and E16.6[d]	Complex events related to medulloblastoma	High dose worse on day E13.5, low dose worse on day E16.5. Different repair pathways predominate at different embryo ages		[T12]
ONCOGENE OVER-EXPRESSING ANIMALS						
Mouse *CCSP-rtTA/Ki-ras,* 8 week-old	*Ras* overexpression in lung induced by DOX	X-radiation "CT scan quality" Dose: 5, 15, 25 mGy per fraction once a week for 4 weeks[g]	Lung cancer burden	80–160 mGy correlates with increased number of tumours per mouse but not their size; no dose dependence and no tumours if DOX was not used		[M46]
Mouse AKR/J, 7 week-old	Murine leukaemia viral gene, female	Gamma radiation (^{137}Cs) Dose: 2.1, 4.5 Gy Dose rate: 0.7, 0.8 Gy/min, respectively, over 130 days[e]	Progression of thymic lymphoma	Earliest cases in low dose rate mice, but they still live longest of all	T- and B-cell activation genes	[S38]
Mouse AKR/J, 8 week-old	Murine leukaemia viral gene, female	Gamma radiation (^{137}Cs) Dose rate: 0.7 mGy/h for 130 days total dose 2.1 Gy or acute[e]	Gene expression	Most apoptosis in low dose exposed mice	Increased CDP-diacylglycerol synthase 1 in low dose rate mice; integrin-α4, integrin-β1 and Myc in both groups	[B49, B50, B51]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse C57B/6 *Lck-Bax; Lck-Bax38 & 1*, 4–9 week-old	Thymocyte-specific *Bax*	Gamma radiation (^{137}Cs) Dose: 100, 1 000 mGy Dose rate: 0.35Gy/min Si or Fe ions Dose : 100, 1 000 Gy Dose rate: acute radiation over 1 minute[d,f]	Lymphoma onset	*Lck-Bax1* shortened latency in females only; Fe ion exposure shortens latency		[J6]
OTHER TRANSGENIC ANIMALS						
Mouse *pKZ1*$^{Tg/+}$		Tritiated water Dose rate: 10 kBq/L, or 1, 20 MBq/L[f]	LacZ inversions	No changes		[B12]
Mouse *pKZ1*$^{Tg/+}$	Male	X-radiation Dose : 0.001, 0.005, 0.01, 0.02, 1, 10, 1 000 mGy[d]	LacZ inversions in prostate 3 days after ionizing radiation	"Triphasic" response – inversions decreased at 1 and 10 mGy but increased at doses above and below these		[H35, Z5]
Mouse *pKZ1*$^{Tg/+}$	Male	X-radiation Dose : 0.001, 0.01, 1, 10 mGy[d]	Challenge doses 4 hours later 1 or 4 Gy	Several dose combinations modulated inversions		[D6, D8]
Mouse *ApoE*$^{-/-}$ on C57BL/6J; 8 week-old	*ApoE*$^{-/-}$; female	Gamma radiation (^{60}Co) Dose: 25–2 000 mGy Dose rate: 1, 150 mGy/min[d]	Heart pathology and aortic atherosclerosis	Adaptive response at doses up to 0.5 Gy; increased capillary density	Decreased inflammatory vascular markers; changes of collagen IV and Thy-1 tissue levels	[M23]
Mouse *ApoE*$^{-/-}$ on C57BL/6J; 2 or 8 month-old	*ApoE*$^{-/-}$; female	Gamma radiation (^{60}Co) Dose: 25–500 mGy Dose rate: 1, 150 mGy/min[d]	Pathology of aortic atherosclerosis	Disease slowed down by 25 or 50 mGy delivered at 1 mGy/min		[M37]
Mouse *ApoE*$^{-/-}$ on C57BL/6J; 7–8 week-old	*ApoE*$^{-/-}$; male	Gamma radiation (^{137}Cs) in vitro delivery Dose: 4, 20, 100 kBq/L[f]	Pathology of aortic atherosclerosis	Plaque stability increased, inflammatory profile reduced		[L8]

Type of animal, strain, age[a]	*Genetic or chemical modulation, sex*[b]	*Irradiation conditions*	*Outcomes recorded, time post-exposure*	*Definitive findings*	*RNAs, proteins, metabolite, epigenetics*[c]	*Reference, comments*
Mouse *ApoE*$^{-/-}$ on C57BL/6J; 8 week-old	*ApoE*$^{-/-}$; male	Gamma radiation (^{60}Co) Dose: 69, 161 mGy Dose rate: 12, 28 µGy/h[e]	Pathology of atherosclerosis	Macrophages and gene expression show adaptive response	Cytokines (Il-4, Il-10, Il-13 and Il-18), catalase and SODs up-regulated	[E1]
Mouse C57BL/6J, Tg2576, APP with the Swedish mutation, PS19 tau with the P301S mutation, 10 week-old	WT, or APP or tau; female	X-radiation Dose : 100 mGy[d]	Pathologies like Alzheimer's monitored	No changes		[W2]
Mouse MRL-*lpr/lpr*, 5 week-old	Female	Gamma radiation (^{137}Cs) Dose rate: 0.35, 1.2 mGy/h[d]	Life expectancy	Best improvements in life expectancy 1.2 mGy/h 5 weeks, some with 0.35 mGy/h		[I6]
Mouse Transgenic *TNF-α*		X-radiation Dose : 500 mGy[d]	Control of arthritis	Apoptosis of synoviocytes, reduced expression of inflammatory phenotype		[D15]

[a] At time of exposure or experiment initiating age.

[b] When nothing is indicated both were used.

[c] Indicated if recorded.

[d] Low-dose radiation.

[e] Low-dose-rate radiation.

[f] Internal emitters or high-LET.

[g] Fractionated exposure.

IV. INTEGRATION OF DATA AT DIFFERENT LEVELS OF ORGANIZATION AND MODELLING OF CANCER MECHANISMS

411. This section presents mathematical models that were developed to describe different levels of organization and to analyse mechanisms of carcinogenesis. Articles from 2006 and later were selected that the Committee considered informative regarding the effect of ionizing radiation on the process of carcinogenesis and its potential consequences. The framework of the models was required to be sufficiently far developed with a robust scientific basis. The models should only depend on a limited number of parameters so that the implications would not depend on too many assumptions. The models should inform about cancer mechanisms that might be generalizable beyond the specific data that they were applied to. Three areas with different description levels were identified. Firstly, biologically-based mechanistic models of carcinogenesis were applied to epidemiological cohorts and the effect of exposure on cellular processes and consequences for radiation risk were investigated (tables 8 and 9). Secondly, models of the bronchial airway were also developed to assess the impact of radon inhalation on cellular and tissue effects in the lungs. Thirdly, models of intercellular signalling were applied to apoptosis and bystander mechanisms.

412. The UNSCEAR 2000 Report, annex G, "Biological effects of low radiation doses" [U5] included a section on biologically-based modelling of radiation carcinogenesis. It was concluded that the modelling of epidemiological data tends to favour the view that, in general, cancer risk at low doses rises as a simple function of dose. The report recognized that the further development and validation of biologically-based models would have an important role in the future work of the Committee.

A. Biologically-based mechanistic models of carcinogenesis

413. Based on the idea that cancer develops from several successive cellular changes, Armitage and Doll [A17] developed a mathematical model that was applied to human cancer rates. Using the observations from Fisher and Hollomon [F12] and Nordling [N18] that cancer rates appeared to increase with about the sixth power of age, Armitage and Doll proposed a multistage process that included seven stages. Since initiated (or pre-malignant) cells, i.e. cells in an intermediate stage to cancer, may have a growth advantage compared to normal cells, in a subsequent publication Armitage and Doll [A18] proposed a two-stage model in which the cells in the first stage were allowed to grow exponentially and become malignant after a second mutational event. On these grounds and using the hypothesis by Knudson that certain cancers such as retinoblastoma require two mutational events [K30], Moolgavkar and Venzon [M40] developed an entirely stochastic model of carcinogenesis with two stages in which both normal cells and cells at an intermediate state were allowed to grow, differentiate and die. This model was named the two-stage clonal expansion (TSCE) model and is an important representative of biologically-based models of carcinogenesis.

414. These types of biologically-based models aim at describing the long-term process of carcinogenesis by a series of molecular changes in cells towards malignancy that ultimately result in cancer. The complex process of cancer development is reduced to a few basic steps. The parameters of the models can be derived from fits to epidemiological cohorts and biological input. With the assumption that the model and its parameters capture underlying long-term carcinogenic processes, the model can then be used to estimate radiation risk for exposures different from the epidemiological study. Historically, the most frequently used model is the TSCE model that includes two basic steps from healthy stem cells towards malignant cells. The first stage, the initiation, involves a transition, induced by genetic or epigenetic

events, from healthy cells towards initiated cells. These initiated cells have a growth advantage, possibly related to driver gene mutations, and can clonally expand. This process of clonal expansion is often referred to as "promotion". Further mutational events can induce malignant transitions from the initiated cells towards cancer cells, referred to as "transformation", that may manifest in a phenomenologically detectable cancer after a certain lag time. Extensions of this model include models with several stages, or the inclusion of additional pathways, see Rühm et al. [R22] for a review on these models.

415. Such mechanistic models provide a general framework for the long-term development of carcinogenesis already in the absence of radiation. In such models, radiation can act on existing pathways, either inducing transitions between different stages, in particular on the initiation or transformation step, or can accelerate clonal expansion. Furthermore, radiation-induced carcinogenesis might proceed along different pathways compared to spontaneous cancer. By application of these models to radio-epidemiological cohorts the type and form of radiation action can be assessed, and the shape of dose response can be derived. While descriptive models of excess relative risk or models of excess absolute risk are usually given as a function of total dose, in contrast, mechanistic models depend directly on dose rate. The model parameters change during exposure and usually return to their spontaneous values after exposure. The corresponding changes in the incidence/mortality rates and consequences for the dose–response relationship can then be calculated.

416. Following the pioneering works, the mathematical structure of the models was studied in greater depth and they were applied to various radio-epidemiological cohorts. Different endpoints after low-LET radiation were studied in particular for the atomic bombing survivors of Hiroshima and Nagasaki (Life Span Study cohort). Little [L33] studied 2-, 3- and 4-mutation models for solid cancers and leukaemia [L33]. Cancers of the stomach, colon and lung were analysed with the TSCE model by Kai et al. [K4] with particular focus on the dependence of the excess relative risk with age at exposure and time since exposure. Different two-stage and multistage models were compared for all solid tumours and several tumour sites by Heidenreich et al. [H19, H20]. For high-LET radiation, lung cancer after radon exposure was studied in several cohorts. Luebeck et al. [L46] analysed lung cancer mortality in the Colorado uranium miners with the TSCE model including smoking information, and found a highly significant effect of radon on the clonal expansion of initiated cells. Subsequently, Little et al. [L32] extended the analysis of the Colorado miners to models with three stages. Significant effects of radon on clonal expansion were also found in analyses of lung cancer with the TSCE model in the Yunnan tin miners by Hazelton et al. [H17] and of miners from the Czech Republic, France, China, and Colorado by Heidenreich et al. [H22]. Furthermore, mechanistic models were used to study liver cancer in thorotrast patients [H21, L16] and bone and head sinus cancers in radium dial painters [L15]. Additional background information can be found in Little [L36] and Rühm et al. [R22].

417. The following discussion describes the efforts since 2006 to extend the range of applications of the biologically-based models and to strengthen their link to biological processes. For low-LET radiation, several studies were performed for the atomic bombing survivors of Hiroshima and Nagasaki. Further investigations were carried out for occupationally, environmentally or medically exposed persons, often with protracted low-dose-rate exposures. For high-LET radiation, a number of studies on lung cancer after radon exposure among miners and on lung cancer after plutonium exposure in the Mayak workers were performed. Tables 8 and 9 provide a summary of the findings. The mechanistic models were also applied to non-radiation data, such as the Surveillance, Epidemiology and End Results registry, with the aim to gain more insight on the underlying processes and model structure, in particular on biological effects such as genomic instability. This can improve the basis for implementation of radiation effects in these models. Additional mechanistic studies also included analyses of animal data.

1. Application of mechanistic models of carcinogenesis to low- linear energy transfer radio-epidemiological cohorts

418. Incidence of cancer in the atomic bombing survivors has been analysed by Heidenreich et al. with the TSCE model for stomach, colon, liver, lung and all solid tumours and for a collection of nine tumour sites with a follow-up period of 1958–1997 [H24]. Radiation was allowed to act either as initiating event or on clonal expansion. The age-time patterns of risk suggested that both mechanisms play a role in radiation-induced carcinogenesis. Assuming that additional time-limiting steps were needed to obtain intermediate cells with a growth advantage did not improve the quality of fit.

419. Jacob et al. [J3] analysed the Life Span Study mortality data for all solid, stomach and liver cancers with a follow-up from 1950 to 2000. In addition to the effect of radiation on the mutation rates the influence of radiation-induced cell inactivation was studied. Cell survival data for liver cells, stomach cells and a mixture of cells from different organs together with experimental data on low-dose hypersensitivity were implemented in their TSCE model. A significant effect of radiation on the initiation rate, i.e. the transition rate from a healthy cell towards a pre-malignant cell, was found for all endpoints. Additional effects of radiation on the clonal expansion rate and the transformation rate towards malignant cells were seen. Without consideration of radiation-induced cell inactivation the TSCE model would predict a linear dose–response curve. However, if radiation-induced hyper-sensitivity influenced cell inactivation then the initial slope at low doses of the resulting dose–response curve was steeper than at high doses.

420. Shuryak et al. [S42, S43] have described cancer development involving a short- and long-term component. The authors developed a model framework to describe the processes on both time scales independently, and to integrate both formalisms. The short-term part of the model considered initiation, inactivation, and repopulation of normal and pre-malignant stem cells during the radiation regimen and a recovery period of about a month. Niche takeover by pre-malignant cells was also considered a short-term process. These short-term processes were assumed to be essentially completed by a few months following exposure, before the long-term processes of spontaneous initiation in the absence of radiation and niche replication, which operate on the time scale of multiple years, start to have an appreciable effect. Cancer incidence was assumed to be proportional to the total number of pre-malignant cells in all niches, shifted by a lag time. These models were applied to the example of radiotherapy, to estimate risk for nine solid cancer types using data on radiotherapy-induced second malignancies, on Japanese atomic bombing survivors, and on background United States cancer incidence.

421. While excess relative risk for solid cancers in the Life Span Study decreased with increasing age at exposure during childhood, Shuryak et al. [S44] observed that this pattern seemed to be different for exposures during adult ages with constant or even increasing risk. Using data of excess relative risk from the atomic bombing survivors 1958–1998 of all solid, liver, colon, lung, breast, stomach, and bladder cancer, the authors applied the biologically-based model of radiation carcinogenesis derived in Shuryak et al. [S43]. With the aim to assess whether such a potential age at exposure dependence would be biologically plausible, radiation was allowed to act both on the initiation stage and on clonal expansion. The authors concluded that the observed patterns of radiation induced cancer risks as a function of age at exposure were not consistent with models of radiation carcinogenesis in which radiation solely initiated pre-malignant cells but were consistent with models of radiation carcinogenesis that included both radiation-induced initiation and clonal expansion.

422. Breast cancer incidence in the Japanese atomic bombing survivors was studied by Kaiser et al. [K5] with the TSCE model. The follow-up included the years 1958–1998 and 1,038 breast cancer cases. Radiation was assumed to act linearly with dose on the initiation, clonal expansion and transformation stages of the TSCE model. Three different models were found to describe the data similarly well. The

first model included an effect of radiation on the initiation rate, the second a permanent increase of the clonal expansion rate, and the third a permanent simultaneous increase of the mutation rates for initiation and transformation. Permanent radiation enhancements of the mutation rates or the clonal expansion are indications for long-lasting radiation effects, e.g. in the case of radiation-induced genomic instability. Risk for women at age 70 years, exposed at age 30 years, was estimated to 10 (90% CI: 8.2, 13) cases per 10,000 person-years at 1 Gy for the excess absolute risk, and 1.2 (90% CI: 0.72, 1.9) at 1 Gy for the excess relative risk.

423. Colon cancer proceeds along two principal pathways. The majority of colon cancers show chromosomal instability related to defects in the tumour suppressor gene APC. The second pathway is related to microsatellite instability, e.g. induced by defects in DNA mismatch repair genes such as *MLH1*. Kaiser et al. [K6] developed a mechanistic model including the two pathways, and applied it to incidence data 1958–1998 of the atomic bombing survivors of Hiroshima and Nagasaki with 1,508 colon cancer cases. Biological information was used to estimate several model parameters, e.g. the number of stem cells in the colon or cell division rates in adenoma and early carcinoma. In their preferred model, ionizing radiation acted on the initiation rate among both sexes, in both pathways, while in addition it acted on the clonal expansion rate of chromosomal instability cells among males, in the chromosomal instability pathway. Hence radiation acted by enhancing APC mutations, *MLH1* methylation or suppression of cell death. The latter effect might be caused by inflammation. The model allowed the prediction of the share of microsatellite instability and chromosomal instability-type tumours for the Life Span Study colon cancers as a function of attained age. In the future, if molecular measurements from the Life Span Study colon cancer cases were available these models predictions could be validated. On average, the model predicted a share of microsatellite instability-type tumours of 25%, which is remarkably close to the share of 15–20% reported for the clinically observed colorectal tumours in molecular studies of American colon cancer patients (from TCGA, the Cancer Genome Atlas).

424. Hazelton et al. studied lung cancer incidence among Canadian radiation workers with individual annual dosimetry for protracted low-dose occupational gamma and tritium radiation exposure [H18]. Within the follow-up time 1969–1988, 322 male lung cancer cases were observed among 95,439 males. The TSCE model and extensions of the model with up to 10 initiation steps were applied to the data. Dose response to initiation, clonal expansion or malignant transformation in the TSCE model was parametrized as a power law. In the TSCE model, dose response was significant for both clonal expansion and malignant transformation, while it was only insignificant for initiation. The dose response of excess relative risk had a downward curvature with an inverse dose-rate effect. For the multistage models, the best fit was found for three initiation stages. However, clonal expansion was found to be highly significant in all of these models.

425. All solid cancer incidence and mortality in the Techa River cohort was analysed with the TSCE model by Eidemüller et al. [E11, E14]. In total, 1,889 incident solid cancers in the period 1956–2003 were observed among 17,069 persons, and 2,064 solid cancer deaths among 29,771 individuals for the period 1950–2003. For the incidence data, it turned out that when changes in the investigated rates were only allowed during radiation exposure, initiation was the only process that was affected while the processes of clonal expansion and transformation were not affected. When a permanent effect of radiation exposure on clonal expansion was added the fit to the incidence data was significantly improved. For the mortality data, again a direct effect on initiation and a permanent effect on clonal expansion were preferred, while a model with a smaller direct effect on initiation before a critical age of 24 years and a stronger direct effect on initiation at older ages described the data only slightly worse. The analysis showed a significant effect of ionizing radiation on the initiation stage, and good indication for an additional effect on clonal expansion. However, no radiation effect on the transformation stage was seen.

426. Breast cancer incidence from 17,200 female patients of the Swedish haemangioma cohort was analysed by Eidemüller et al. [E13]. Exposure was at infancy mainly by ^{226}Ra applicators. Within the period 1958–2009, a total of 877 breast cancer cases was observed. Based on previous work [E10], the authors had indications that radiation-induced cancer proceeded along a different pathway than spontaneous cancer, and that this pathway hinted to long-lasting radiation-induced effects such as genomic instability. Therefore, a structurally more advanced model was developed with a separate pathway possibly related to genomic instability. The analysis showed that the two-pathway model was a significant improvement compared to the TSCE model. Radiation was also found to induce the second pathway with a linear dose–response relationship. Using biologically plausible assumptions on some parameters, the authors derived relationships between the spontaneous pathway and the one possibly related to radiation-induced genomic instability. The speed of cancer development was increased in the radiation-induced genomic instability path and the model suggested that in particular the rate of mutations from non-initiated cells towards initiated cells with a growth advantage was substantially elevated.

427. A number of studies provided evidence that overexpression of the *CLIP2* gene on transcriptomic and proteomic levels in tissue of PTC from patients undergoing surgery before 20 years of age served as a marker for the radiation-induced origin of the disease [H27, S23, S24]. To integrate the *CLIP2* findings into radio-epidemiological risk assessment, Kaiser et al. [K7] developed a model for two molecular pathways pertaining either to sporadic PTC development or to radiation-induced pathogenesis which features *CLIP2* overexpression as a radiation marker. The model was applied to the Ukrainian-American (UkrAm) cohort of 13,183 subjects with 115 PTC who were exposed as children and adolescents to ionizing radiation from ingested ^{131}I after the Chernobyl accident. The authors showed that the dose response for the probability of finding the *CLIP2* marker in PTC tissue is directly related to the radiation risk of the UkrAm cohort. Furthermore, the mechanistic model was applied to explore the impact of radiation on molecular alterations such as mutations, genomic copy number alterations or altered mRNA and protein expression. The authors provided a comprehensive characterization of PTC carcinogenesis for a number of clinical and molecular properties.

428. The effect of smoking and radiation on molecular pathways of lung adenocarcinoma was studied by Castelletti et al. [C8]. To obtain a model structure reflecting the main pathways towards lung adenocarcinoma, the authors analysed molecular profiles from patients of the United States and China. Grouping the clinical data by driver mutations revealed two main distinct molecular pathways to lung adenocarcinoma. The two-path model was applied to incidence data of Japanese atomic bombing survivors that contain information on radiation and smoking but lack molecular genotyping of lung cancer tissue. Consistent with the model structure, the best fit of the incidence data was achieved with a model containing two pathways: one unique to transmembrane receptor-mutant patients that displayed published signatures of radiation exposure and one shared between submembrane transducer-mutant patients and patients with no evident driver mutation that carried the signature of smoking. For both agents, radiation and smoking, net clonal expansion was identified as the main biological target. The derived model strongly suggested that smoking and radiation in the development of lung adenocarcinoma act on different pathways without noticeable synergy. These findings point to additive absolute risks of radiation and smoking with little biological interaction.

Table 8. Mechanistic models applied to low-linear energy transfer studies

Cohort (low-LET)	*Endpoint*	*Model*	*Analysis/Findings*	*Study*
Life Span Study	Incidence 1958–1998 of stomach, colon, liver, lung and all solid tumours and for a collection of nine tumour sites	TSCE	Evidence for radiation action both on initiation and clonal expansion	[H24]
Life Span Study	Mortality 1950–2000 for all solid, stomach and liver cancer	TSCE	Modelling of cell inactivation in clonal expansion and consequences for dose–response relationship	[J3]
Life Span Study	Incidence 1958–1998 of stomach, lung, colon, rectal, pancreatic, bladder, breast, central nervous system and thyroid cancer	Short- and long-term model	Combining short-term radiation effects with long-term cancer development	[S42, S43]
Life Span Study	Incidence 1958–1998 of all solid, liver, colon, lung, breast, stomach and bladder cancer	Biologically-based initiation-promotion model	Risk assessment for exposure at middle ages	[S44]
Life Span Study	Incidence 1958–1998 of breast cancer	TSCE	Combination of models with different radiation targets using multi-model inference	[K5]
Life Span Study	Incidence 1958–1998 of colon cancer	Two-path model adapted to molecular pathways	Separate models for two molecular pathways of chromosomal instability and microsatellite instability, evidence for epigenetic radiation action (hypermethylation)	[K6]
Life Span Study	Incidence 1958–1999 of lung adenocarcinoma	Two-path model adapted to molecular pathways	Distinct molecular pathways for radiation and smoking. Both radiation and smoking affect clonal expansion	[C8]
Canadian radiation workers	Incidence 1969–1988 of lung cancer	TSCE and multistage	Radiation effect on clonal expansion and malignant transformation	[H18]
Techa River population	Incidence and mortality 1956 (1950)–2003 of all solid cancer	TSCE	Radiation effect on initiation, evidence of additional long-term effect on clonal expansion	[E11]
Swedish haemangioma	Incidence 1958–2009 of breast cancer	Two-path model	Evidence for radiation-induced transition towards genomic instability	[E13]
Ukrainian-American	Incidence 1998–2008 of papillary thyroid cancer	Two-path model including biomarker status	Integration of a radiation biomarker into a mechanistic model and risk analysis	[K7]

2. Application of mechanistic models of carcinogenesis to high-linear energy transfer radio-epidemiological cohorts

429. Jacob et al. [J2] used the TSCE model to analyse lung cancer mortality among the Mayak workers from plutonium incorporation and external exposure [J2]. In the period 1948–2002 a total of 301 lung cancer deaths were observed among 6,293 male workers. In a previous analysis of this cohort the preferred TSCE model allowed for a dependence of the clonal expansion rate both on smoking status and internal plutonium dose, while external exposure modified the initiation step [J5]. Based on these results, the authors studied the Mayak data with TSCE models that included smoking behaviour and a potential radiation-induced bystander effect. The bystander effect was assumed to influence the initiation rate of the model in a non-linear way. Under this assumption, the data were found to be incompatible with a detrimental bystander effect. Models with a protective bystander effect did not improve the quality of fit compared to models without a bystander effect. Smoking was found to act on the clonal expansion rate. The best models included a radiation action both on initiation and clonal expansion. While the radiation action on initiation was linear, it acted on clonal expansion in a non-linear way with a levelling at large plutonium exposure rate. The preferred TSCE model was sub-multiplicative in the risks due to smoking and internal radiation, and more than additive. In a subsequent analysis with an updated data set for the male Mayak workers, the authors applied a TSCE model that included a dose threshold for the bystander effect [J4]. The preferred model suggested an effect of plutonium exposure with a dose threshold both on initiation and clonal expansion.

430. Lung cancer mortality from radon exposure in the Wismut cohort was analysed with the two-mutation carcinogenesis model by van Dillen et al. [V2]. The cohort comprised 35,084 workers among whom 461 died from lung cancer in the follow-up period 1955–1998. The two-mutation carcinogenesis model is structurally similar to the TSCE model with two stages and a net clonal expansion of intermediate cells. Assuming that radiation can only affect initiation or transformation, but not the net clonal expansion rate, the authors found a significant cell killing contribution in the second mutation rate and a large value of the lag time of 13–14 years from a malignant cell to an observable tumour. To estimate the effect of missing smoking information in this cohort, van Dillen et al. [V3] applied an imputation technique for smoking based on Monte Carlo resampling. Smoking data from a case-control study were randomly projected onto the corresponding cohort. A sensitivity analysis based on a set of 200 independent projections with subsequent model analyses yielded narrow distributions of the free model parameters, indicating that parameter values were relatively stable and independent of individual projections.

431. The TSCE model was used by Heidenreich et al. [H25] to analyse lung cancer mortality of European miners from the Czech Republic, France and Germany from exposure to radon and its progeny. All three cohorts indicated a highly significant action of the alpha particles on clonal expansion. An initiating action of the radiation only played a minor role and was not significant in the French cohort. A radiation action on the transformation stage was tested but was not found to be significant. The pattern was not changed when exposure uncertainties were included.

432. Lung cancer mortality from radon exposure was studied by Eidemüller et al. [E12] for the Eldorado miners. The cohort included 16,236 male uranium miners and mill workers. In the follow-up period 1950–1999 a total of 618 lung cancer deaths were observed. The model showed a strong action of the alpha radiation from radon progeny on the clonal expansion rate. The best fit of the TSCE model resulted in a strong increase of the clonal expansion rate until an exposure rate of about 20 working level month (WLM) per year, while this increase became smaller but still depended linearly on exposure rate, above 20 WLM per year. The authors speculated that this behaviour could be due to a bystander effect in which at exposure rates lower than 20 WLM per year more cells than just those hit by alpha particles are affected

by the radiation, while at higher exposure rates this effect saturates, and most of the observed effect is due to directly hit cells.

433. The Mayak workers were investigated by Zöllner et al. to study lung cancer mortality from plutonium exposure [Z21]. Three hundred eighty-eight lung cancer deaths among 8,604 males occurred within the follow-up period 1948–2008. Only workers with full information on internal dose were included. Plutonium remains in the lung and has a long physical half-life, and exposure conditions are therefore different from radon. Information on smoking status and alcohol status was available for all individuals in the cohort. The analysis was performed both with a standard mechanistic TSCE model, and with more complex three-stage models with two phases of clonal expansion. For the TSCE model, the alpha exposure from plutonium increased the rate of clonal expansion. The change of the clonal expansion rate was linear at low dose rates and saturated at higher annual dose rates. On the other hand, no effect of radiation on initiation or malignant transformation was found. Similar to radiation, smoking affected only the clonal expansion process. Among the possible three-stage models, three models were found to provide a plausible fit to the data. In all of these models a highly significant effect of radiation on the clonal expansion phases was seen. Furthermore, in all models the clonal expansion rates showed a linear increase at lower dose rates with a levelling at higher dose rates. It was not possible to find a model where radiation affected only the transition rates between the different stages. Depending on the model, smoking could affect either clonal expansion or the mutation rates. Consequences of the models for the shape of dose response were investigated. The preferred three-stage model predicted that at low doses the dose response curve started linearly with an initial slope considerably flatter than predicted by the TSCE model, then increased exponentially with dose and steeper than predicted by the TSCE model, and finally levelled off. The dose response curves of the two other three-stage models were compatible with the dose response of the preferred three-stage model. The findings give strong indications that the influence of plutonium on the carcinogenic process is closely related to clonal expansion.

434. The TSCE model was used by Zaballa and Eidemuller [Z1] to study lung cancer mortality from radon exposure among 58,695 male workers employed by the Wismut company in Germany for uranium mining [Z1]. In the follow-up period 1946–2003, a total of 2,996 lung cancer deaths were observed. Adjustment for exposure to silica dust was performed. The best model included a highly significant effect of exposure on the clonal expansion rate. The rate increased linearly with annual exposure at low dose rates, and then saturated at higher exposure rates of about 100 WLM per year. No indication for additional radiation effects on the initiation or malignant transformation rate was found.

Table 9. Mechanistic models applied to high-linear energy transfer studies

Cohort (high-LET)	*Endpoint*	*Model*	*Analysis/Findings*	*Study*
Mayak workers	Lung cancer mortality, 1948–2002 (Plutonium)	TSCE	Modelling bystander effect on mutation rates. Interaction of smoking and plutonium exposure	[J2]
Mayak workers	Lung cancer mortality, 1948–2004 (Plutonium)	TSCE	Model with bystander effect and dose threshold for plutonium exposure	[J2]
Wismut miners	Lung cancer mortality, 1955–1998 (Radon)	Two-mutation carcinogenesis	Radiation action on mutation rates with cell killing contribution. Radiation action on clonal expansion not investigated	[V2, V3]
Miners from the Czech Republic, France and Germany	Lung cancer mortality (Radon)	TSCE	In all cohorts strongest effect of radon exposure on clonal expansion with linear-levelling.[a] Additional effect on initiation. Inclusion of measurement errors	[H25]
Eldorado miners	Lung cancer mortality, 1950–1999 (Radon)	TSCE	Radiation action on clonal expansion with stronger increase for lower exposure rates, and smaller increase for higher exposure rates	[E12]
Mayak workers	Lung cancer mortality, 1948–2008 (Plutonium)	TSCE and three-stage model	Radiation action on clonal expansion with linear-levelling[a] both in two-stage and three-stage models	[Z21]
Wismut miners	Lung cancer mortality, 1946–2003 (Radon)	TSCE	Radiation action on clonal expansion with linear-levelling.[a] Correction for silica exposure	[Z1]

[a] Linear-levelling: Clonal expansion rate increases linearly at lower exposure rate, and levels off at higher exposure rate.

3. Application of mechanistic models of carcinogenesis to non-radiation epidemiological data

435. To gain more insight into the underlying model structure and implement biological effects, more complex mechanistic models including destabilizing mutations were developed. Based on previous work [L35], Little et al. set up a general model framework with a potentially arbitrary number of carcinogenic mutations [L34, L35]. Each stage corresponds to increasingly carcinogenic cells with a higher number of genetic or epigenetic mutations. These pre-malignant cells can clonally expand. Finally, a pre-malignant cell can acquire a malignant transformation towards a malignant cell that ultimately results in cancer. In addition, healthy or pre-malignant cells can experience one or several destabilizing mutations towards genomic instability. These models were applied to colon cancer incidence data for the period 1973–2002 from the Surveillance, Epidemiology and End Results registry [L34, L35]. Several models described the colon cancer incidence as a function of age rather well. The rates of carcinogenic mutations in the pathways related to genomic instability were several orders of magnitude higher than in the pathway without destabilizing events. It was commented that without further biological information, and only based on age-incidence data, it is difficult to single out a specific model for genomic instability in colon cancer carcinogenesis.

436. Multistage clonal expansion models with several carcinogenic mutations, but without separate pathways for genomic instability, were applied to spontaneous incidence of four gastrointestinal malignancies [L47]. The Surveillance, Epidemiology and End Results registries for the period 1975–2008 were used to describe colorectal cancer, gastric cancer, pancreatic cancer, and oesophageal adenocarcinomas. The analysis found a phase that increased exponentially with age up to an age of about 60 years, and a second phase that increased linearly at older ages. The model included an initiation step on normal tissue stem cells involving a two-hit process and leading to pre-malignant progenitor cells. These two hits may involve inactivation of tumour suppressor genes such as *APC* in the case of colorectal cancer. The authors were able to derive different time scales of the carcinogenic process: The mean time required for a pre-malignant cell until occurrence of the first malignant cell (between 15.0 and 53.2 years), the mean time from a pre-malignant cell to appearance of the first malignant cell of a surviving malignant clone (between 37.9 and 58.6 years), and the mean time of persistent preclinical cancers from the first malignant cell to time of cancer diagnosis (between 0.6 and 12.2 years).

4. Further mechanistic studies and studies including animal data

437. While cancer risk at high and medium doses can be estimated from radio-epidemiological cohorts, cancer risk estimates at low and very low doses rely on extrapolations. Brenner [B54] used biophysical arguments to discuss the plausibility for a linear non-threshold extrapolation from low (about 10 mGy) to very low doses (about or less than 1 mGy) [B54]. At doses of about 10 mGy each cell is traversed by one or only a few radiation tracks. Reducing the dose, the number of traversed cells decreases linearly with dose, however, the induced physical damage of each traversed cell would not change. This argument suggests a biophysically-based rationale for a linear non-threshold extrapolation from low to very low doses. Limitations to this biophysical argument include possible confounding effects of intercellular communication, potential differential effects of immunosurveillance when the number of (pre)malignant cells is low, the possible impacts of stem cell competition, and the possibility of different types of biological damage, and damage responses, dominating at very low doses, compared to low doses.

438. Heidenreich et al. [H23] determined doubling dose rates for lung cancer induction in male and female wild-type Janus mice for acute and fractionated gamma and fission neutron exposure. The model considered initiation and promotion and included the notion that relative risk mounts gradually until irradiations are completed for protracted exposures while it only drops off for acute exposures. Lung cancer doubling dose rate for males exposed to gamma rays was 13 mGy per day, and for neutrons 1.4 mGy per day; equivalent values for females were 7.1 mGy per day for gamma rays and 0.53 mGy/d for neutrons.

439. Risk extrapolation based on animal data that directly compares exposures of interest can be used to evaluate the robustness of models used. For example, direct comparison of life expectancy for mice exposed to acute or protracted external beam radiation with total doses below 1.5 Gy showed that such comparisons have to be made in order to evaluate how risk is modulated by dose fractionation. Conversely, following the BEIR VII approach[1] and extrapolating risk from data only for acute exposures lead to overconfident estimates that became broader with inclusion of comparisons between experimental data [H7].

[1] Biological effects of Ionizing radiation, the BEIR VII report (see https://www.nap.edu/catalog/11340/health-risks-from-exposure-to-low-levels-of-ionizing-radiation

440. To assess bone cancer mortality from ^{239}Pu, Bijwaard and Dekkers [B35] combined studies of ^{239}Pu and ^{226}Ra exposed animal data with radium dial painter data. They applied the same two-mutation carcinogenesis model on the different data sets with the aim to find similarities in the risk transfer from radium to plutonium. The animal data consisted of mouse, rat and beagle data sets with information on exposure and the occurrence of fatal osteosarcoma. It was assumed that radiation could only affect initiation or transformation, but not the net clonal expansion rate. While the parameter sets varied among the different studies, the ratio of the strength of the radiation action between plutonium and radium was similar across the studies with a factor of about eight. Using this factor, and applying the two-mutation carcinogenesis model to the radium dial painters, the authors were able to suggest a model that could predict bone cancer mortality from plutonium in humans that was in good agreement with published risk estimates based on other epidemiological and animal data. The authors argued that the higher toxicity of ^{239}Pu compared to ^{226}Ra might come from different metabolic behaviour of the radionuclides. Whereas radium diffuses in the bone volume, plutonium remains at the bone surface where the sensitive cells are located.

441. Dekkers et al. [D14] applied the two-mutation carcinogenesis model to radiation-induced acute myeloid leukaemia mouse data [D14]. From experimental evidence that radiation-induced murine acute myeloid leukaemia develops through two key steps that involve a deletion of one copy of the *Sfpi1* gene, followed by a mutation in the remaining copy, the authors adjusted the two-mutation carcinogenesis model to several data sets of X-rays- and fission neutron-exposed CBA/H mice. All mice underwent single acute exposure at young age. The model allowed for a radiation effect on the first or second mutation rate, but not on net clonal expansion. Since the exposure was at young age where only a small number of premalignant cells are present, no radiation effect on the second mutation could be determined. The dose response at the first mutation rate was linear with a potential cell-killing effect at higher doses. The obtained models provided good fits to the data. A comparison between the predictions for neutron-induced and X-ray-induced first mutation rates yielded a relative biological effectiveness for 1 MeV fission neutrons of approximately three. The authors noted that the model could be considered as a first step towards a model for human radiation-induced acute myeloid leukaemia.

442. Based on observations that more-rapidly renewing tissues could be at higher cancer risk because of the larger number of cell divisions and consequently mutations [T17], Little and Hendry [L38] developed generalizations of three recent cancer models that account for the joint maintenance and renewal of stem and transit cells. A particular focus of these mathematical models was the assessment of the probability of cancer associated with radiation or smoking. All three models demonstrated substantial variation in cancer risks, spanning over 20 orders of magnitude. However, it was shown that if cancer occurred the chance that it was caused by a dominant mutagenic exposure was within an order of magnitude. In these models few cancers arose from mutations occurring solely in stem cells rather than in a combination of stem and transit cells. However, for cancers arising from 2–3 mutations, many had at least one mutation derived from a mutated stem cell. The analysis suggested that the probability of a cancer being mutagen-induced correlates significantly with the cumulative number of stem cell divisions.

5. Summary and conclusions

443. Mechanistic models of carcinogenesis provide a way to integrate biological information into cancer risk assessment. While the structure of the models used so far is relatively simple compared to the complex process of cancer development, the models aim at describing essential rate-limiting steps. These might be related to driver gene mutations. Most analyses were performed with the TSCE model including two stages. However, several recent studies used more complex models, including three or more stages, or additional pathways. The use of more complex models involves a trade-off between more realistic descriptions of cancer development and parameter identifiability.

444. The mechanisms of radiation-induced carcinogenesis are reflected in the age and dose dependencies of the epidemiologically observed cancer incidence rates. For example, radiation action on initiation, clonal expansion or transformation leads to different age and time-since-exposure dependencies of radiation risk. The mechanistic models can therefore be used to generate and test hypotheses on the type and form of radiation action. The predictive power of the models improves with increasing biological knowledge on the underlying model structure and model parameters. Nevertheless, such model studies can only test whether the underlying assumptions on radiation carcinogenesis are compatible with the epidemiological data. They do not provide a proof that the assumed biological processes are correct [R22]. However, if the outcomes of a model study that has been carried out correctly are incompatible with data, this does provide proof that the assumptions in the model are incorrect.

445. While the structures of mechanistic models are, in principle, informed by biological processes, the direct identification of the model stages in experiments is a difficult task. Most data are derived from cancer tissue which represents the final stage of the models. So far, little experimental data exist for the pre-cancer stages from radiobiological cohorts. For example, for thyroid cancer it was possible to directly include a radiation biomarker into radiation risk assessment [K7]. For characterization of the key events and estimation of the magnitude of the model parameters also studies of non-irradiated persons can be used. Further data from radiation effects on human, animal, and organoid culture systems might then be used to refine the models and assess the impact of radiation.

446. Low-LET studies included the Life Span Study as well as several other cohorts for different endpoints of solid cancer. Almost all studies found evidence for a radiation action on either initiation, clonal expansion or both initiation and clonal expansion. A radiation effect on the transformation stage seemed to be only of minor relevance. Recent studies extended the TSCE model towards models with different pathways, and the effect of radiation on these pathways was analysed. Such pathways could be related to genomic instability. A number of studies using biologically-based models, e.g. those used by Kaiser et al. [K5, K6] and Castelletti et al. [C8], report reduced cell inactivation as the effective mechanism to stimulate clonal growth of pre-neoplastic lesions. This mechanism has been detected recently in the oesophagus of irradiated mice where p53-mutant cells could clonally expand due to resistance to radiation-induced oxidative stress [F10].

447. Lung cancer studies from high-LET radiation were mainly based on miners exposed to radon. In addition, lung cancer from plutonium inhalation was analysed. Plutonium has further potential chemotoxical and biological effects, however, such effects could not be taken into account. In almost all studies, the radiation action showed a remarkable consistency and was different compared to the low-LET studies. It acted dominantly on the clonal expansion, both for radon and plutonium exposure. Furthermore, the dose response of the clonal expansion rate was strongly non-linear. At low exposures the clonal expansion rate showed a steep linear increase with exposure rate, while for higher exposure rates it either increased with a smaller slope or levelled off. Radiation-induced bystander effect and inflammation have been suggested as cause for such kind of dose response. In the mechanistic model framework, the complex dose and age dependencies observed in miner studies, i.e. the inverse dose-rate effect and the dependence of risk on time since exposure and attained age, are a consequence of this distinctive form of radiation action. Since no residential cohorts were analysed with mechanistic models it is not possible to compare the model structure and dose response directly to studies with low-dose high-LET exposures.

448. In most studies a fixed lag time between the appearance of the first malignant cell and clinical symptomatic cancer was used. However, the progression from a potentially malignant cell towards cancer is a complicated biological process. It includes the development of dormant tumours that may remain dormant, become extinct, or continue with invasive growth after some delay time. The immune system can play an important role in this process. Implementing such mechanisms, the modelling study by Fakir

et al. [F5] found a substantially increased lag time with large variations. Currently it is unclear if such mechanisms would lead to modifications of the conclusions drawn from the mechanistic models, since these effects can be partially compensated by higher initiation rates to account for the loss of malignant cells. A detailed biological understanding is necessary to provide a more robust theoretical framework.

449. The mechanistic models are based on parameters that describe key steps in the carcinogenic process. Advances in omics technologies provide new possibilities to link these parameters to molecular measurements. For example, using two whole-exome sequencing data sets for lung adenocarcinoma and colorectal cancers, Tomasetti et al. [T16] analysed the number of driver gene mutations as rate-limiting steps necessary for cancer development. It was inferred that only a relatively small number of driver gene mutations appeared to be required for the development of advanced cancers of the lung and colon. In both cases the most likely number of required mutations was three. The effect of ionizing radiation on key pathways for the acquisition of tumour hallmark capabilities should be identified and quantified. Results of network analysis could support model design and development. While tissue microenvironment plays an important role in carcinogenesis, this has not been well described by mechanistic models and constitutes an important area of future research. An integration with other modelling approaches at various levels of disease development, and the integration with molecular data from radio-epidemiological cohorts would represent an important step forward.

450. An added value of these models is that they provide a way to translate radiation-induced cancer dynamics into risk assessment. If the assumed dynamics correlates with the real biological processes, the models can suggest ways to extrapolate risk observed in an epidemiological cohort with specific exposure conditions to different exposure situations, e.g. towards lower doses, for protracted exposures or for different ages at exposure and attained age. The studies have demonstrated that potential non-targeted effects such as radiation-induced genomic instability or bystander effect can affect the age and dose dependencies of risk. Improved understanding of these non-targeted effects, combined with an integration into mechanistic models, might add new insights into radiation-induced health consequences.

451. While the mechanistic models do not allow conclusive results on the shape of dose response, some general observations can be drawn from the current evidence. For low-LET, the majority of studies found a radiation action on initiation or on clonal expansion consistent with a linear dependence on dose rate, though there are limitations in terms of the data available at dose rates of 1 mGy/h and above with uniform irradiation. This is compatible with a linear dependence of risk at low total doses, however, different forms of dose response cannot be excluded. Noteworthy, radiation action on initiation or clonal expansion leads to different temporal risk dependencies. For initiation, radiation risk would increase only after a relatively long lag time but then remains elevated during lifetime. Radiation risk that originates from an increase in clonal expansion would start earlier but become smaller with increasing time since exposure or attained age. Little evidence was seen for radiation acting on the last transformation stage towards a malignant cell. The latter effect would manifest in a peak of radiation risk relatively short after exposure. For high-LET, lung cancer risk of miners after radon exposure depends in a complex way on attained age, age at exposure and time since exposure. Furthermore, the risk depends inversely on exposure rate. Within the mechanistic models, these effects result from the specific non-linear dependence of the clonal expansion rate on exposure rate. Nevertheless, for low exposure rates the clonal expansion rate is consistent with a linear increase. An improved mechanistic understanding of the underlying processes and robust parameter values among several studies and cohorts could provide a good basis for the transfer of risk to other exposure scenarios.

B. Bronchial airway deposition of inhaled radon progeny and cellular and tissue effects

452. Lung cancer from radon progeny poses an important radiogenic risk for the population and for workers in places with elevated radon concentration. Because of the complex lung tissue structure and the particular deposition patterns it can be expected that the carcinogenic mechanisms and, consequently, the dose–response relationship and temporal dependencies of risk are different compared to other solid cancers after low-LET irradiation. The short-lived alpha particles emitted by radon progeny ^{218}Po and ^{214}Po are deposited in the bronchial airways of the lung. The alpha particles from the airway surfaces can hit the basal and secretory cells in the bronchial epithelium and induce or accelerate the bronchial carcinogenic development.

453. Because of the biological variability of the lung structure, and the inhomogeneity of the local radionuclide distributions within the airway bifurcations, local dose distributions are highly variable. Cells located at carinal ridges receive highly elevated doses, also depending on particle size and mucociliary clearance [B10, H31]. Furthermore, because of the short range of alpha particles, the epithelial structure plays an important role when calculating the dose to sensitive target cells, and various cell types in the bronchial epithelium are located at different depths in different airway generations [I4, M30]. Hofmann et al. [H32] modelled energy deposition and cellular radiation effects from radon progeny for basal and secretory cell nuclei at different depths, and related these to inactivation, mutation and transformation frequencies using in vitro radiobiological data. Fakir et al. [F3, F4] performed microdosimetric calculations in the bronchial airway bifurcations with a Monte Carlo code and related these to transformation frequencies in sensitive target cells. More background information on lung models and radon progeny deposition patterns is provided in Hofmann el al. [H33]. In the following, the modelling efforts since 2006 are described to relate radon progeny deposition to potential mechanisms of radiation-induced lung carcinogenesis.

454. Based on experimental evidence that the deposition pattern of radon progeny is highly non-uniform within the airways of the lung, Szoke et al. applied a computational fluid and particle dynamics model to quantify local deposition patterns of inhaled radon progenies [S76]. For selected low-dose exposures, microdosimetric quantities for all epithelial cells were calculated. A mathematical model of the cellular structure of the bronchial epithelium was constructed with the aim to estimate the cell death and cell transformation probabilities. The deposition distributions of the attached radon progenies were highly non-uniform both in mine and indoor exposure situations; however, the inhomogeneity was stronger in the case of mines. For the unattached fraction, the molecular diffusion caused a more uniform deposition distribution in both cases. The number of cellular hits and the hit probability were estimated for both types of exposure for the same total amount of inhaled radon decay products. Exposure rates in mines were significantly higher, and one week of exposure in homes was compared to 4.6 min of exposure in mines. The numbers of cells which receive multiple hits and the maxima of radiation burdens were significantly higher in mines.

455. Balashazy et al. [B11] simulated cellular hit probabilities of alpha particles emitted by inhaled radon progenies in sensitive bronchial epithelial cell nuclei at low exposure levels characteristic of homes and uranium mines [B11]. Based on a bronchial airway deposition model, the results suggested that in the vicinity of the carinal regions of the central airways the probability of multiple cellular hits by alpha particles can be quite high, even at low average doses, and increases non-linearly with radon concentration. It was speculated that the high exposure burden in these hot spots might induce non-uniform dose effects or inflammation.

456. With the aim to compute the spatio-temporal distributions of cell nucleus hits, cell killing and cell transformation events of inhaled radon and radon progeny, Farkas et al. [F8] simulated radioaerosol deposition patterns in the central bronchial airway bifurcation with a human bronchial epithelium model [F8]. The results indicated that the preferential radionuclide deposition at carinal ridges plays an important role in the space and time evolution of the biological events. While multiple hits were generally rare for low cumulative exposures, their probability might be quite high at the carinal ridges of the airway bifurcations. Likewise, cell killing and transformation events also occurred with higher probability in this area. It was commented that the site-specificity of radionuclide deposition might impact not only direct effects, but also non-targeted radiobiological effects due to intercellular communication. Regarding time distributions, the probability of multiple hits in case of a uniform surface activity was low and the hits were well separated in time, while they might be quite high for the cell nuclei located in the deposition hot spot even at low doses.

457. A mathematical model of the bronchial epithelium was developed by Madas and Balashazy [M1] to study the biological effects of alpha particles emitted by inhaled radon progeny [M1]. Cell pillar types consisted of basal, indeterminate, goblet, other secretory, ciliated and preciliated cells. Assuming that cell survival probability decreased exponentially with the number of nuclear traversals, the number of inactivated and surviving progenitor cells could be estimated. Assuming equilibrium between cell death and cell division, cell division rates could be estimated at different exposure rates. The mean number of induced mutations per progenitor cell per day was calculated applying a mathematical model for mutation induction which considers both radiation-induced DNA double-strand breaks and cell divisions as events which contribute to mutagenesis. The results indicated that the mutagenic effect of chronic exposure to densely ionizing radiation is dominated by the indirect increase in cell division rate of progenitor cells due to cell inactivation in order to maintain homeostasis and not by DNA damage in surviving cells. This suggested that radiation burdens of non-progenitor cells play a significant role in mutagenesis in case of protracted exposures to densely ionizing radiation, because of the acceleration of cell turnover due to cell inactivation that increases mutation frequency. Mutation rates as a function of dose rate were found to exhibit a convex shape below a threshold, indicating the exhaustion of the tissue regeneration capacity of local progenitor cells. Further, it was suggested that progenitor cell hyperplasia occurs beyond the threshold dose rate, giving a possible explanation of the inverse dose-rate effect observed in lung cancer among uranium miners.

458. To study the effects of hyperplasia, bronchial epithelium models were developed by Madas et al. with increased number of basal [M3] and goblet cells [M4] mimicking the hyperplastic epithelium in the deposition hot spots. Basal and goblet cells were selected because histological and experimental studies showed that these cell types can increase in number upon exposure to different drugs and irritants. It was assumed that the hyperplastic epithelium is thicker than the normal one, and that the thickness of the mucus layer covering the epithelium is proportional to the number of goblet cells. It was shown that the average tissue dose resulting from radon exposure and the average hit number and dose of basal cells decreased with the increase of both basal and goblet cell number. Exposure history of the tissue might thus affect the microdosimetric consequences of present exposures. In this sense, hyperplasia induced by radon exposure could be considered as adaptive tissue response. Goblet cells can discharge mucus within milliseconds upon exposure to ROS, and the increase in mucus thickness also significantly reduced the radiation burden of the tissue.

459. To study the possible effects of radon progeny on different stages in the TSCE model, Madas et al. used a biophysical model for induction of mutations, i.e. the initiation and transformation steps, and of clonal growth [M2]. Based on previous work for dose distributions and a mathematical model for the bronchial epithelium [M1], the authors estimated the mutation and clonal expansion rates by the number of non-repaired DNA double-strand breaks and by a geometric growth model, respectively. Cell survival probability was supposed to decrease exponentially with cell nucleus hits. The number of cells in a clone

increased quadratically with dose rate and was limited to the periphery of the clone, and its perimeter increased linearly with dose rate and time. It was found that rates of initiation and transformation increased monotonically with dose rate, whereas the effective clonal expansion rate decreased with time but increased sublinearly with dose rate. The authors suggested that effects of radon exposure on both mutational events and clonal growth are significant and should be considered in mechanistic models of carcinogenesis applied for analysing epidemiological data.

460. In an attempt to link cell-based processes to cancer development and epidemiological findings, Drozsdik and Madas [D20] investigated whether progenitor cell hyperplasia can explain the levelling in clonal expansion rates above a given exposure rate observed by applying the TSCE model to epidemiological data of lung cancer among uranium miners. As hyperplasia reduces the local dose consequences of radon exposure, and its measure is expected to increase with exposure rate, hyperplasia should diminish the effects of exposure rate. A main assumption of the model was that the clonal expansion rate obtained from epidemiological data is directly proportional to cell division rate quantified by previous models to describe the bronchial epithelium, the alpha particle dosimetry and cell survival. While the authors found a parameter set resulting in a cell division rate – exposure rate relationship corresponding to the plateau in clonal expansion rate, the simulation results were highly dependent on the chosen model parameters and had a high uncertainty. The work provides an example how models at different levels of description, cell and tissue-based radon models and mechanistic model of carcinogenesis, can be integrated to investigate consequences of underlying processes on radiation risk.

Summary and conclusions

461. In summary, the mechanisms of radon on lung carcinogenesis involve effects not only at cellular level, but also at higher levels. Alpha particles from radon and its progeny have a high cell killing efficiency. Replacement of the killed cells increase the mutation rates in the descendants of the neighbouring cells. These additional cell divisions may be a significant source of mutations and may exceed the mutations induced by DNA damage. Furthermore, already mutated cells with a growth advantage might replace the missing cells faster. Cell killing may also change the tissue structure especially in case of chronic exposures, e.g. by the induction of progenitor cell hyperplasia. Hyperplasia reduces the local dose consequences of subsequent radon exposures and may in this sense be regarded as an adaptive response. Such a mechanism might be a source of differences in risk regarding acute compared with chronic effects, or regarding different dose rates. Radon progeny deposition in the bronchial airways can be highly inhomogeneous. The consequences of this heterogeneous dose distribution for cancer risk are not clear. Doses and dose rates at a macroscopic level might be very different to the microscopic situation and might also be different for exposure situations in mines and at home. Epidemiological studies of lung cancer risk from miners and residences find compatible risk per unit exposure, however, the dependence of risk on time since exposure, age at exposure or attained age are still under discussion [U12]. High local radon progeny concentrations may also lead to immune responses and result in inflammation.

462. These considerations imply that it will likely be very difficult to understand radon-induced carcinogenesis at a purely cellular level. Different levels of biological organization, from cellular to tissue level, may be involved and interrelated in the carcinogenic process. First attempts tried to link the cell and tissue-based bronchial airway models with mechanistic model of carcinogenesis to better understand the particular patterns of radon-induced lung cancer risk. Future progress may be achieved by improved integrated modelling at different levels of organization, together with experimental data that could guide such model development.

C. Intercellular communication

463. A model for cell-to-cell communication including effects of ionizing radiation was developed by Mariotti et al. [M14]. The study focused on cytokine signalling, and in particular the dynamics of IL-6 cell releases. The model was applied to experimental data in which primary human AG01522 fibroblasts were irradiated with ^{60}Co gamma rays with a dose rate of 0.83 Gy/min. The seeding medium was completely replaced with fixed volumes of freshly made complete medium before collection started. In the case of experiments with radiation, the medium was replaced with fresh complete medium before irradiation. IL-6 concentrations in cell culture supernatants were determined with solid-phase sandwich ELISA. The applied doses were 0.1, 0.25 and 1 Gy. In the experimental set-up, the net release rate of IL-6 molecules in the control cells without irradiation was a truncated bell-shaped function of time after medium change that reached its maximum release value at two hours and returned to near zero at 10 hours. Each cell emitted around one cytokine molecule per second at the time of maximum release. For cells irradiated with a moderate dose of gamma rays at 0.25 Gy, the maximum release peaked at three hours. The radiation-induced response was around one third of the response induced by the medium changes with about 0.3 cytokines per cell and second and might contribute to radiation-induced bystander effects. The study was extended including the role of ROS and the effect of alpha radiation [M15]. The analysis indicated that ROS are constitutively important to produce IL-6, playing a crucial role for the modulation of the signal emission after irradiation. Alpha radiation from an ^{241}Am source led to a similar overall qualitative behaviour as for gamma irradiation, albeit with higher maximum IL-6 release.

464. A kinetic-based model of radiation-induced intercellular signalling was developed by McMahon et al. [M27] with the aim to study signal production and response kinetics in bystander experiments [M27]. In this model, irradiated cells generate a signal for an extended time period proportional to the delivered dose, which is spatially- and temporally-dependent. Exposure to this signal above a certain threshold concentration can lead to a damaging response in cells, both in hit and non-hit cells, with a probability related to the time the cell is exposed to the signal above this threshold, rather than total signal exposure. The model was applied to different experimental studies, including media transfer experiments and modulated field exposures, and generally showed good agreement. The model suggested that bystander effects play a significant role in determining cellular survival and that the inclusion of intercellular communication might be essential to produce robust models of radiobiological outcomes in clinically relevant in vivo situations. Using a similar model, Kundrát and Friedland [K51] estimated that ionizing radiation activated the emission of bystander signals from the hit cells for about a day, while the signal itself decayed with a lifetime of about an hour, in medium transfer experiments with WTK1 lymphoblastoid cells [Z8].

465. When co-cultured with normal cells, oncogenic transformed cells are subject to the selective removal by apoptosis induced via intercellular signalling [B23, B24]. Oncogenic transformed cells are used as an in vitro model system that mimics early-stage carcinogenesis. They do not obey contact inhibition. Kundrát et al. [K50] presented a detailed analysis of the intercellular signalling involving cytokines and ROS/RNS such as superoxide anions, nitric oxide and hydroxyl radicals [K50]; through inducing membrane damage, the mitochondrial pathway of apoptosis was triggered. The selectivity of this process was given by the short range of superoxide anions released by the transformed cells. Numerous in vitro studies demonstrated that this process is active for normal and transformed cells of varying types, indicating the generality of this phenomenon. Hence, this selective removal of transformed cells upon signaling with their normal neighbours was suggested to represent a natural anti-carcinogenic mechanism [B23, B24]. This suggestion was supported by mechanistic modelling, which predicts that this phenomenon may stop the growth of a population of transformed cells and induce a kind of dormancy in early-stage carcinogenesis. This may precede and/or enable the action of the immune system [K50].

466. While radiation is known to increase cancer risk, it was shown to enhance the selective removal of transformed cells in vitro, i.e. to act in an anti-carcinogenic way. In an effort to explain this discrepancy, Kundrát and Friedland [K52] developed an extended signalling model to describe the selective removal by apoptosis. Radiation-induced enhancements of the releases of superoxide, nitric oxide and/or peroxidase from transformed or normal cells increased the induction of apoptosis under in vitro conditions. However, the involved ROS/RNS possess much shorter lifetimes and the cell densities are considerably higher in vivo than in vitro. The modelling study predicted that when a small population of transformed cells is in proximity to a larger number of normal cells, as is the case in early-stage carcinogenesis, ionizing radiation reduces rather than enhances the induction of apoptosis in transformed cells. Thus, in addition to its well-known pro-transforming action, ionizing radiation loosens the growth limit on the early-stage transformed clones. Small lesions that would be eradicated by apoptosis are predicted to escape this control mechanism upon irradiation [K53]. The signalling behaviour was predicted to be highly non-linear, arising due to spatial aspects of the underlying signalling scheme.

Summary and conclusions

467. In summary, several studies analysed in detail intercellular signalling and associated bystander effects and apoptosis induction. Although the radiation-induced modulations of the intercellular signalling are transient only, their sequelae might be of permanent nature [K52]. Importantly, the investigated signalling processes can be highly non-linear in radiation dose, time and space. It is unknown how relevant these potential non-linear effects are for radiation-induced carcinogenesis. A big challenge is the transfer of these mechanisms and results from in vitro to in vivo conditions. Further theoretical and experimental research is needed to elucidate this issue. On the one hand, this includes experiments to test model predictions for conditions close to those in vivo that still can be realized in vitro, for instance densely seeded cells with increased levels of antioxidants in the growth medium. On the other hand, the respective models shall be further developed, in particular to study the effect of radiation exposure on these processes. A promising way to infer on the consequences of radiation-modulated intercellular signalling processes for cancer risk is the implementation of these processes and their models into mechanistic models of carcinogenesis.

V. IMPLICATIONS FOR CANCER RISK INFERENCE

468. In chapter IV recent findings relevant to mechanisms of radiation carcinogenesis at low radiation doses have been considered. In this chapter the intention is to summarize the implications of these findings relevant to the inference of radiation cancer risks at low doses and low dose rates. These will be taken by section as presented earlier.

A. DNA damage

469. The basis of cancer risk inference at low doses and low dose rates currently draws upon the knowledge that DNA damage is induced in linear proportion to radiation exposure and any damage site has a small but finite probability of leading to mutations of DNA sequence. Some such mutations may modify the function of key genes controlling cell proliferation, alter transcription of key genes and expression of encoded proteins, or other hallmark of cancer characteristic, thus increasing the risk of

cancer presentation in the individual sustaining such a mutation. The forms of DNA damage considered of most importance following ionizing radiation exposure are DSBs and sites of clustered damage that may include any induced lesion type in close proximity to other induced lesions. An important role for ROS and other reactive species is apparent at low dose exposures.

470. Substantial data are available from nuclear foci assays involving chromatin and core repair proteins (e.g. 53BP1, γH2AX) that are taken to reflect the frequency of induced DNA double-strand breaks. These substantiate findings that DNA double-strand breaks are induced in linear proportion to dose down to 1 mGy at least. While double-strand breaks are of importance, other forms of DNA damage are likely to contribute to the effects of radiation exposure, these include ROS-mediated damage, single-strand breaks and base damages. Various assays (alkaline comet assay, specific ROS stains) point to linear induction of these types of DNA damage.

471. The chromatin foci assays are not direct measures of DNA double-strand breaks but rather reflect the early stages of DNA damage signalling (γH2AX) or repair (53BP1). Physical measurements of DNA strand breakage are generally less sensitive than the foci assays but are direct measurements of damage. Among the most sensitive of the physical assays for DSBs is the neutral comet assay, and the available data are consistent with the conclusion that DSBs are formed in linear proportion to radiation dose.

B. DNA damage signalling, chromatin remodelling and epigenetics

472. The main molecular mechanism that drives DNA damage signalling, is post-translational modification of specific proteins, including ATM, particularly by phosphorylation. Based on limited data relating to phosphorylation of key proteins in the signalling pathway following acute exposures to ionizing radiation (X- and γ-rays), DNA damage signalling appears to be linear with dose over a 10 mGy to 1 Gy dose range.

473. The activation of the DNA damage response has been investigated using restriction enzyme generated DSBs or breaks induced by other enzymatic systems as a model for radiation-induced breaks. These enzyme-generated breaks are simpler in end structure than radiation-induced breaks. Nonetheless single and multiple DSBs generated by restriction enzymes do effectively trigger a DNA damage response as assessed by the initiation of p53-dependent processes and the formation of repair complexes (e.g. [B6, W14, Z19]) and thus represent a valid model system for radiation-induced breakage. While drawn from a model system, these findings are consistent with activation of the DNA damage response by a single DSB per cell and the notion that the repair of such breaks utilizes similar pathways as does the repair of radiation-induced DSBs, and tend to confirm the linear induction of phospho-ATM.

474. A general conclusion is that loss of functional chromatin remodelling and modification impairs the repair of DSBs and confers radiosensitivity to cells. The existence of radiation- and cancer-prone human disorders associated with single DNA damage response gene mutations and the radiosensitivity and cancer proneness of mouse models carrying mutations in single DNA damage response genes, provide evidence for crucial importance of functional DNA damage response genes for cancer risk. Most likely this also holds for low-dose exposures based on outcome of model systems carrying a single DSB. Evidently the cohort of genes that participate in the response to ionizing radiation-induced DNA damage, is substantially larger than previously anticipated and this may have implications for the proportion of the population carrying heterozygous mutations in these genes and consequently is at increased risk of cancer after exposure, and this may impact on the shape of cancer dose-response curves.

475. Collectively, existing data suggest that radiation-induced effects on DNA methylation depend on tissue/cell type, sex, and species. Moreover, radiation type or quality, dose, and time after exposure have to be taken into consideration as well as the technologies to determine DNA methylation. The data also suggest that dose–response relationships are complex. The evidence from the scarce number of studies suggests that low-dose radiation is capable of causing changes to DNA methylation and that the methylation changes might differ between low and high dose. The methylation data are inconclusive when related to transcriptional responses. To address the latter point, future work should focus on the use of high-resolution technologies to analyse simultaneously DNA methylation and transcription.

476. Epigenetic endpoints can be difficult to study because of the many different factors that influence epigenetics to an equal extent as low doses of radiation and it can therefore be difficult to segregate different influences from one another. A possible approach to deal with this complication was suggested in the work by Tang et al. [T10]. This study, looking at lncRNAs compared responses to low doses of radiation in two strains of mice after removing from the complete list those lncRNAs associated with physiological states such as oestrus cycle.

C. Effects on signal transduction pathways

477. The effects of low-dose radiation exposure on intracellular signalling pathways have been addressed mainly in immune cells, either purified from exposed animals or exposed in vitro after purification. Low-dose radiation exposure effects on signalling pathways can be seen in all cell types examined: human purified monocytes, human B- and mast cell lines, endothelial cells, murine and rat mast cells, splenic $CD4^+$ T-cells, splenocytes.

478. In these cells, radiation modulates the activity of several kinases and transcription factors belonging to different signalling pathways. Distinct signalling pathways may be activated after exposure to low- and high-dose radiation in the same cell type. A given pathway can be activated or inhibited by low-dose radiation exposure in different cell types.

479. A radiation dose as low as 1 mGy can activate antioxidant and innate immune signalling pathways in murine splenocytes. The kinetics of these effects are different according to the dose delivery (acute or in three fractions).

480. The downstream consequences of signalling pathway activation/suppression in relation to carcinogenesis has received little attention. Therefore, conclusions on relevance of these observations to cancer risk inference cannot be drawn.

D. Gene and protein expression

481. There are many studies of gene expression responses to radiation exposure, many at high and moderate doses and a few that include low doses. Much of the work has been carried out with a main purpose to improve biological dosimetry and as such uses peripheral blood lymphocytes and their derivatives.

482. While some studies have observed a different set of genes or proteins responding to radiation, there is no consensus on what defines a gene set that is uniquely responsive to high dose as opposed to an exclusively low-dose responsive gene set. This may in part be due to inherent technical variability in the

method predominantly used to date (i.e. gene expression analysis using microarrays), the variety of methods that have been applied and the selected time points which vary between studies. In the future the application of more readily controlled methods including digital PCR may lead to improvements in consistency and reproducibility, but it remains possible that the biological responses are in fact inherently variable, depending on precise cells of origin, donor and probably other factors.

483. As has been stated, cancers can result from altered differentiation of cells leading to uncontrolled proliferation and other hallmark characteristics, and patterns of gene expression are a substantial driver of differentiation state. Therefore, gene expression patterns are of relevance to carcinogenesis, and radiation has been shown to affect the expression of cancer-related genes, notably p53-responsive genes. However, the majority of available data concern gene and protein expression alterations 24–48 hours after radiation exposure, with relatively little information available on the persistence of such alterations.

484. The data available from gene and protein expression studies are insufficient to draw substantive conclusions on the relevance of these studies for low-dose radiation cancer risk inference due to variable findings between studies and the generally short time scale of reported studies.

E. DNA repair and effects on somatic cells

485. Several studies have shown that the damage revealed by chromatin/repair foci can be effectively repaired by normal cells over the course of a few hours, the repair is generally observed to be complete with foci level returning to control levels. A potentially important exception to this finding concerns repair after very low doses of one to a few mGy low-LET exposure in cultured human fibroblasts. A number of studies report that foci levels in this situation are not found to be fully repaired, these studies report that small residual level of excess foci remain, around one additional focus per 10 cells. Evidence points to the lack of sufficient ROS after these very low doses to induce repair. Some evidence indicating that base damage repair can be induced by low radiation exposures is also available. In respect of the implications of this incomplete/inducible repair for cancer risk, the interpretation is dependent on having a clear understanding of the subsequent fate of the excess damage bearing cells. Some studies suggest that the cells with excess residual damage enter apoptosis after one or a few cell cycles; this effectively removes the cells from the pool of potential cancer cells. In contrast to the above-mentioned results, in vivo studies on lymphocytes from individuals undergoing computed tomography examination are not consistent with incomplete repair and so drawing general conclusions that apply to all cell types is not currently possible. There is overwhelming evidence that DNA repair pathways NHEJ and HRR contribute to repair of low dose induced DSBs, depending on cell cycle phase, except in mitosis. The HRR pathway is less prone to errors and therefore plays a role in reducing cancer risk. It is notable that some evidence indicates that HRR is the predominant pathway on stem cell populations, likely protecting the stem cell pool from the accumulation of induced mutations.

486. The repair assays based on chromatin/repair foci also revealed inter-individual variation in DSB foci parameters in primary fibroblasts exposed to low doses of X-rays in a small survey of apparently normal people suggesting that hypomorphic genetic variants in DNA damage response may contribute to differential susceptibility to cancer after low-dose radiation.

487. The consequences of DNA damage in somatic cells can be observed in the form of DNA sequence mutations and chromosomal damage. The induction of micronuclei, a sensitive endpoint that reflects chromosomal breakage and whole chromosome loss events, has been observed to be linear with dose in the low dose range down to 10 mGy both in vivo and in vitro. A plausible mechanism for the linear non-

threshold induction of micronuclei by X-rays is the observation that DSB repair does not function in the mitotic phase of the cell cycle, even not in repair proficient cells. It is conceivable that the mitotic period without repair of DSB in wild-type (repair proficient) cells would allow the generation of micronucleus in a frequency directly related to the initial frequency of DSB induction (which is linear). The formation and subsequent re-integration of micronuclei appears to be an important pathway for the generation of chromothripsis, highly localized and extensive genome re-arrangement that has been observed in several forms of cancer (though not specifically radiation-induced cancers). Whereas the induction studies suggest that micronuclei are formed in linear proportion to dose, little is known on the dose relationship for the re-integration of micronuclei, although it has been observed that cells carrying micronuclei can undergo at least some cell divisions. Furthermore, low dose exposures have been reported to stimulate the integration of DNA into chromosomal locations.

488. LOH is commonly observed in cancer genomes and can be generated, among other mechanisms, through chromosomal translocations and deletions. Available data indicate that LOH is caused by radiation in linear proportion with dose down to 50 mGy low-LET radiation. The age-dependence of induced chromosome aberration yields where some studies suggest that in the young yields are lower than in adults, may have an impact on dose-response shapes in populations. However, such age-dependent variation is not commonly observed in stem cells, and thus may not be a significant factor in determining cancer risk at different ages.

F. Genomic instability, bystander effects, damage/effects on non-nuclear cellular components, adaptive response and hyper-radiosensitivity

489. Thus far, the focus has been on direct damage to DNA and the consequences that directly follow. However, radiation causes damage to all cellular components and observations indicate that there are non-DNA targeted responses to radiation exposure.

490. Substantial attention has been given to effects of radiation exposure on mitochondria and mitochondrial function. Mitochondrial damage can mediate apoptosis independently of damage to nuclear DNA. Low-dose and fractionated exposures have been reported to lead to both mitochondrial fusion and fission. Conflicting findings on the effects of radiation on ROS levels, mitochondrial mass and cell proliferation have been reported. Nonetheless there appears to be a consensus that radiation exposure even at low doses, e.g. 20–100 mGy, low-LET radiation can affect energy metabolism. Such changes may be of relevance to cancer risk inference given the hallmark characteristic of cancer of altered cellular energetics, and generally a shift to glycolysis [Z22].

491. More recently available studies on the non-targeted effects of transmissible genomic instability and bystander phenomena do not affect or substantially challenge the Committee's prior judgements as most recently set out in the UNSCEAR 2012 White Paper [U9].

492. Notwithstanding the above considerations, cancer risk modelling has demonstrated the potential importance of transmissible genomic instability and bystander phenomena for cancer risk inference. Thus, it remains important to generate more definitive data on the operation of these processes after radiation exposure in vivo, particularly at low dose and low dose rate.

493. Adaptive response studies remain difficult to interpret and bring into any low-dose radiation risk assessment framework. There remains no consensus on the adapting dose range or duration of the

adapting signal, and this is further complicated by the lack of a consistent mechanistic basis for the phenomena. Additionally, some negative studies continue to be reported.

494. Given that all humans live in an environment with natural background radiation, it may be that everyone is, to an extent, adapted. Also, if adaptive responses were a major modulator of cancer risks in exposed populations, one might expect differences in the magnitude of cancer risks assessed in occupational (chronic low/moderate exposures with changes in dose rate) studies and those in the atomic bombing survivor studies (acute and frequently high-dose exposures), with the occupational studies finding lower risk – this is not what is observed. Furthermore, given that radiation is not the sole, or usually predominant cause of cancer, and there are multiple contributory risk factors, any modulation by radiation adaptive response is likely to be very small and therefore masked by the effects of the other contributory risk factors such as diet, smoking and exposures to other agents. A broadly similar argument can be made in relation to the small risks associated with low-dose and low-dose-rate exposures.

495. Hyper-radiosensitivity has been observed in cell culture systems and to a limited extent in vivo, and most notably in stem cells in vitro and in vivo. The loss of cells carrying damage at low doses is expected to serve to protect against cancer in that potentially induced mutation-carrying cells will be lost through cell death and cannot therefore contribute to cancer development. However, the depletion of stem cell pools can cause an increase in proliferation of the stem cells to replenish the pool, this carries the risk of accumulation of replication-associated mutations. It is therefore difficult to interpret the impact of the hyper-radiosensitivity phenomenon on cancer risk.

G. Stem cells and target cell populations for radiation carcinogenesis

496. Stem and early progenitor cells are viewed as main target cell populations for radiation cancer due to their long lifespan in the organism. Stem cell populations in several tissues have been shown to respond to low doses of radiation, and in terms of DNA damage response they appear to respond in a similar fashion to other somatic cells, and this holds true for mutation frequencies that do not strictly correlate with replication cycles traversed. The phenomenon of hyper-radiosensitivity has been described in stem cell populations, as noted previously the impact of this in relation to cancer risk is not certain.

497. However, some stem cells, most notably embryonic stem cells, but also induced pluripotent stem cells, depend more on HRR of DNA damage than NHEJ. The HRR pathway preference likely serves to preserve genome integrity and avoid mutation in the presence of DNA damage given the higher fidelity of recombinational repair compared to NHEJ. This is likely therefore to act to protect stem cells from oncogenic mutations, much as the retention of parental DNA strands through DNA replication and cell division that has been observed in some stem cell populations.

498. The basal and secretory cells are target cells for radon induced bronchogenic cancer. The alpha emitting radon decay products are located on the bronchial epithelium about 30 μm above the cells and the high-LET alpha particles can produce direct DNA damage in the target cell populations.

499. Some stem cell populations enter apoptosis readily following radiation exposure, possibly due to their hyper-radiosensitive phenotype, and this likely serves to protect from cancer. Loss of cells through apoptosis though may lead to substantial proliferation of stem cell populations to restore tissue that brings risk of replication-associated mutation, at low doses this is expected to be a minor consideration.

500. A recent study has demonstrated how low dose (50 mGy) radiation exposures can lead to the preferential expansion of cells and clones carrying mutations in the *Trp53* gene [F10]. This supports a role of low doses in promoting the growth of pre-existing pre-neoplastic clones.

H. Effects at the whole organism level

1. Immune system and cytokine responses

501. In section III.H.1 recent work on the effects of low-dose/low-dose-rate radiation exposure on the immune system was considered. This section includes studies considering in vivo human exposure, including atomic bombing survivors and environmental, occupational or accidental exposure, and experimental exposure of cells and mice to low-dose, low-dose-rate and/or repeated low-dose exposure.

502. There remains no consensus from published studies whether low-dose radiation exposures serve to stimulate or reduce immune functions. This most probably stems from the nature of the immune system, which is an integrated network of highly specialized/differentiated cells. Radiation exposure has different effects on different cell types, and it is difficult to infer the effects on the protective functions of the immune system at the organismal level. Low-dose radiation exposure effects may depend on the immune status at time of exposure, with exposure on an inflammatory background reducing inflammation and exposure on a "neutral" background inducing inflammatory responses.

503. However, the association of certain alleles of immune genes with breast cancer development in radiological technologists suggests that dysregulation of immune functions may allow the development of cancers after protracted low-dose exposure. In addition, there is evidence that low-dose radiation exposure can reduce the efficiency of transplantation of cancer cells in immuno-competent mice. However, given the mixed nature of the findings, it is not possible to draw a conclusion on the effects of low-dose/low-dose-rate exposure on cancer immunity, and especially cancer immunosurveillance/ immuno-editing.

504. Available studies on the immune status and immune functions of those living in areas with high natural background radiation levels or professionally exposed individuals do not allow any consistent conclusions to be drawn at that time. Some of the late effects of radiation exposure in vivo appear to accelerate the normal process of immunological aging. Premature immunosenescence may increase the susceptibility to cancer and non-cancer diseases.

505. Clearly the immune system, and particularly cancer immunity, could be a very important factor in modulating post-radiation cancer risk. However, the available data are not sufficiently consistent or robust to draw conclusions relating to the use of such data in the inference of cancer risk.

2. Carcinogenesis

506. Two themes that relate to low dose and carcinogenesis depend on the proliferation of stem and/or lineage-specific cells. While moderate and high doses of radiation "exhaust" stem cell pools and lead to accelerated aging, low doses of radiation seem to have the opposite effect. An extreme example of this is

a transgenic model of accelerated aging homozygous mouse model, klotho knockout mice where exposure to low doses significantly extended lifespan of animals.

507. Cells responsible for the development of new blood vessels proliferate following low/moderate doses of radiation leading to increase of neovascularization in vivo. Several cancer and non-cancer studies support this concept pointing to an alternative mechanism by which low/moderate exposures may contribute to carcinogenesis.

508. Other endpoints that relate to later stages of cancer development such as cell migration, cell invasiveness and EMT have not been studied in great depth following low-dose radiation exposure, and the available evidence is mixed. There is the potential for low doses to affect these endpoints that relate to tumour metastasis, and there are reports of low-dose exposure reduction of migration and invasion.

509. Both of these types of cell proliferation (i.e. stem cell proliferation and proliferation of vascular cells and the growth of the vasculature) can be associated with carcinogenesis, both at the onset and during cancer development.

510. Overall, the most serious obstacle in data interpretation from wild-type animal studies is the fact that the age of animals has great effects on all aspects of their health. This makes room for data variation. Statistically significant findings are most often observed in studies where only animals of the same age were used.

511. Not surprisingly, low-dose radiation studies of mutant animals where the mutation is relevant for carcinogenesis, are far more affected by the presence of a mutation than by differences in animal age. For that reason, studies on mutant homozygote or heterozygote animals exposed to low-dose radiation often produce the most robust data. Some of these studies are focused on tumour suppressor genes where LOH leads to cancer development.

512. Another group of studies explored animals with overexpression of different oncogenes. Variable effects were observed ranging from increased to decreased to no effect on radiation induction of cancers. While studies in mutant mice produce the most robust data – increase or decrease in cancer incidence depends on what the exact mutation is and whether other mutagens are present in the environment at the same time. Finally, studies focused primarily on DNA damage resolution – mutations introduced by low-dose radiation exposures in animals with intact repair are the most robust in those cases when animals of same age were used. While none of these studies could be directly related to carcinogenesis at this time, there remain examples of animal studies where only apparently beneficial effects of low-dose exposures were registered.

513. Because of the complexity of multicellular organisms and differences between cells and tissues it should be appreciated that the same epigenetic modifications and mutations lead to different consequences in different parts of the body. Thus, an animal may show no life shortening or even a lifespan extension after exposure to a specific low dose, even if some of its non-proliferating cells accumulate mutations that could under different circumstances lead to cancer development. With the decrease of dose, ionization events became progressively less frequent and their burden on the organism as a whole may cease to be stress to the organism.

514. As noted above, while many effects are context dependent, it is difficult to reach an overall conclusion on the damaging compared with beneficial effects of radiation as reported in in vivo studies. All animal experiments dealing with low doses and cancer induction suffer from the potential difficulties of limited statistical power, and this could be contributing to the mixed evidence available.

515. For most responses high-LET radiation is more damaging than the low-LET radiation nevertheless for carcinogenesis the response is complex. It depends in many cases on whether the exposure is external or internal, on the exact cancer, the tissue type of cancer, the sex and age of exposure of the animal, dose and dose rate and many other factors.

I. Integration of data at different levels of organization and modelling of cancer mechanisms

516. In chapter IV, approaches to modelling and inferring cancer risk were considered. It is evident that the available models can incorporate information drawn from biological/mechanistic studies, although the extent to which this has been successfully demonstrated varies with the study objective. The general approach of integrating biological data into cancer risk models clearly holds promise for the improvement and refinement of cancer risk inference.

517. Broadly, the conclusions to date from modelling approaches suggest *(a)* low-LET radiations could act to initiate or promote carcinogenesis; *(b)* high-LET radiations for lung cancer act largely through promotion/clonal expansion. This difference relating to the predominant stage at which radiation acts has substantial implications on the dependence of radiation risk on age at exposure, attained age and time since exposure. The inverse exposure-rate effect observed for lung cancer after radon exposure in miner studies and the particular form of high-LET radiation action might be related to the lung-specific tissue architecture and exposure situation.

518. Modelling of lung airway radiation dose deposition from radon inhalation suggests non-linear effects due to the different levels of biological organization involved and the highly inhomogeneous radon deposition in the bronchial airways. It is anticipated that cell repopulation and/or inflammation might play an important role in radon-induced lung carcinogenesis.

519. Models for intercellular signalling suggest processes that can be highly non-linear in radiation dose, time and space. It is currently unknown whether these effects might affect dose–response relationships for cancers. A critical issue is the transfer of these mechanisms from in vitro to in vivo conditions.

520. To benefit most from the modelling approaches incorporating biological data, there are a number of specific data requirements. These and other research needs are considered in chapter VI.

VI. DIRECTIONS FOR FUTURE RESEARCH

521. It is apparent that there remain many gaps in the evidence and knowledge on the biological mechanisms relevant for low-dose radiation cancer risk inference. Beside these gaps, there are areas where findings need consolidation or replication to provide the robust evidence. It is noted particularly that while many studies include consideration of effects of a 100 mGy low-LET exposure, relatively few consider doses below that point. There remains a need for studies that explore the sub-100 mGy region more thoroughly and include moderate doses for the purpose of comparison at different dose levels and extrapolation between dose levels. The following paragraphs summarize the key areas where the Committee considers that further research is required. The order below reflects the order in which topics were considered in the main body of the annex and does not reflect the Committee's view on the relative importance or urgency of the different areas of research.

522. In the area of DNA damage, it is important to provide better quantitative data on the induction and frequency of complex DNA damage sites, and to develop more sensitive approaches to provide physical quantification of DNA double-strand breaks. Additionally, further information on the induction of damage to mitochondria by ROS, and the specific targets within the mitochondria could be useful.

523. Concerning DNA damage signalling, epigenetic effects and chromatin remodelling, the Committee encourages research to:

(a) understand the dose/dose-rate/quality dependence of epigenetic alterations caused by radiation exposure and if dose–response relationships and/or persistence of induced changes vary substantially from those for the formation of mutations and chromosomal changes as this could have substantial impact on judgements on the model used for low-dose risk inference;

(b) understand the dynamics of post-translational modifications (especially phosphorylations/ dephosphorylations) by cutting-edge proteomics, identification of the relevant kinases and phosphatases and integration with de novo transcriptional analysis. This understanding would provide a basis to determine cell fate after radiation;

(c) gather information on sites/genes that low-dose exposures methylate/demethylate, or otherwise epigenetically alter and the impact of these alterations on transcription.

524. The Committee considers that research is needed to improve the reproducibility and inter-comparability of results from gene/protein expression studies to determine what, if any, are the differences in response to radiation at differing dose levels, dose rates and with irradiations of different types and qualities. As noted above, steady-state analyses should be complemented by analysis of de novo transcriptional response. Information on the duration of any alterations is required. Additionally, it is important to follow any changes over longer time periods to help ascertain their relevance for diseases or adverse outcome pathways of importance.

525. With respect to DNA repair and effects on somatic cells, the Committee encourages research to:

(a) Provide a better understanding of the persistence of residual DNA damage and the fate of cells carrying these after milligray-level exposures;

(b) Understand if radiation exposures do increase the occurrence of chromothripsis and the relevant exposure–response relationships, this will help elucidate the relative importance of the pathway for carcinogenesis compared to conventional mutation/ chromosomal alteration/epi-mutation induction;

(c) Provide greater insight into the generation and persistence of ROS and the consequent cellular effects under physiologically realistic low O_2 conditions;

(d) Provide information on the frequency of integration of DNA fragments into the genomes of irradiated cells to allow a better assessment of the contribution of chromothripsis to low-dose radiation carcinogenesis.

526. In relation to genomic instability, bystander effects, damage/effects on non-nuclear cellular components, adaptive response and hyper-radiosensitivity, the Committee encourages research to:

(a) Determine whether in vivo exposures lead to persistent elevation of mutation/chromosome aberration/epi-mutation/chromothripsis frequencies that drive carcinogenesis in human or animal model systems, and to confirm if thresholds for the induction of genomic instabilities exist;

(b) Ascertain if bystander induction of cancer occurs in humans after ionizing radiation exposure and whether bystander effects are generally cancer risk enhancing or risk reducing;

(c) Assess the relative importance of adaptive response by comparison with the influences of other contributory risk factors.

527. The immune system has potentially a substantial impact on cancer risk modulation. The Committee, therefore, encourages research to determine how inflammation and immune functions are affected by low-dose and low-dose-rate exposures in vivo (environmental, occupational and experimental) and how any such effects modulate cancer risk.

528. In relation to carcinogenesis, the Committee considers that further data are required from human and animal model radiation cancers, and organoid culture systems to build up a picture of the key events that convert normal cells to cancer cells, and the dose- and dose-rate–effect relationships, particularly in the low to moderate dose range for these events occurring prior to presentation of disease. Further work that considers the impact of low-dose and low-dose-rate exposures on later stages of carcinogenesis is also required, considering the preliminary information available on neovascularization, and endpoints related to tumour metastasis. These data will be crucial for the further development, assessment and validation of mathematical modelling approaches to integrate biological with epidemiological information for cancer risk inference. Such work would be facilitated by the availability of validated biomarkers of radiation disease/effects that could be integrated into epidemiological investigations. Mathematical models provide a framework in which biological and epidemiological data can be combined for risk inference. Important issues that could be addressed using models include: relative importance of genomic instability/bystander effects/adaptive response, relative importance of mutation/epi-mutation, etc. (when data of exposure dependence is available for each).

529. As stem and early progenitor cells are generally taken to be the target cells for radiation carcinogenesis, the use of appropriate model systems is important as such cell populations may respond differently to low-dose exposures. Notably, further research to provide a better understanding of competition of stem cells in some tissues after milligray and sub-milligray exposure levels (i.e. sparse hits of incremental dose) is likely to be of importance for risk inference.

530. To obtain more robust evidence in the areas above, the Committee encourages the close multidisciplinary working of radiobiology/epidemiology/mathematical modelling that has the potential to generate the critical data required to develop predictive models for risk inference that make use of and capitalize on all available robust and reliable knowledge of biological mechanisms and apply the knowledge to risk inference. It will be important that mechanisms defined using in vitro conditions are translated to in vivo conditions in humans; both experimental and theoretical approaches can be expected to be informative.

VII. CONCLUSIONS

531. Returning to the questions that were set out to guide this evaluation (see chapter I), below the Committee summarizes the evidence and findings of this review relevant to each of the issues:

For which biological mechanisms and pathways is there evidence that indicates that they can affect the frequency of cancers following exposure to ionizing radiation, including at low doses and dose rates?

532. There is very robust and reliable evidence that following the induction of DNA damage incomplete, failed or otherwise dysfunctional DNA damage responses contribute to induced mutation and chromosome damage and thereby affect the occurrence of cancers after exposures at all doses and dose-rates studied, these responses relate to *(a)* direct damage to DNA in the form of DSBs and complex lesions; and *(b)* damage attributable to the generation of reactive oxygen and related species through effects on mitochondria. DNA repair activities can serve to reduce yields of mutations and rearrangements, but they are not 100% effective, do not operate in mitosis and some repair pathways may be induced or regulated by exposure. While not the main focus of this review, it is noted that those carrying certain variants of DNA damage response genes may be radiosensitive and at increased risk of cancer spontaneously and after radiation exposures.

533. Additionally, there is evidence that variants of genes involved in chromatin remodelling also affect cancer risk, indicating that chromatin remodelling pathways are likely of relevance for radiation cancer risk as well, though evidence relating to low dose/dose-rate exposures is not available; the same may apply to epigenetic regulatory genes also. Modelling studies with some support from experimental work indicate that low dose exposures can promote the growth of pre-existing pre-malignant cells and clones in tissues. This represents a pathway in addition to the direct induction of mutations in cells by radiation whereby low dose exposures are anticipated to increase cancer risk. Limited evidence from occupational exposure of medical workers indicates that gene variants relating to immune functions can modulate cancer risk, whether radiation exposure per se can stimulate or suppress such cancer immunity is unclear. There is an emerging understanding that radiation exposure, a least at moderate dose levels may stimulate tumour angiogenesis; while data at low dose levels are limited, if substantiated it may serve as a pathway to promote carcinogenesis. Some experimental studies suggest that low doses can impact on endpoints related to tumour metastasis but the available results are mixed and inconclusive at this stage.

What are the differences in utilization and/or activation of these pathways and mechanisms at low doses compared with moderate doses?

534. DNA damage response operates at all dose levels, but with differences in utilization of specific pathways (for example at low doses responses related to reactive oxygen damage are likely to predominate as that form of damage is considerably more frequent than DNA double-strand breaks). The relative importance of complex/clustered damage sites and their repair is greater at higher dose levels (and after high-LET exposures) as such lesions are more common, nonetheless complex/clustered damage does occur after low-dose exposure to low-LET radiation. Some pathways of DNA repair show inducibility such that they are up-regulated after certain exposure levels, and this may apply to DSB repair and base damage repair, it is not clear if this extends to complex/clustered damage sites. There are known thresholds for the G2/M phase cell cycle checkpoint which is triggered only following doses in the range 200–500 mGy and above for low-LET radiation. Some studies of gene expression following radiation exposure suggest differing responses at low as opposed to moderate/high doses. However, there is no clear consensus on the pathways specifically regulated at differing exposure levels and the alterations are generally reported at only short times after irradiation, their relevance for carcinogenesis is therefore not clear. The dose range over which the potential promotional effects of radiation on pre-malignant cells

operates is unclear. The dose range over which cancer immunosurveillance operates is not known, but evidence arises from low dose/chronically exposed occupational groups.

What evidence is available on the form of the dose–response relationships for these mechanisms?

535. The dose–response relationships for mutation (LOH) and micronuclei are linear in form in the low dose range down to at least 50 and 10 mGy low-LET radiation, respectively. Similarly, the dose-response for DNA damage response activation is linear down to 10 mGy low-LET radiation. The form of dose-response for the cancer immunosurveillance is not known, but the process has been observed to protect from cancer in occupationally-exposed groups, and in mice, immune system activation signalling operates at 1 mGy low-LET radiation and above. The potential promotional effects of radiation appear to operate at 50 mGy low-LET radiation and above, but further data are required.

Considering the mechanisms identified above, can any conclusions be drawn as to their overall influence on the dose–response relationship between cancers associated with radiation exposure at low doses compared with moderate doses?

536. The knowledge of the mechanisms that affect cancer risk at low doses noted above suggests that an overall threshold for cancer induction is unlikely, and there is evidence that the known mechanisms operate at least down to 10 mGy. Furthermore, the mutational mechanism would imply a dose-risk relationship without a threshold. At the lowest dose levels, where DNA double-strand breaks are induced in say 1 in 10 or fewer cells (around 2 mGy low-LET exposure), ROS mediated effects are likely to predominate, and these include the potential promotional action of radiation.

Are there ways to link information on the biological processes and mechanisms found to be relevant to human cancer and existing epidemiological data on incidence of disease in exposed populations?

537. The answer is positive, and the evidence considered in this review points to two ways in which biological information can be linked to existing epidemiological data. The first is through the application of radiation-related disease biomarkers in epidemiological investigations of risk. Such markers have the potential to reduce the time taken to obtain epidemiological data through the use of robust surrogate markers of disease when and where available and improve its accuracy by means of reducing the impact of confounding by competing causes of death and co-morbidity. The second approach entails the integration of qualitative and quantitative biological data into mechanistic modelling of cancer risk. The accuracy of models can be enhanced by inclusion of data on the specific steps in carcinogenesis, particularly where quantitative data are available. Furthermore, the modelling approaches can be used to help inform on judgements on the relevance of specific pathways for carcinogenesis.[2] Arguably the mechanistic modelling of radiation risk as reviewed in this annex indicates that quantitative mechanistic modelling of radiation risk is more advanced than that in the chemicals arena, though more work is required to provide the qualitative and quantitative information for low dose and low dose-rate mechanistic modelling.

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[2] The Committee notes that other researchers and organizations recognize the value of integration of epidemiological and biological data through mathematical models [Preston, 2015; Chauhan et al. 2019], notably the National Council on Radiation Protection and Measurement that has published recently in its Report 186 "Approaches for integrating radiation biology and epidemiology for enhancing low dose risk assessment" [N11]. These publications advocate the application of adverse outcome pathway approaches for low-dose radiation risk assessment as they are utilized in chemical risk assessment.

Is there evidence for tissue-specific variation in the mechanisms of response to ionizing radiation that relate to the differing sensitivity of tissues to radiogenic cancer?

538. There is long-standing evidence that the number of mutational steps required for leukaemia is less than in the case of solid cancers, and this impacts on the time to presentation of leukaemia by comparison with solid cancers. Skin stem cell populations have been found to have different responses to radiation in terms of apoptosis and this appears to relate to the higher risk of basal cell carcinoma. More generally stem cell populations appear to have a greater dependence on HRR than NHEJ, this may serve to provide a relative degree of protection of stem cell populations from induced mutation.

Are the mechanisms that operate and can be associated with disease development similar following low- and high-LET exposures?

539. Given the higher frequency induction of complex/clustered damage after high-LET exposure, the DNA damage response to these lesions will play a greater role than following low-LET exposures, independent of the high-LET radiation dose rate. The repair of complex/clustered damage is more challenging for the cell and often results in the reorganization of lesions into repair centres. Evidence indicates that high-LET exposures readily trigger the G2/M checkpoint independent of dose. Modelling studies for lung cancer suggest that high-LET exposures may have a greater promotional effect on pre-malignant cells and clones; however, low doses (50 mGy) of low-LET radiation act to promote the growth of Trp53 mutation carrying clones and cells in oesophageal epithelium in one study. Modelling that indicates an inhomogeneous distribution of radon (and progeny) in the lung leads to a protracted high-dose-rate exposure of a small population of cells, and this protracted irradiation is likely to impact on lung carcinogenesis and radon-related risks. Further insight into the effectiveness of high-LET radiation may be gained by a better mechanistic understanding of the resulting DNA damage at a microdosimetric level together with consideration of the impact of spatial aspects of dose distribution on DNA misrepair.

540. Some general conclusions may be drawn in addition to the above responses to the review questions originally set out. In the paragraphs that follow, a brief summary of the findings of this report is provided. In this annex, the Committee has undertaken a comprehensive evaluation of the biological mechanisms that are considered to contribute to or modulate carcinogenesis following radiation exposure, particularly at low exposure levels (100 mGy and below for low-LET radiations and at dose rates of 0.1 mGy/min and below). A complete understanding of the mechanisms and modulators of carcinogenesis following low-dose and low-dose-rate radiation exposures is not yet available. Additionally, an appendix considering principles and criteria for ensuring the quality of the Committee's reviews of experimental studies of radiation exposure is provided.

541. Little in the way of robust data could be identified that would prompt the need to change the current approach taken for low-dose radiation cancer risk inferenceas used for radiation protection purposes and for the purpose of comparison with other risks. The potential contributions of phenomena such as transmissible genomic instability, bystander phenomena and adaptive response remain unclear. It is notable that since the Committee's last major evaluation of contributory mechanisms for radiation cancer [U7], there have been substantial new data on low-dose and low-dose-rate risk from epidemiological investigations, particularly of occupational and medical cohorts. These studies have added to the epidemiological evidence underpinning low-dose and low-dose-rate cancer risk estimation.

542. The Committee concluded that there remains good justification for the use of a non-threshold model for risk inference given the robust knowledge on the role of mutation and chromosomal aberrations in carcinogenesis. That said, there are ways that radiation could act that might lead to a re-evaluation of the use of a linear dose-response model to infer radiation cancer risks. Some studies indicate that low-dose and low-dose-rate exposures can extend lifespan and possibly reduce tumour burdens in experimental

animals. Generally, there is insufficient mechanistic understanding of these observations, however. This situation may be improved for example, if low-dose exposures were shown consistently and unequivocally to stimulate DNA damage response/repair, or immune responses preventing cancer development, such a consistent evidence base has not been found in this review. In this case, some elements of risk reduction may have to be taken into consideration alongside the established DNA damage–mutational, and potential promotional pathways. Another example where additional evidence would help assessment of risk are the findings relating to stimulation of tumour vascularization by low-dose exposures, where there is greater consistency and coherence of the available data. Stimulation of tumour vascularization would serve to promote tumour development.

543. The implications of the studies on the induction of transmissible genomic instability, bystander effects and adaptive responses are still not clear. Some studies suggested thresholds for the induction of transmissible instability and bystander effects at around 100 mGy low-LET radiation; if true, this would indicate the phenomena are not relevant for low-dose cancer risk inference. Further, adaptive response studies remain without a confirmed mechanistic basis and are of mixed outcome; similarly studies of samples from persons inhabiting areas with high natural background radiation levels that are interpreted by some as providing evidence for adaptive response are insufficiently coherent to adopt for risk assessment purposes.

544. Looking to the future, the recommended approach to combine mechanistic understanding of low-dose radiation carcinogenesis with epidemiological studies is to use mathematical modelling integrating data from experimental systems (e.g. dose–response data for induction of key mutations or epimutations). There exist good multistage model frameworks for this purpose that have the flexibility to include data on somatic events and germ line influences on risk. These approaches may be used to test hypotheses and provide further insights for risk inference. Consideration should be given to the use of adverse outcome pathway approaches to help define and formalize key mechanistic steps in carcinogenesis following low-dose exposures. Additionally, experimental investigations may identify cancer risk indicators, that when validated could be integrated into epidemiological investigations to improve statistical power, or be used for population screening.

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In drafting the appendix on principles and criteria for ensuring the quality of the Committee's reviews of experimental studies of radiation exposure, the Expert Group have drawn extensively on the UNSCEAR 2017 Report, annex A, "Principles and criteria for ensuring the quality of the Committee's reviews of epidemiological studies of radiation exposure" [U11], which was an extremely valuable source for this work.

Members of the Expert Group

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APPENDIX A. PRINCIPLES AND CRITERIA FOR ENSURING THE QUALITY OF THE COMMITTEE'S REVIEWS OF EXPERIMENTAL STUDIES OF RADIATION EXPOSURE

I. INTRODUCTION

A1. Evidence from experimental radiobiological studies forms an important part of the scientific evaluation of radiation effects conducted and reported by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). In many UNSCEAR evaluations, experimental studies are reviewed, and their results used to inform the assessment of health risks of radiation, particularly in dose and dose-rate ranges where direct epidemiological evidence is not available. In common with radiation epidemiological studies, experimental studies have strengths and limitations, and each study requires careful and systematic assessment to gauge its contribution to the topic being addressed. The Committee's UNSCEAR 2017 Report, annex A, "Principles and criteria for ensuring the quality of the Committee's reviews of epidemiological studies of radiation exposure" [U11] marked an important strengthening of the robustness and transparency of its evaluations, and there was a clear need to develop similar principles and criteria for experimental investigations, and there are many similarities in the consideration of quality of epidemiological and experimental publications.

A2. The Committee's evaluations commonly need to cover a broad array of experimental studies conducted at the level of molecules, cells and whole organisms. Such studies are frequently conducted with widely differing motivations, objectives, designs and results. Clear and transparent criteria that define the processes and decisions for the inclusion or exclusion of individual studies are essential to ensure that its evaluations meet the key requirement of objectivity, i.e. to use sound evidence in an undistorted manner, regardless of the composition of the group of experts conducting the evaluation for the Committee´s scrutiny, with assessment unaffected by non-pertinent features of studies.

A3. The principles of systematic review are clearly applicable and fundamental for the Committee's evaluations. Compiling, summarizing and comparing the results from all relevant and sound evidence is essential for achieving the goal of the Committee in producing high quality evaluations. In common with the quality criteria developed for epidemiological studies, this requires that the question to be addressed is clearly defined, a search of all evidence is carried out, relevant studies fulfilling the inclusion criteria are identified, their results compiled, and conclusions drawn from the evidence.

A4. The UNSCEAR 2017 Report [U11] on quality criteria for epidemiological investigations noted that systematic reviews are an important tool for providing guidelines in medicine, largely due to the evidence-based medicine movement since the 1990s [E2, S1]. Evidence-based medicine offers broad principles for translating research into policies and practice. The need for it arose largely from conflicting guidelines prepared by professional societies and other bodies, and also from difference in the quantity of scientific literature available. A key tenet of evidence-based medicine is the requirement that practice of medicine should be based on the best available scientific evidence. The concept of science-based medicine was not first introduced with evidence-based medicine, but it emphasizes that the evidence must be directly applicable, i.e. based on real patients and outcomes of treatment. For health effects of radiation

exposure, this would translate into underscoring the importance of human epidemiological studies for which the UNSCEAR 2017 Report is available [U11].

A5. However, it is recognized that human data are not always available for specific exposure situations, notably at low doses of exposure where epidemiological investigations may not have sufficient statistical power to detect health effects. In these situations, supporting evidence from biological investigations of radiation effects is valuable for the Committee's evaluations. Furthermore, the Committee considers the role of specific biological mechanisms relevant for health risk evaluation. Recent examples of the Committee's evaluations of biological and mechanistic data include, annex C, "Non-targeted and delayed effects of exposure to ionizing radiation" and annex D, "Effects of ionizing radiation on the immune system" of the UNSCEAR 2006 Report [U7].

A6. Health risk assessment frameworks have also been developed, combining several lines of evidence from toxicology, epidemiology and other disciplines for estimating the health impact of exposure to potentially harmful substances. In general, health risk assessment is a process consisting of hazard identification, exposure assessment, dose-response assessment and risk characterization [I9] and it provides the basis for risk management and risk communication. Health risk assessment has been widely applied by expert committees mainly for chemical agents [I8]. Across the world, systematic reviews are increasingly becoming the basis for these assessments [F9].

A7. The Committee's evaluations synthesize studies of effects of radiation exposure relevant to human health to guide decision-making, assist in preparing regulations and inform the scientific community and the public. The reports comprise evidence summaries and aim to be comprehensive and systematic to provide a definitive evaluation of the current scientific knowledge and provide evidence-based conclusions. The Committee has previously stated, in its UNSCEAR 2012 Report, that a balanced evaluation involves avoiding unjustified causal associations (false positives) and also unjustified dismissal of real health effects (false negatives) [U10]. In common with the quality criteria for epidemiological studies, the approach to ensure the robustness of evaluation of biological investigations requires several sequential steps:

(a) Clearly define the topic and objectives of the specific evaluation;

(b) Develop a search strategy and perform a search that allows identification of all studies with potential to contribute to the evaluation and its defined objectives;

(c) Apply a uniform approach to critically appraising the quality of the studies;

(d) Synthesize the available evidence from the studies by summarizing their results from those studies meeting criteria for inclusion (providing relevant, high-quality evidence);

(e) Develop overall conclusions drawn from the systematically retrieved, assessed and summarized studies.

A8. This appendix extends the UNSCEAR 2017 Report, annex A, that provided guidelines to judge the quality of epidemiological studies [U11]. It aims to recommend procedures for assessing experimental radiobiological studies and criteria for conducting scientific reviews of biological studies for the Committee's evaluations. These criteria make use of the experience of development of the criteria for epidemiological evaluations, which in turn drew on evidence-based medicine approaches. Furthermore, this annex also re-iterates the importance of adhering to the Governing Principles of the Committee[3],

[3] https://www.unscear.org/docs/GoverningPrinciples_ADOPTED_20150605.pdf

notably to its process in the selection of experts according to defined terms of reference, qualifications and competence, and the declarations of potential conflicts of interest are essential steps in securing quality and scientific excellence of the Committee's evaluations. All conflict of interest statements collected in the framework of the Committee's work are available at the Committee's secretariat.

II. OVERVIEW OF EXPERIMENTAL RADIOBIOLOGICAL STUDY TYPES

A9. There is a wide range of biological and mechanistic study types that the Committee may consider in its evaluations. Potentially relevant studies may be conducted at the molecular, cellular or whole organism level. All investigations examine the effect a given (and usually well defined) radiation exposure has on a biological endpoint. There is a very wide range of endpoints that might be considered in relevant studies, including but not restricted to reproductive outcomes, specific diseases such as cancers or circulatory diseases, tissue damage, cell killing, damage to DNA/chromosomes, damage to mitochondria and other cellular components, enzymatic activities and molecular interactions.

A10. Irrespective of organizational level or endpoint under consideration, there are some common features required in all studies:

(a) Definition and characterization of experimental model in which effects are to be investigated;

(b) Definition and documentation of radiation exposure;

(c) Definition and documentation of the endpoint under consideration;

(d) Determining the magnitude and variability of the effect (if present), including power calculations and robust statistical analysis where required;

(e) Studying dose–response relationships;

(f) Review the results generated and their relevance to human health.

A11. A critical difference between experimental studies and epidemiological investigations is the ability to control the conditions of an experiment. Inclusion of appropriate positive and negative controls in experiments is essential for correct interpretation of results. Evaluation of experimental outcomes in blind fashion is an important contributor to the avoidance of bias. All investigations require the inclusion of exposed and non-exposed subjects and will often include investigation of dose-, dose-rate or radiation quality-dependence of the effect under investigation.

A12. Careful consideration of the experimental model selected is of great importance and impacts on the extent to which findings and conclusions are generalizable. In the case of studies relating to human health, a close relation to the human in evolutionary terms and a close relation to the disease in question is important. More fundamental work might be investigating specific biological processes at the cellular or biochemical/molecular level and the relevance of these investigations to human health may only emerge later.

A13. Biochemical and molecular level investigations can provide information on the effects of radiation exposure on fundamental biological processes. These may not necessarily relate directly to a

radiogenic disease. As an example, a great deal of mechanistic detail of DNA repair pathways has been gained through biochemical and molecular approaches, sometimes utilizing modelled DNA damage (e.g. restriction enzyme breaks as a model of radiation-induced DSBs).

A14. Investigations at the cell level are perhaps the most common form of study, but these can include a wide range of cell types of varying relevance to human health. It is intuitive that human cells are the best model for human disease-related studies, and a similar consideration would apply if the research related to a given non-human species. Primary cells at early passage are generally taken to be more similar to cells in vivo. At later passages damage, mutational and epi-mutational changes may accumulate in culture. Specific adaptions to allow permanent culture such as viral transformation can present substantial difficulties for cancer-related investigations and less weight should be given to studies with these cell type. The more recent advent of cell lifespan extension through TERT transformation is considered to have fewer effects on normal cell function.

A15. Cellular studies need to provide a cell culture environment close to the in vivo situation. Differences between in vivo and in vitro oxygen levels can be substantial and impact on the results observed. In general, 3D culture models are thought to more closely mimic the in vivo tissue environment than classical 2D cell culture. Heterotypic cultures including more than one cell type should be a better mimic of the in vivo situation by comparison with homotypic cultures. Both 3-D and heterotypic cell culture methods remain at relatively early stages of development, and improvements can be anticipated.

A16. Studies at the whole organism level, by definition, bear a closer physiological relationship to individual humans but there are many constraints. In common with cellular studies, the use of organisms with a close evolutionary relationship to the human is important. The range of available animal models is in principle wide, but most utilize rodents, particularly mice, as a well characterized mammalian system where both inbred and outbred strains are available. There is increasing use of genetically modified mouse models where genes are inactivated or mutated in the laboratory. Such models can provide useful mechanistic insights into the pathways of disease pathogenesis, but relatively rarely reflect genetic variation within the human population. The use of models with closer relationship to the human, primates for example, is possible though relatively rare due to the housing requirements, lifespan, and ethical considerations. Biologists have additionally developed lower eukaryote models such as *Drosophila* and the nematode *Caenorhabditis elegans*, yeast and bacterial models have also been used among others.

A17. Irrespective of the experimental model selected, a sufficient sample of observations is required to ensure statistical significance of the investigation. Statistical power calculations can be performed to inform on suitable sample sizes if there is sufficient prior information available on the endpoint/ outcome measure (see chapter III below).

A18. The outcome measure selected for study is another critical factor, with those most proximal to a disease or effect being preferred. The appropriate selection of the outcome measure is highly dependent on the current state of knowledge on the disease/effect of interest. For example, there is a very large knowledge on the mechanisms of carcinogenesis summarized in the “hallmarks of cancer” [H10], while less is known of mechanisms contributing to cataract formation [H8]. The sensitivity of an endpoint and its variability due to technical variables or biological response variation is an important consideration.

A19. All investigations require careful characterization and documentation of the radiation exposure regime. The doses and dose rates used should closely parallel those of the natural/actual exposure under consideration. But limitations are present due to the sensitivity of the endpoint under consideration. In principle the use of relevant doses/dose rates and their adequate documentation is easy in a laboratory situation, but not all papers clearly document the radiation source used, doses and dose rates employed.

A20. Both qualitative and quantitative investigations can be of use but in general quantitative investigations are more informative. Quantitative studies require statistical evaluation of their significance. The statistical robustness of a study can be affected by the sample size (see above), inherent and technical variability of the endpoint. Technical variability can be assessed through the inclusion of technical replicates (i.e. utilization of the same sample with independent tests/assays). Assessment of biological variability requires the use of fully independently generated biological samples.

III. MAIN FEATURES AFFECTING THE QUALITY OF EXPERIMENTAL BIOLOGICAL STUDIES

A21. Assessment of research evidence needs to consider the quality of studies available. This chapter discusses the core features influencing the quality of experimental biological studies. All these issues are relevant when reviewing study quality and should not be viewed separately. For studies of radiation effects that can inform health risk assessment, the experimental model system selected and its relevance to human and the experimental endpoint/outcome measure and its relevance to the disease/health effect under consideration are of particular importance.

A. Experimental models system

A22. As noted above this is of importance for studies that aim to improve human health risk assessment. The model should have a close evolutionary relationship to human and as closely as possible recapitulate the in vivo physiological situation. Thus, studies utilizing mammalian models would be viewed as more directly relevant than say studies using *Drosophila*. There are of course ethical considerations that need to be taken into account as well as husbandry issues. Obtaining ethical approval for experiments on primates, while closer to human in evolutionary terms, is more difficult. In addition, the housing requirements and lifespan are likely to present constraints. Genetically modified models can be of use to determine if a given pathway is relevant to a disease, direct translation of findings to human needs to be carried out with caution as rarely do similar genetic mutations exist in populations.

A23. Cell models are ideally of human origin, but a wider range of mammalian cell lines have been used to inform human disease mechanisms. 2D culture is not a realistic representation of tissue architecture, and so 3D heterotypic cell models/organoids can be seen as more relevant. One factor that has often been overlooked in cell culture experiments is the oxygen concentration. In vivo concentrations of oxygen in tissue are lower than ambient concentrations. The use of low physiologically relevant oxygen conditions can have a substantial impact on study outcomes and should be preferred.

A24. When considering biochemical investigations, the use of proteins and other biochemicals of human origin can be viewed as more directly relevant. Nevertheless, the above considerations potentially relevant information can and has been derived from a wide range of experimental model systems at all levels of organization and a wide range of species. Observations at one level or in one species can be extended to a more relevant model following initial observations and demonstration of principles.

B. Radiation exposure

A25. By contrast with epidemiological investigations, exposure to radiation can be carefully controlled in experiments. The source used should be well characterized and exposure conditions well documented. Dose and dose rate are of course important considerations and should be of relevance for actual human exposures where possible. However, it is rarely possible to exactly mimic human exposure in terms of duration or dose. Based on current understanding, to be of relevance to the assessment of risk of low-dose exposures, doses should be of 100 mGy and less for low-LET exposures, and low-dose-rate exposures should be 0.1 mGy/min or less.

C. Endpoint/outcome

A26. Depending on the level of biological organization selected for an investigation, the endpoint/outcome may be a disease or a known or speculated intermediate step on a disease pathway. Thus, many experimental rodent studies of radiation carcinogenesis are available. Here the disease in general is clearly of relevance but some rodent cancers bear closer histopathological similarity to human disease than others.

A27. If an investigation concerns endpoints prior to disease presentation their relevance needs to be established. Many mechanistic investigations are undertaken to try to establish the relevance of an endpoint or pathway to a particular disease while others are conducted simply to observe and describe effects of radiation on the experimental system. Useful information for the Committee's evaluations can be found in both types of investigation.

D. Experimental conditions

A28. The protocol adopted for any investigation requires to be documented and the fundamental principles of experimental design must be respected. Controls, both positive and negative should be included, independent biological and technical replicates should be included, sample sizes should be sufficient to ensure suitable statistical power. Documentation of procedures must be sufficient to allow for independent replication. Where an effect has been observed to occur using multiple analytic approaches it can be viewed to be more firmly established.

A29. Any experimental study of the effects of radiation is limited by the timeframe under consideration. All biological processes are dynamic and so selection of a suitable timepoint(s) for evaluation of outcome endpoints is important. Studies that take multiple timepoints are likely to be more informative than those with one or a few. For example, studies of the effectiveness of DNA double-strand break repair will depend critically on the timepoints for analysis selected, and caution should be taken in analysis where any given process is not observed to come to completion.

A30. A finding that has been replicated in an independent experiment in an independent laboratory is more robust than a one-off reported finding. Exact replication of a given study may not always be available but supporting findings help to provide a more robust evidence on which to base conclusions.

E. Analysis

A31. The analysis of results should be based on a study plan developed in advance. A clear and transparent description of experimental results is necessary. To improve the transparency of research findings, making the original (non-patient/participant identifiable, if human samples are used) data publicly available is encouraged, for example, by the open science framework and many journal editorial policies. There are also well-established databases for genomic and transcriptomic data. Confidentiality requirements have to be kept in mind when making study data available to the scientific community.

F. Variation

A32. Some consideration of the variation in response between individuals or populations will be of relevance to drawing conclusions on the general relevance of findings. Some variation in biological response is expected – usually taken to be around 10%, but important inter-individual or inter-group differences may be apparent. For example, the induction by radiation of delayed transmissible genomic instability has been reported in cells from some but not all individuals, and this may have a genetic basis (e.g. [K3]). As well as variation in response due to genetic factors, age may be of importance (e.g. [B4, G7, O8]). Similarly, sex may be a factor that affects individual response.

G. Reporting

A33. While a study may have been conducted with great accuracy and care, the written report may not necessarily be of the same quality due to using selective reports or omitting details of the study plan and procedures. Reporting standards for a wide range of study types are available, e.g. qualitative studies [O1], the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines for quantitative PCR studies [B60].

A34. It is sometimes the case that a research report is found to be erroneous and has to be withdrawn. This may not always be apparent from literature searches, and so those reviewing studies should take steps to ascertain if individual reports have been withdrawn.

A35. The PRISMA[4] guidelines [M39] support transparent and comprehensive reporting of systematic reviews and meta-analyses. While the focus of PRISMA is on healthcare interventions, the checklist items can be applied with minor adaptation to systematic reviews of observational epidemiological studies or experimental studies [M39].

[4] Referred reporting items for systematic review and meta-analysis protocols.

IV. PROCESS OF SYNTHESIS OF RESEARCH RESULTS

A36. For radiation risk assessment, experimental/mechanistic studies can provide supporting evidence to quantitative epidemiological assessments of risk. For traditional narrative reviews of experimental/mechanistic studies, no rigorous methodological standards have been established. Any such review would aim to provide assessment of weight or strength of evidence, i.e. judgement of value or contribution of studies commonly reporting a wide range of results, some contradictory. However, the degree of credibility assigned to each study and formulation of summary measures does not tend to follow formal procedures. Hence, the appraisal of the balance of evidence is heuristic and generally based on ad hoc criteria. This methodological "softness" makes such reviews susceptible to judgemental bias, however not intentionally.

A37. For literature synthesis and evidence assessment, which are both essential components for the Committee's evaluation of experimental studies, appraising their quality of evidence is a key task. Two levels of quality are to be considered. The first is the quality of any individual study used for the topic. As noted in chapter III, many factors can affect the quality of experimental biological investigation.

A38. The next step in the assessment of quality pertains to the totality of the evidence, i.e. all the studies used for reporting on the topic of interest for the Committee's evaluation. In the overall assessment of the available evidence, low-quality studies and those using less relevant model systems or endpoints should be assigned less weight than high-quality studies, or even excluded if the quality limitations are critical.

A39. For a scientific evaluation of a defined research topic according to established scientific procedures and values (thus following Governing Principles of the Committee), a systematic approach is recommended, including the following steps:

- Step A: Definition of the topic for which evidence is required;
- Step B: Transparent and systematic collection of information, based on a clearly defined scope, objectives and protocol;
- Step C: Abstraction of relevant data from selected studies or other sources of information;
- Step D: Assessment of individual study quality following unambiguous and consistent standards;
- Step E: Synthesis of information;
- Step F: Drawing of conclusions and consideration of future research needs.

A40. Well-conducted and reported studies relevant to the topic in question utilizing relevant models and endpoints should always form the main input into the Committee's evaluations. Lower quality studies can and should be identified and evaluated with a transparent decision either to exclude them or to include them with a lower weight assigned to their results. Conclusions and summary statements should rest primarily on the best quality studies. The lower quality studies can be assessed to attempt to determine whether their results are generally consistent with or strongly deviate from the summary results.

A41. The different steps in the process of research synthesis, including the definition of the study question (topic), systematic evidence search, study identification, quality assessment, synthesis and derivation of conclusions have been described in the UNSCEAR 2017 Report, annex A [U11]. Very similar considerations apply to experimental studies.

A. Definition of the topic for which evidence is required

A42. A crucial issue at the planning stage of a scientific evaluation is a clear and precise definition of the question or topic the evaluation seeks to cover. The scope of the planned work should be clearly outlined in a document plan, ideally with precise and answerable study questions organized by experimental model, exposure type, comparison, and outcome/endpoint. A protocol giving a clear definition and description of exposure, including dose levels of interest and further exposure details, should be produced. Likewise, the outcomes/endpoints of interest ideally need to be specified in advance, and eligible study designs and outcome measures outlined. This preparatory work should result in a thorough and detailed work plan. Changes to the protocol and to the agreed scope/direction of the evaluation should be avoided. Any necessary changes should be clearly documented and endorsed by the Committee or the scientific experts charged with an evaluation by the Committee.

B. Collection of information: searching the literature

A43. Systematic reviews address a clearly defined objective, use transparent and reproducible methodology and include a systematic literature search for all studies that meet predefined eligibility criteria. The systematic literature search results in a set of research reports consequently used for the overall evidence assessment and research synthesis. Systematic reviews should depend mainly on primary peer-reviewed publications rather than reviews.

A44. The substantial growth of new journals, particularly those with an open-access model, over recent years has changed the scientific publishing landscape. There are many (online) journals with non-transparent quality control measures that nevertheless claim to be peer-reviewed and reputable. This development makes research synthesis more difficult and calls for even more emphasis on strict quality assessment of publications that are to be included in evidence synthesis.

A45. There are many ways to find relevant literature. A systematic search of one or more electronic databases for specified key words should provide for completeness of information and extend the knowledge base, minimizing the possibility of selective inclusion of research reports. Nowadays, several databases of scientific literature are used, such as Medline/PubMed, Scopus or EMBASE. These can provide a good coverage of the published literature, but augmentation by expert databases and expert knowledge of the respective literature is often warranted particularly for very specific topics that may not be identified through keyword searches. A standard procedure is to also cover the reference lists of included publications and of related research overviews. Through its revision and discussion process, the Committee additionally ensures that further literature contained only in expert databases can be screened for relevance and inclusion. Unpublished research ("grey literature") is more difficult to find, and while evidence-based medicine systematic reviews nowadays do include unpublished research, the Committee usually does not use unpublished literature. However, reference lists of unpublished work may hint to further published research, and at times such work may be useful for hypothesis generation or for highlighting previously unconsidered facts. Even if English is the predominant language in scientific publishing, studies published in other languages can also contribute to the knowledge base and should, as a rule, be identified and evaluated. Expert groups often comprise members from several different countries and can thus potentially provide for expertise in different languages.

A46. For searches in electronic databases, a translation of the review topic into the language of the database (e.g. in terms of specific keywords) is required, and also a clear and concise use of search terms for the model(s), endpoint(s) and exposure(s) of interest, for the comparisons (e.g. in relation to dose,

dose rate or radiation quality) and the outcomes (cell level, molecular level or in vivo model system). Searches may be limited to particular models/endpoints or publication types using suitable index terms. Spelling variants and natural language variants of text terms should be considered [E18]. Overlap between publications because of several publications deriving from the same material needs to be identified and taken into account.

C. Abstraction of relevant data from selected studies or other sources of information

A47. Documentation of the literature search and selection process is needed so that the search can be repeated or updated at a later stage. Methods used for retrieval of information and data in the report should be described, including evidence abstraction (e.g. was it based on titles and abstracts, review of full texts?), criteria for inclusion, and quality assessment. The UNSCEAR 2017 Report, annex A, "Principles and criteria for ensuring the quality of the Committee's reviews of epidemiological studies of radiation exposure" considers the presentation of systematic review protocols based on the PRISMA guidelines [U11].

D. Assessment of study quality

A48. Once the studies to be included have been identified, core features are abstracted for further assessment, as described in the UNSCEAR 2017 Report, annex A, "Principles and criteria for ensuring the quality of the Committee's reviews of epidemiological studies of radiation exposure" [U11]. In the case of experimental studies, these features usually include study identifier (e.g. author, year), publication type (e.g. peer-reviewed, review etc.), relevance of publication to review questions being addressed, radiation exposure characteristics – quality, dose rate, dose, and summary of main findings. Further comments or details relevant to the respective study can be added. Study quality and relevance are also assessed. Study quality assessment aims to provide a clear view of the robustness of the reported findings (statistical considerations, methodology, replication or verification by independent investigators). Study quality assessment forms an integral part of evidence synthesis, and must be performed in a transparent and reproducible fashion.

A49. Risk of bias or quality assessments are focused on the methodological quality of the study, and not on issues such as magnitude of effect or applicability of results. It should be noted that study size or statistical power to identify effects (if existing) are not necessarily included in the quality assessment but need to be considered in the overall assessment of the evidence. For the Committee's work, the following domains should be addressed: *(a)* exposure delivery; *(b)* methods for measuring endpoint/outcome; *(c)* sources of bias specific to the study design; *(d)* methods to control for confounding; *(e)* statistical methods; *(f)* study reporting; and *(g)* statement on conflict of interest of authors.

A50. Based on the above assessments and information abstracted from publications a judgement can be made on its relevance and importance for the review, and a decision made as to include or exclude consideration of the publication in the Committee's reports. A quality weighting approach can be used but is not essential – such an approach would have to consider the relevance of the experimental model, the endpoint/assay reported, relevance of the radiation exposure, quality of the experimental design, quality of the statistical analysis, study reproducibility.

A51. The result of the quality assessment/inclusion/exclusion for each study is documented, and the review should then describe how the quality rating approach is used in the further steps of the review. There is little use in assessing quality without utilizing its results. Studies with critical risk of bias or methodological failings should generally be excluded. This also pertains to studies with seriously inadequate dosimetry or endpoint assessment.

A52. Applying the approach recommended by the Committee will ensure that research overviews will be more informative as they identify and summarize the best and most relevant studies on a given topic and put less or no weight on flawed or non-relevant investigations. Providing transparency in quality assessment as outlined here will form a good scientific basis for the Committee's discussions on the merits of study-specific scientific evidence used for the Committee's evaluations.

E. Synthesis of studies: meta-analysis and narrative approaches

A53. The subsequent step of the literature review process is the actual study synthesis and interpretation. Narrative synthesis involves description and summary of the core findings from the included studies of varying structure and method. This involves grouping of the studies, for example according to study design, population or outcome, and providing a verbal account of the body of evidence relevant for the review question. On the basis of focused description of the individual studies, the evidence across the different studies is summarized and jointly assessed. When few studies are available or are very diverse in terms of important characteristics, a narrative review may be more appropriate than meta-analysis of data that are not truly compatible. When large numbers of relevant studies are available tabulation of the key features of each study can be very valuable. Such tabulations should document *(a)* the experimental model – species, cell or tissue type; *(b)* radiation exposures used, the radiation quality, and both dose range and dose rate; *(c)* reported effect/endpoints; and *(d)* publication reference.

A54. An important concern is the potential for publication bias, i.e. the selective reporting of studies, often those with statistically significant findings. Publication bias can seriously affect the synthesis of evidence, and several methods to detect and evaluate publication bias have been developed for use in meta-analyses [E9, J22].

A55. Unlike the situation when synthesizing epidemiological evidence, rarely is it possible to undertake mathematical analyses of risk (or other relevant) values, thus meta-analysis and statistical pooling is seldom employed. These analyses will need to be independently reviewed.

F. Reaching an overall conclusion from the evidence synthesis

A56. The final part of the evaluation of experimental studies should address and provide insight into the systematic review questions posed at the outset. As noted, only very rarely is it possible to undertake numerical analyses as one can in epidemiological studies of risk. Thus, reviews are generally of the narrative type giving some indication of weight of evidence and confidence in the review findings.

A57. As described in the UNSCEAR 2017 Report, annex A [U11], a more articulated approach with wider assessment of various aspects of evidence has been developed within the Grading of Recommendations Assessment, Development and Evaluation (GRADE) collaboration for the Cochrane Library and has also been adopted by some international institutions involved in guideline development, including the World Health Organization [W9]. The GRADE categorization [G9] is usually specific to an outcome, for example cancer site-specific incidence. Application of such criteria, or variants thereof in narrative reviews is possible but not essential.

A58. Transparent organization and reporting of evidence is also an important step towards reducing differences of understanding and subsequent interpretation between expert evidence synthesis, the way the Committee uses this synthesis, and public understanding of the topic. To support transparency and clarity, standardized vocabulary and terminology with clear definitions are required. This would also help to ensure that reports can be translated accurately into other languages.

A59. The Committee's draft evaluations, including syntheses of experimental evidence, have always been subject to close scrutiny (independent critical review) and discussion at its annual sessions. This is an important step in continuous quality control and encompasses all steps of scientific report development, with critical revisions from experts with different scientific backgrounds. The Committee also assesses the consistency and plausibility of experimental evidence synthesis with results from epidemiology and indicates research needs arising from the overall assessment. The identification of uncertainties regarding the respective topic and possible approaches to their reduction will usually be part of the Committee's research recommendations.

V. CONCLUSIONS

A60. Individual reported experimental studies on biological mechanisms relevant for the inference of cancer risks from low-dose radiation provide the basic evidence on radiation effects, and this annex provides guidance on assessing the quality of individual studies and of the synthesis of evidence from several studies. Methods of evidence synthesis have evolved considerably during recent decades. The current methodological standards define procedures for literature search, evaluation of quality, combining research results and grading the overall strength of evidence. The current methods of evidence synthesis are systematic reviews, which are regarded as the state-of-the-art scientific standards for pooling research evidence and superior to traditional narrative reviews.

A61. The Committee will benefit from adopting a framework that is informed by these developments. However, the specific nature and scientific contents of experimental radiobiological studies make application of generic quality criteria problematic. Therefore, this annex provides the Committee's approach to radiation experimental study quality assessment and to synthesis of findings across studies. The approach provides for increased methodological rigour, which could enhance the degree of coherence, transparency and objectivity in assessments.

A62. The evidence synthesis approach outlined in this annex is focused on evidence from experimental radiobiological studies; previously the Committee has published similar quality criteria for epidemiological studies [U11]. The Committee draws on these fields and others to reach conclusions on the assessment of radiation health risk.

A63. Overall, the Committee aims to continue working with a quality-oriented systematic review approach for its evaluation wherever applicable, based on the concepts described in this appendix. While

the focus on study quality and the explicit review of strengths and limitations of experimental radiobiological studies is a long-standing feature of the Committee's work, using well documented quality criteria in a systematic way as outlined here has not been always applied to the Committee's evaluations. In summary, a high degree of transparency, quality and a systematic approach to collecting, analysing and interpreting information for the Committee's evaluations and assessments will help to maintain the high scientific standard necessary for its widely appraised reports.

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GLOSSARY

Note: The glossary is mainly based on the glossary included in the UNSCEAR 2012 Report [U10] and other relevant UNSCEAR reports to ensure consistency of terms and definitions.

Abscopal effect: A biological consequence induced by radiation that is observed at a distance from the irradiated tissue.

Absolute risk: Excess risk attributed to an agent and usually expressed as the numeric difference between exposed and unexposed populations (e.g. five cancer deaths over a lifetime per 100 people, each irradiated with 1 Sv).

Absorbed dose, D: The fundamental dose quantity given by:

$$D = \frac{\mathrm{d}\bar{\varepsilon}}{\mathrm{d}m}$$

where $\mathrm{d}\bar{\varepsilon}$ is the mean energy imparted to matter of mass $\mathrm{d}m$ by ionizing radiation. The SI unit for absorbed dose is joule per kilogram (J/kg) and its special name is gray (Gy).

Activity: The rate at which spontaneous transformations occur in a given amount of radioactive material. (Strictly, the expectation value of the number of nuclear transformations occurring in a given quantity of material per unit time.) It is measured in becquerels (Bq); 1 becquerel equals one transformation per second.

Acute exposure: Exposure received within a short time period (see also "Protracted exposure" or "Chronic exposure").

Adaptive response: Refers to the phenomenon by which cells irradiated with a sublethal dose of ionizing radiation (an "adaptive" dose of a few centigrays) become less susceptible to subsequent exposure to high doses of radiation (a "challenge" dose of several grays).

Adjustment: The process of statistically accounting for effects of differences between groups or populations under comparison in order to control confounding. Adjustment is frequently performed when estimating effect measures from epidemiological data, for example by stratification or multivariate regression analysis.

Allele: One of several alternative forms of a gene (or DNA sequence) at a specific chromosome allocation. At each autosomal locus, an individual possesses two alleles, one inherited from the father and one from the mother.

Aneuploid: The chromosome number is not an exact multiple of the haploid number; an individual with an aneuploid chromosome number; usually refers to an absence (monosomy) or an extra copy (trisomy) of a single chromosome.

Antioxidants: Substances preventing oxidation.

Apoptosis: Cell death caused by an intracellular pathway which can be activated by external stimuli.

Attained age: Age at observation or during the follow-up, usually refers to age as time-dependent characteristic in a cohort study.

Becquerel (Bq): The SI unit of activity, equal to one transformation per second. As the unit is so small, multiples are frequently used such as megabecquerels (MBq) which is 10^6 or a million becquerels (1 GBq is 10^9 Bq; 1 TBq is 10^{12} Bq; and 1 PBq is 10^{15} Bq).

Bias: A statistical estimation procedure is "biased" if the expected value of the estimate of the quantity of interest is not equal to the true value of the quantity. The "bias" of the procedure is the difference between the expected and true values.

Biodosimetry: The use of biological (or chemical, physiological) markers of exposure to reconstruct acute or protracted radiation doses.

Bystander effect: A non-targeted cellular action of radiation exposure meaning that not only the cell that was actually hit shows radiation consequences, but also neighbouring cells.

Candidate gene: Any gene by virtue of its property (function, expression pattern, chromosomal location, structural motif, etc) is considered a possible locus for a given disease.

Carcinogen: An agent, chemical, physical, or biological, that can act on living tissue in such a way as to cause a malignant neoplasm.

Solitary or complete: The agent does not need additional action of further exogenous cancer risk factors to cause a neoplasm.

Indirect or pre-carcinogen: The agent has to be transformed to its active molecular form (ultimate carcinogen) in the metabolism.

Case-control study: An epidemiological study type, where cases with the health outcome of interest are selected from the population and compared in terms of exposure with control free of the outcome, who represent the exposure distribution in the population. The effect of exposure on the outcome in a case-control study is expressed as an odds ratio.

Causation: The relationship between cause (here, radiation exposure) and outcome (radiogenic health effect or associated risk). The term can also mean the action of causing something.

Chromosomal aberration: A change in chromosome structure or number from the normal chromosome complement of an organism.

Chromothripsis: A form of deleterious genome rearrangement where one chromosome is fragmented and multiple parts are re-integrated into the genome of a somatic cell, observed in some cancers by whole genome sequencing.

Chronic exposure: Exposure persisting in time. (See also: "Acute exposure" or "Protracted exposure".)

Co-factor: A substance or agent that acts with another substance to bring about certain effects; e.g. coenzyme, a low-molecular entity needed for enzymatic activity of the apoenzyme.

Coding sequence: Those parts of a gene from which the genetic code is translated into amino acid sequences of a protein.

Cohort study: An epidemiological study type, where people free of the health outcome of interest are selected and subdivided into groups based on exposure of interest. The occurrence of the health outcome (typically incidence or mortality) is recorded for the entire study population and the effect of exposure on the risk of the outcome is expressed as a rate ratio, risk ratio or hazard ratio. Follow-up for development

of disease cases since start of study period can be prospective or retrospective (collection of information of the cases that have already occurred).

Confounding factor or confounder: A confounding factor is a variable that is correlated with both the exposure (e.g. radiation exposure or dose) and the outcome variable (e.g. risk of lung cancer) and, if not controlled for analytically, may distort the conclusions. For example, occupation may be a confounding factor in a study of the relation between lung cancer incidence among non-smokers (dependent variable) and medical radiation exposure (independent variable). For instance, air crew are exposed to higher levels of radiation due to their employment (correlation with radiation exposure) while staff working in certain recreation industries are often occupationally exposed to cigarette smoke (correlation with outcome lung cancer). This confounding might be controlled by introducing into the analysis an indicator for the occupational group.

Cytokines: Small secreted proteins that mediate intercellular communication and signalling.

Deletion: Loss of a portion of a gene or chromosome; a type of mutation; a synonym for deficiency.

Diploid: A full set of genetic material, consisting of paired chromosomes – one chromosome from each parental set; most animal cells except the gametes have a diploid set of chromosomes (compare haploid).

DNA methylation: The addition of methyl groups to the 5-position of cytosine by DNA methyltransferases (Dnmts). High level methylation of genes or gene regulatory sequences usually indicates a transcriptionally inactive state. A form of epigenetic modification.

DNA sequence: The relative order of base pairs, whether in a fragment of DNA, a gene, a chromosome, or an entire genome.

Dose: A measure of the energy deposited by radiation in a target. Dose can be used as a shorthand for absorbed dose and effective dose when the context is clear.

Dose rate: Dose relative to time during which the dose is received. In experimental studies dose rate is often expressed per minute, while in epidemiological studies of long-term exposure dose per year is frequently used.

Doubling dose (DD): An important concept used in genetic risk estimation. It is defined as the dose of radiation required to produce as many mutations as those which occur spontaneously in a generation. It is calculated as a ratio of the average spontaneous and induced rates of mutations in a defined set of genes.

EAR: See “Excess absolute rate/risk”.

Effective dose, E: The sum over all tissues and organs of the equivalent doses weighted by the tissue weighting factor wT, which represents the contribution of that organ or tissue to the total detriment resulting from uniform irradiation of the whole body. *E* has the unit joule per kilogram, which is given the name sievert (Sv). The recommended values of wT are given in ICRP Publication 103 [I15].

Epigen, epigenetic: Changes in an organism brought about by alterations in the expression of genetic information without any change in the genome itself; the genotype is unaffected by such a change but the phenotype is altered.

Epigenetic modification: The modification of DNA or associated chromatin proteins that leads to altered expression of genes. DNA methylation, histone acetylation and methylation are among the epigenetic marks currently known.

Equivalent dose: Fundamental dosimetric quantity for the purposes of radiological protection defined as the product of the absorbed dose in the tissue or organ and the appropriate radiation weighting factor for the type of radiation giving rise to the dose; measured in sieverts (Sv).

Equivalent dose, H_T: The averaged absorbed dose in tissue or organ T, modified by the radiation weighting factor, w_R. The unit of equivalent dose is joule per kilogram, and it is given the special name sievert (Sv). The recommended values of w_R are given in ICRP Publication 118 [I2].

ERR: See "Excess relative risk/rate.

Error: The difference between an observed or estimated value and the true (but unknown) value. Since the true value is unknowable, error cannot be quantified. Error can be contrasted with uncertainty, which can be estimated by repeated measurements or Bayesian inference.

Excess absolute risk/rate (EAR): The difference between the hazard rate in an exposed population and the "baseline rate" in that population. This is often a function of dose, age (or some other measure of time) and other factors (effect modifiers). Excess absolute rate is often called "excess absolute risk" or "excess rate". Strictly risk would apply to a prospective estimate inferred from the data and reasoning, while rate would be the direct statistic calculated from the data.

Excess relative risk/rate (ERR): The "relative risk/rate" minus one. The excess relative rate is strictly a statistic calculated from observed frequencies/rates, while the excess relative risk is a prospective estimate inferred from the data and reasoning. The ERR is often considered as a function of dose and other factors.

Excess risk/rate: A measure of the statistical relationship between a given risk factor and a specific outcome. Depending on the context it can refer to some characterization of the influence on rates such as the "relative risk", "excess relative risk", or "excess (absolute) rate", or, perhaps most appropriately, to estimates of the risk over some period of time (lifetime risk) associated with an exposure of interest. The excess rate is strictly a statistic calculated from observed frequencies/rates, while the excess risk is a prospective estimate inferred from the observations and reasoning.

Exon: A region of a gene containing a coding sequence. Most genes have several exons separated by introns (non-coding) which are usually longer.

Exposure: Concentration, amount, or intensity of a particular physical or chemical or environmental agent that reaches the target population, organism, organ, tissue, or cell, usually expressed in numerical terms of substance concentration, duration, and frequency (for chemical agents or microorganisms) or intensity (for physical agents such as radiation). In the radiation field, exposure may also denote the electrical charge of ions caused by X- or gamma rays per unit mass of air; however, the term is used in its more general sense as described here.

γH2AX foci: Nuclear foci or spots detected by immunofluorescence using antibodies specific for the phosphorylated form of histone γH2AX. An indicator of DNA double-strand breaks. A number of other nuclear proteins from foci after DNA damage.

Gene: The fundamental physical and functional unit of heredity. A gene is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a functional product.

Genome: The complete DNA sequence of an organism.

Genomic instability: An all-embracing term to describe the increased rate of acquisition of alterations in the genome. As compared with the direct actions of radiation, i.e. those consequence directly induced

by energy deposition, radiation-induced instability is observed in cells at delayed times after irradiation and manifests in the progeny of exposed cells multiple generations after the initial insult.

Genotype: *(a)* the genetic constitution of an individual; *(b)* the types of alleles found at a locus in an individual.

Gray (Gy): Unit of absorbed dose, equal to 1 joule per kilogram (J/kg).

Hazard: In epidemiology and statistics, a measure of occurrence obtained as time integral of risk and interpreted as risk intensity. With respect to aspect of health and safety, the term "hazard" refers to any source of potential damage, harm or adverse health effects.

Health effects (of radiation) (also radiogenic health effects):

> **Deterministic (health) effect:** A health effect of radiation for which generally a threshold level of dose exists, which varies with person and circumstance, above which the severity of the health effect is greater for a higher dose. The ICRP has introduced a term "tissue reactions" to describe a group of health effects comprising deterministic effects and some health effects (such as cataracts and fibrosis) that are not determined solely at the time of irradiation but can be modified after radiation exposure [II15, I16].
>
> **Stochastic (health) effect:** A radiation-related health effect, the probability of occurrence of which depends on radiation dose and the severity of which (if it occurs) is independent of dose. In radiation protection the so-called LNT-model is used (linear non-threshold) meaning that the assumption is made that one can linearly extrapolate from moderate/high doses to low and very low doses without a threshold. Note that this should not be confused with the stochastic actions or processes of radiation interaction at the molecular or cellular level.

Heterozygote: An individual with different alleles at some particular locus.

High dose: Doses above 1 Gy are high doses [U10], though the threshold value is necessarily arbitrary.

Homologous recombination repair: One of the pathways of DNA double-strand break repair that requires an undamaged homologous stretch of DNA to provide a template for repair. Restricted to S and G2 phases of the cell cycle.

Homozygote: An individual with the same allele at the corresponding loci on the homologous chromosomes.

Imputation: A statistical technique for generating a plausible value to replace a missing value.

In vitro measurement: A procedure used to determine the nature, activity, location or retention of radionuclides in the body by analysis of material excreted or otherwise removed from the body.

Inference: The process of drawing conclusions from scientific observations, evidence and reasoning in the presence of uncertainty. While this report is focussed on prospectively inferring risk, note that estimating an assigned share (or probability of causation) is also inference, but retrospective.

Interaction: Refers to the situation in which the magnitude of the influence of one risk factor on disease rates depends upon the magnitude of one or more other risk factors (see "Effect modification" or "Joint effects").

Internal dose: *Radiation:* Dose from radioactive material deposited in the body.

Chemicals: (a) Amount of a chemical recently absorbed; measured, e.g. as metal concentration in blood; *(b)* amount of chemical stored in one or several body compartments or in the whole body (body burden); used mainly for cumulative toxicants; *(c)* in the case of ideal biological monitoring, amount of active chemical species bound to the critical sites of action (target dose; e.g. carbon monoxide binding to haemoglobin).

Inverse dose (or exposure) rate effect: A form of effect modification, where the risk per unit cumulative dose is increased under circumstance of lower exposure intensity (dose rate).

Latency (period): The period between exposure and manifestation of a health effect. This is also the period after which statistically significant increases in frequency of occurrence of the health effect in a population have been seen; theoretically, there might be an undetectable increased frequency of occurrence of the health effect in an exposed population during the presumed latency period, but this possibility becomes vanishingly small in the period shortly after exposure because there is a finite time required for damaged cells to replicate in an uncontrolled manner and manifest as a cancerous growth.

LET (linear energy transfer): The average linear rate of energy loss of charged particle radiation in a medium, i.e. the radiation energy lost per unit length of path through a material. That is, the quotient of dE by dl where dE is the mean energy lost by a charged particle owing to collisions with electrons in traversing a distance dl in matter.

$$L = \frac{\mathrm{d}E}{\mathrm{d}l}$$

The unit of L is J/m, often given in keV/μm.

Locus (plural: loci): A unique chromosomal location defining the position of an individual gene or DNA sequence.

Low dose: Doses below 100 mGy are called low doses [U10], though the threshold value is necessarily arbitrary.

Low dose rate: The Committee has defined "low dose rate" as 0.1 mGy per minute, averaged over one hour or less, for radiations such as external X-rays and gamma rays.

Meta-analysis: In statistics, a meta-analysis combines the results of two or more studies that address a set of related research hypotheses, for example by estimating a certain unknown parameter or parametric function common to two or more data sets, while controlling or adjusting for differences in other parameters unrelated to those of immediate interest. The purpose is to obtain an estimate that is more informative than any obtainable from a single study, while ensuring that other data do not corrupt or bias the result. The term "meta-analysis" is also used for statistical pooling of results available in published form, in contrast with a *pooled analysis* of original data from two or more studies.

Micro RNA (miRNA): A class of small non-coding RNAs that are involved in the epigenetic regulation of gene expression.

Mitochondria: Sub-cellular organelles that are the main site of energy production. Mitochondria contain a small circular DNA molecule that encodes some of their constituent proteins.

Mitochondrial DNA: DNA distinct from nuclear DNA in that it is mostly unique sequence DNA and codes for proteins that reside in mitochondria.

Model: An analytical or physical representation or quantification of a real system and the ways in which phenomena occur within that system, used to predict or assess the behaviour of the real system under specified (often hypothetical) conditions. This is in contrast to a relationship, which is simply the way two or more factors are connected or related (i.e. relationships include causal relationships, those based on models, and those that simply fit the observed data).

Moderate/medium dose: Doses between 100 mGy to 1 Gy are called moderate doses [U10], though the threshold value is necessarily arbitrary.

Molecular epidemiology: This epidemiological approach uses the currently available biomarkers (not radiation-specific) combined with classical epidemiological methods. Central to this are the –omics (genomics, transcriptomics, proteomics, metabolomics and others) which allow the detection of individual characteristics on various molecular levels (genes, transcripts, proteins, metabolism and others). The frequency of occurrence of these individual characteristics in exposed populations are then studied using classical epidemiological methods.

Mutagen: A substance that can induce heritable changes (mutations) of the genotype in a cell as a consequence of alteration or loss of genes or chromosomes (or parts thereof).

Mutation: A hereditary change in genetic material. A mutation can be a change in a single base (point mutation) or a single gene or it can involve larger chromosomal rearrangements such as deletions and translocations.

Non-homologous end joining: A DNA double-strand break repair process that is the predominant pathway of repair in mammalian cells, predominately active in G1.

Nucleotide: A subunit of DNA or RNA consisting of a nitrogenous base (adenine (A), guanine (G), thymine (T) or cytosine (C) in DNA, adenine, guanine, uracil (U) or cytosine in RNA.

Occupationally exposed: Any person who is employed, whether full time, part time or temporarily, by an employer, and who has recognized rights and duties in relation to occupational radiological protection. The Japanese regulations use another similar term, radiation worker.

Oncogene: A gene, or more forms of which is associated with cancer; many oncogenes are involved, directly or indirectly, in controlling the rate of cell growth.

Plausible: This relates to an argument or statement that seems reasonable or probable, without it necessarily being so. For the purposes of this annex, the term is used to constrain the number of possibilities to those that currently appear to be supported by sound scientific reasoning, e.g. in the case of health effects and inferred risks, those that can be reasonably supported by arguments based on observed or known radiation action and response in biological systems. Other responses not supported by mechanistic reasoning or other observations may be possible, but without such reasoning are not considered plausible.

Pooled analysis: A combined analysis of original data from two or more data sets bearing on a common question of interest. The analysis may include parameters that distinguish between the different data sets. (Contrast with "*meta-analysis"*).

Precursor: Substance from which another, usually more biologically active, substance is formed.

Prediction – well-founded and conditional: The conventional meaning relates to foretelling that a specified outcome will occur in the future or will be a consequence of an action, event or set of conditions. In this annex, a distinction is made between well-founded prediction and conditional prediction, both

being included within the concept of science-based inference. A well-founded prediction is one that is based on a hypothesis that is deemed proven by the application of scientific method; a conditional prediction is one that is based on a hypothesis that is currently not deemed proven but is based on scientific reasoning including the use of models and assumptions.

Progression: Increase in autonomous growth and malignancy; used in particular to describe the transition from benign to malignant tumours and the progression of malignancy. There are probably numerous stages of progression during neoplastic development. The process of progression features in the general model of carcinogenesis as well as in the multi-stage model.

Promoter: Risk factors of cancer that are capable of triggering preferential multiplication of a cell changed by initiation. Often, following initiation, a long-term action on the target tissue is necessary. Promoters often cause enzyme induction, hyperplasia, and/or tissue damage. The essential primary effects are considered to be reversible. As a rule, promoters do not bind covalently to cell components and do not exert an immediate genotoxic action.

Proteome: The complete complement of proteins within an individual cell—investigated using the techniques of proteomics.

Protracted exposure: Exposure received over a long time period (compare with "Acute exposure" and "Chronic exposure").

Radiogenic health effects: See "Health effects (of radiation)".

Radionuclide: A radioactive isotope of an element. Different isotopes of an element have the same number of protons but different numbers of neutrons and hence different atomic masses. If there are too many or too few neutrons, the nuclei of the isotope tend to be unstable and transform into the nuclei of another element and in the process emit radiation.

Radiosensitivity: Susceptibility of cells, tissues and organisms to effects induced by ionizing radiation, especially, but not exclusively, in relation to radiation-associated carcinogenesis. The term "radiosensitivity" with regard to cancer induction refers to the rate of radiogenic tumour induction and does not refer to the degree of malignancy.

Radon levels: Radon concentration in air. The special unit for radon level is Becquerel per cubic metre (Bq/m^3).

Rate: In general, the ratio of two quantities, where the denominator is usually a function of the period of time at risk. Rates of interest in epidemiology (and radiation risk estimation) are of the form:

$$\frac{e}{\mathrm{PY}}$$

where e is the number of events over some time period of interest in a study population and PY (expressed as person-years) is the sum of time at risk during this time period for each person in the study population. Rates for an event of interest can vary with such factors as age, sex, and dose. Characterization of how rates depend on dose (or exposure) is central to radiation risk estimation. See also "Hazard function", "Risk", "Relative risk", "Excess rate", and "Relative risk", and "Excess relative risk".

Recessive: A trait that is expressed in individuals who are homozygous for a particular allele.

Recessive mutations: Those that produce a specific phenotype when present in the homozygous (autosomal recessive) or in hemizygous state (X-chromosomal recessive).

Relationship: The way two or more factors are connected or related (i.e. relationships include causal relationships, those based on models, and those that simply fit the observed data).

Relative risk (RR): Ratio between the cancer cases in the exposed population to the number of cases in the unexposed population. A relative risk of 1.5 indicates a 50% increase in cancer due to the agent under consideration. Excess relative risk (ERR) is RR – 1.

Shielding: The absorbing property of material between a radiation source and a receptor which results in reduced exposure.

Sievert (Sv): Unit of equivalent dose and of effective dose, equal to 1 joule per kilogram (J/kg).

Signalling: Transmission of activatory or inhibitory signals in the form of association and/or dissociation and/or modification of molecules that affect biological processes within a cell. Signalling can be intra-cellular or inter-cellular. Disruption of signalling can contribute to disease pathogenesis.

Single nucleotide polymorphisms: Variants of the genome at individual base pairs which can be useful in genetic mapping of diseases or susceptibles.

Somatic cells: All cells in the body except gametes and their precursors.

Statistical power: The expected probability of being able to detect an effect of a specified magnitude, estimated before the start of a study. It depends on study size, baseline risk and effect size. Statistical power reflects only random error in outcome occurrence, while ignoring measurement error and typically assumes no bias in the study.

Statistical significance: Statistical significance testing is a technique originally developed for decision making, where the compatibility of the observations is contrasted with that expected in the absence of any difference (null hypothesis). An alternative or study hypothesis needs to be specified positing a difference, usually evaluating difference to any direction (called two-sided test), or to a specific direction (one-sided test). Conventionally, 0.05 has been used as the limit (critical value, alpha level or type I error) for significance, corresponding to a frequency of 1/20 for occurrence of a difference equally large or larger as observed in the absence of any true difference (under the null hypothesis). The choice of the cut-off level is; however, arbitrary and statistical significance should not be seen as the sole criterion for interpretation of the findings.

Target (biological): Any organism, organ, tissue, or cell that is subject to the action of a pollutant or other chemical, physical, or biological agent.

Telomere: The end of a chromosome. This specialized structure is involved in the replication and stability of linear DNA molecules.

Threshold dose: The minimum dose that will produce a biological effect. Dose below which no effects occur ("true", mechanistically derived threshold) or are measurable (apparent threshold). For a given agent there can be multiple threshold doses, in essence one for each definable effect.

Tissue dose: Local dose in an organ or a functional or structural entity of an organ. See also Absorbed dose and Internal dose-chemicals.

Toxicity: Capacity of an agent to cause injury to a living organism. Toxicity can only be defined in quantitative terms with reference to the quantity of substance administered or absorbed, the way in which this quantity is administered (e.g. inhalation, ingestion, or injection) and distributed in time (e.g. single or repeated doses), the type and severity of injury, and the time needed to produce the injury.

Transcription: Process by which genetic information in DNA is converted into mRNA ahead of translation into protein.

Transmissible genomic instability: Persistent formation of genetic alterations (commonly mutations or chromosomal aberrations) over many post-irradiation cell generations.

Working level (WL): Any combination of the short-lived decay products of radon in one litre of air that will result in the emission of 1.3×10^5 MeV of potential alpha energy (see also "Potential alpha energy concentration").

Working level month (WLM): The cumulative exposure from breathing an atmosphere at a concentration of 1 WL for a working month of 170 hours (1 WLM=3.54×10^{-3} J h/m^3=6.38×10^5 h Bq/m^3 EEC).

LIST OF GENES AND PROTEINS

By convention gene symbols are italicized while proteins are not. Human genes/proteins are given in capital letters, those of other organisms are generally given with an initial capital letter only

53BP1	p53-binding protein 1
AEN	Apoptosis-enhancing nuclease
AGGF1	Angiogenic factor with G patch and FHA domains 1
AGPAT (AGPAT3, AGPAT9, etc.)	1-acylglycerol-3-phosphate O-acyltransferase (3, 9, etc.)
AKT	AKT serine/threonine kinase, protein kinase B
ALC1	Amplified in liver cancer 1, also known as chromodomain-helicase-DNA binding protein 1-like (CHD1L)
ALP	Alkaline phosphatase
ANGPTL4	Angiopoietin-like 4
ANXA6	Annexin A6
AP-1	Activator protein 1 (transcription factor)
APC	Adenomatous polyposis coli
ATF6	Activating transcription factor 6
ATM	Ataxia-telangiectasia-mutated protein
ATR	Ataxia-telangiectasia and Rad3-related protein
ATRIP	ATR-interacting protein
B4GALT1	Beta-1,4-galactosyltransferase 1
BAALC	Brain and acute leukaemia, cytoplasmic
BACTIN	Beta-actin
BAX	BCL-2-associated X protein
BBC3	BCL2 binding component 3 (also: p53 upregulated modulator of apoptosis)
BCL (e.g. BCL2, BCL-xL)	B-cell lymphoma (apoptosis regulators)
BID	BH3-interacting domain death agonist
Bim	BCL-2 interacting mediator of cell death (also: BCL-2-like protein 11)
Bmf	BCL-2 modifying factor
BMPR2	Bone morphogenetic protein receptor type II
BRCA1	Breast cancer type 1 susceptibility protein
BTG2	BTG anti-proliferation factor 2

CAMK (CAMK1D, CAMKII, etc.)	Calcium/calmodulin-dependent protein kinase (ID, II, etc.)
CCL (CCL5, CCL20, etc.)	C-C motif chemokine ligand (5, 20, etc.)
CCNE1	Cyclin E1
CCR (CCR2, CCR4, etc.)	C-C chemokine receptor type (2, 4, etc.)
CD (CD4+, CD14, CD40, etc.)	Cluster of differentiation (4, 14, 40, etc.)
CDC (CDC6, etc.)	Cell division control protein (e.g. 6, etc.)
CDK2	Cyclin dependent kinase 2
CDKN1A (CDKN1A, CDKN2A, etc.)	Cyclin dependent kinase inhibitor 1A (1A, 2A, etc.)
CDS1	CDP-diacylglycerol synthase 1
CEBP (CEBPA, CEBPB, etc.)	CCAAT/enhancer-binding protein (alpha, beta, etc.)
cGAS/STING	Cyclic GMP-AMP synthase/Stimulator of Interferon Genes
CHD	Chromodomain-helicase-DNA binding protein
Chk (Chk1, Chk2, etc.)	Checkpoint kinase (1, 2, etc.)
c-JUN	Jun proto-oncogene
CLIC-1	Chloride intracellular channel protein 1
CLIP2	CAP-GLY domain containing linker protein 2
COL--A1 (COL1A1, COL13A1, etc.)	Collagen, type -- (e.g. 1, 13, etc.), alpha 1
COX2	Cyclooxygenase-2
CRE	Creatinine
CREB	cAMP response element-binding protein
CRP	C-reactive protein
CtIP	CtBP (C-terminal binding protein) interacting protein
Cx (Cx26, Cx32, etc.)	Connexin (26, 32, etc.)
CXCL (CXCL1, CXCL16 etc.)	C-X-C motif chemokine ligand (1, 16, etc.)
CXCR1	C-X-C motif chemokine receptor 1
CYCSP27	Cytochrome C, somatic pseudogene 27
CYP4XI	Cytochrome P450, family 4, subfamily X, polypeptide 1
DBP	D site of albumin promoter binding protein
DCLRE (DCLRE1A, DCLRE1C, etc.)	DNA cross-link repair (1A, 1C, etc.)
DDB2	DNA damage-binding protein 2
DLC1	Deleted in liver cancer 1
DNA-PKcs	DNA-dependent protein kinase, catalytic subunit
DRAK1	DAP kinase-related apoptosis-inducing protein kinase 1
DUSP (DUSP16, DUSP22, etc.)	Dual specificity protein phosphatase (16, 22, etc.)

E2F	Transcription factor E2F
EB13	Epstein-Barr virus induced gene 3
EFABP	Epidermal-fatty acid binding protein
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGR (EGR1, EGR4, etc.)	Early growth response (1, 4, etc.)
EIF (Eif2, Eif4b, etc.)	Eukaryotic translation initiation factor (2, 4, etc.)
Endo III	Endonuclease III
EPC2	Enhancer of polycomb homolog 2
ERCC (ERCC5, ERCC6, etc.)	Excision repair cross-complementing protein (5, 6, etc.)
ERK	Extracellular signal-regulated kinase
ESM1	Endothelial cell-specific molecule 1
FANCD (FANCD2, etc.)	Fanconi anemia group D protein (D2, etc.)
FasL	Fas ligand
FCGR (FCGR2B, FCGR3, etc.)	Fc fragment of IgG receptor (IIB, III, etc.)
FcεRI	Fc fragment of IgE receptor
FDXR	Ferredoxin reductase
FGF (FGF2, FGF21, etc.)	Fibroblast growth factor (2, 21, etc.)
FIS1	Mitochondrial fission 1 protein
FLJ35725	tRNA methyltransferase 44 homolog
FOLR1	Folate receptor 1
FTH1	Ferritin heavy chain 1
GADD45	Growth arrest and DNA damage-inducible 45 gene
GAP-43	Growth-associated protein 43
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
GJC1	Gap junction gamma-1 protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GNG11	Guanine nucleotide-binding protein subunit gamma-11
GPR124	G-protein coupled receptor 124
GPX1	Glutathione peroxidase 1
GRIK3	Glutamate receptor, ionotropic kainate 3
GRP78	Glucose related protein 78
GSK-3b	Glycogen synthase kinase 3 beta
GSTP1	Glutathione S-transferase P
GTF2H2	General transcription factor IIH subunit 2
H (H3, H4, etc.)	Histone (3, 4, etc.)

H2AX	H2A histone family member X
hCLUp-Luc	Human CLU promoter-luciferase
HDL	High-density lipoprotein
HERPUD1	Homocysteine-responsive endoplasmic reticulum-resident ubiquitin-like domain member 1 protein
HFH-1	Hepatocyte nuclear factor-3 homologue 1
HGF	Hepatocyte growth factor
HIF1A	Hypoxia-inducible factor 1-alpha
HINT-1	Histidine triad nucleotide-binding protein 1
HIST3H2A	Histone H2A type 3
HLA (HLA-A2, HLA-DR, etc.)	Human leukocyte antigen (A2, DR, etc.)
HMGB (HMGB2, etc.)	High mobility group box (e.g. 2, etc.)
HNF4A	Hepatocyte nuclear factor 4 alpha
hNOP56	Human nucleolar protein 56
HO-1	Heme oxygenase 1
HSP (HSP27, HSPA1B, etc.)	Heat shock protein (27, 1B, etc.)
ICAM-1	Intercellular adhesion molecule 1
IFN	Interferon
IFNG	Interferon gamma
Ig (A, E, etc.)	Immunoglobulin (A, E, etc.)
IGFBP3	Insulin-like growth factor-binding protein 3
IGH6	Immunoglobulin heavy chain 6
IL (IL17E, etc.)	Interleukin (17E, etc.)
INO80	INO80 Complex ATPase Subunit, a chromatin-remodelling factor
iNOS	Inducible nitric oxide synthase
IRAK1	Interleukin-1 receptor-associated kinase 1
ISG20L	Interferon stimulated exonuclease gene 20
ISWI	Imitation Switch protein
ITG (ITGA4, ITGB1, etc.)	Integrin (alpha 4, beta 1, etc.)
JNK	c-Jun N-terminal kinase
JUN	Jun proto-oncogene
KRTAP2-1	Keratin associated protein 2-1
lacZ	Beta-galactosidase
Lck	Lymphocyte-specific protein tyrosine kinase
LCN (LCN2, LCN13, etc.)	Lipocalin (2, 13, etc.)
LDH-A	Lactate dehydrogenase A
LIG (LIG3, LIG4, etc.)	DNA ligase (3, 4, etc.)

LILRB3	Leukocyte immunoglobulin-like receptor subfamily B member 3
LINE-1	Long interspersed nuclear element 1
MAP2	Microtubule-associated protein 2
MAPK (MAPK10, etc.)	Mitogen-activated protein kinase (10, etc.)
MAT2A	Methionine adenosyltransferase 2A
MBD4	Methyl-CpG-binding domain protein 4
MBP2	Myrosinase-binding protein 2
MCM2	Minichromosome maintenance complex component 2
MCP-1	Monocyte chemoattractant protein 1
M-CSF	Macrophage colony-stimulating factor
MD2	Myeloid differentiation 2
MDC1	Mediator of DNA damage checkpoint protein 1
MDM2	Mouse double minute 2
MEK	Mitogen-activated protein kinase kinase, it is also known as MapK2
MET	MET proto-oncogene, receptor tyrosine kinase
MHC II	Major histocompatibility complex II
MIR-27B	MicroRNA 27b
MLH1	MutL homolog 1
MMP (MMP1, MMP15, etc.)	Matrix metalloproteinase (1, 15, etc.)
MnSOD	Manganese superoxide dismutase
MOS	Oncogene MOS, Moloney murine sarcoma virus
MRE11	Double-strand break repair protein MRE11
MRN	Mre11-Rad50-Nbs1 complex
MSH (MSH1, MSH2, etc.)	MutS homolog (1, 2, etc.)
mTOR	Mechanistic (or mammalian) Target of Rapamycin
MUC5B	Mucin 5B, oligomeric mucus/gel-forming
MYC	MYC proto-oncogene
MyD88	Myeloid differentiation primary response 88
NBS1	Nibrin
ncRNA PARTICLE	Promoter of MAT2A-antisense radiation-induced circulating long non-coded RNA
NEIL3	Nei like DNA glycosylase 3
NeuN	Neuronal nuclei
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NIK	NF-κB-inducing kinase
NQO1	NAD(P)H quinone dehydrogenase 1

Nr4a2	Nuclear receptor subfamily 4, group A, member 2 (also known as nuclear receptor related 1 protein)
Nrf2	Nuclear factor erythroid 2-related factor 2
OCLN	Occludin
OCN	Osteocalcin
OGG1	8-oxoguanine glycosylase
OK432	Picibanil
OVA	Ovalbumin
p21	Alias for CDKN1A
p38MAPK	p38 mitogen-activated protein kinases
p53	Tumour protein 53
P70S6K	P70 S6 kinase
PAGE1	Prostate-Associated Gene 1 Protein
PAI-1	Plasminogen activator inhibitor-1
PAM	p53-independent protective apoptosis-mediated process
PARP1	Poly [ADP-ribose] polymerase 1
Pax7	Paired box 7
PCNA	Proliferating cell nuclear antigen
PDI	Protein disulphide isomerase
PD-L1	Programmed death ligand-1
pERK	Phosphorylated extracellular signal-regulated kinase
PEX1	Peroxisome biogenesis factor 1
PF4	Platelet factor 4
PHPT1	Phosphohistidine phosphatase 1
PI3K/BTK	Phosphoinositide-3 kinase/Bruton's tyrosine kinase
PIAS1	Protein inhibitor of activated STAT 1
Pim1	Proto-oncogene serine/threonine-protein kinase Pim-1
PKC	Protein kinase C
PLAU	Plasminogen activator, urokinase
PLC	Phospholipase C
PLK2	Polo like kinase 2 (also: serine/threonine-protein kinase PLK2)
POLB	DNA polymerase beta
POLD1	DNA polymerase delta 1
PP (PP2A, PPM1D, etc.)	Protein phosphatase (2A, 1D, etc.)
PPAR	Peroxisome proliferator-activated receptor
PPP2R5A	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit alpha isoform
PSA	Prostate-specific antigen

Ptc1	Alias for PTCH
PTCH (PTCH1, etc.)	Protein patched homolog (1, etc.)
PTGS2	Prostaglandin-endoperoxide synthase 2
PTIO	2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide
PYCARD	PYD (pyrin domain) and CARD (caspase recruitment domain) domain containing
RAD (RAD50, RAD51L1, etc.)	DNA repair protein RAD homolog
RAF1	RAF-1 proto-oncogene, serine/threonine kinase
RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4
RB1	Retinoblastoma 1
RET	Rearranged in transformation
REV3L	Protein reversionless 3-like (translesion DNA polymerase)
RND1	Rho family GTPase 1
RNF (RNF8, RNF168, etc.)	RING finger protein, E3 ubiquitin-protein ligase (RNF8, RNF168, etc.)
RPL10A	Ribosomal protein L10a
RRM (RRM1, RRM2, etc.)	Ribonucleotide reductase catalytic subunit (e.g. M1, M2, etc.)
S100A9	S100 calcium binding protein A9
SAT1	Spermidine/spermine-N1-acetyltransferase 1
SERHL2	Serine hydrolase like 2
SERPINA4-PSI	Serpin family A member 4-presenilin 1
SESN (SESN1, SESN2, etc.)	Sestrin (1, 2, etc.)
SFRP1	Secreted frizzled-related protein 1
SGK3	Serine/threonine-protein kinase SGK3
SIRT (SIRT3, etc.)	Sirtuin (3, etc.)
SLC22A5	Solute carrier family 22 member 5
SMARCA (SMARCA2, SMARCA4, etc.)	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member (2, 4, etc.)
SMC	Structural maintenance of chromosomes
SOD1	Superoxide dismutase 1
Sox9	SRY-Box Transcription Factor 9
ST3GAL5	ST3 beta-galactoside alpha-2,3-sialyltransferase 5
STARD13	StAR-related lipid transfer domain protein 13
SUMO	Small Ubiquitin-like Modifier
Suv39h1	Histone-lysine N-methyltransferase SUV39H1
SWI/SNF	Switch/sucrose Non-Fermentable complex
TERF2	Telomeric repeat-binding factor 2
TERT	Telomerase reverse transcriptase

TFRC	Transferrin receptor
TGF (TGFB1, etc.)	Transforming growth factor (B1, etc.)
TIG3	Tazarotene-induced gene 3
Tip60	Histone acetyltransferase Tip60
TLR	Toll-like receptor
TMPO	Thymopoietin
TNF	Tumour necrosis factor
TNFSF (TNFSF10B)	Tumour necrosis factor superfamily
TP53	Alias for p53
TP53inP1	Tumour protein p53-inducible nuclear protein 1
TRAMP	Transgenic Adenocarcinoma of the Mouse Prostate
TSAP6	Transmembrane protein tumour suppressor-activated pathway 6
UHRF2	E3 Ubiquitin-Protein Ligase UHRF2
UNG	Uracil-DNA glycosylase
USP2	Ubiquitin carboxyl-terminal hydrolase 2
VEGF	Vascular endothelial growth factor
VEGFR (VEGFR1, VEGFR2, etc.)	Vascular endothelial growth factor receptor (1, 2, etc.)
Wnt (Wnt1, Wnt3a, etc.)	Wingless-related integration site (1, 3a, etc.)
XIAP	X-linked inhibitor of apoptosis protein
XP (XPA, XPB, XPC etc.)	Xeroderma pigmentosum (complementation group A, B, C, etc.)
XRCC (XRCC1, XRCC4, etc.)	X-Ray Repair Cross Complementing (1, 4, etc.)
ZAP70	Zeta-chain-associated tyrosine protein kinase 70
ZBTB16	Zinc finger and BTB domain-containing protein 16
γH2AX	Phosphorylated H2A histone family member X

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This publication contains:

VOLUME III

Annex C: Biological mechanisms relevant for the inference of cancer risks from low-dose and low-dose-rate radiation

EVALUATING RADIATION SCIENCE FOR INFORMED DECISION-MAKING

In 1955 the United Nations General Assembly established the Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) in response to concerns about the effects of ionizing radiation on human health and the environment. At that time fallout from atmospheric nuclear weapons tests was reaching people through air, water and food. UNSCEAR was to collect and evaluate information on the levels and effects of ionizing radiation. Its first reports laid the scientific grounds on which the Partial Test Ban Treaty prohibiting atmospheric nuclear weapons testing was negotiated in 1963.

Over the decades, UNSCEAR has evolved to become the world authority on the global levels and effects of exposure to ionizing radiation. UNSCEAR's independent and objective evaluations of the science are to provide for—but not address—informed policymaking and decision-making related to radiation risks and protection.

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