Review

Ionizing radiation biomarkers in epidemiological studies – An update

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Abstract

Recent epidemiology studies highlighted the detrimental health effects of exposure to low dose and low dose rate ionizing radiation (IR): nuclear industry workers studies have shown increased leukaemia and solid tumour risks following cumulative doses of <100 mSv and dose rates of <10 mGy per year; paediatric patients studies have reported increased leukaemia and brain tumours risks after doses of 30–60 mGy from computed tomography scans. Questions arise, however, about the impact of even lower doses and dose rates where classical epidemiological studies have limited power but where subsets within the large cohorts are expected to have an increased risk. Further progress requires integration of biomarkers or bioassays of individual exposure, effects and susceptibility to IR. The European DoReMi (Low (Should read: Low Dose Research towards Multidisciplinary Integration)dose research towards multidisciplinary integration) consortium previously reviewed biomarkers for potential use in IR epidemiological studies. Given the increased mechanistic understanding of responses to low dose radiation the current review provides an update covering technical advances and recent studies. A key issue identified is deciding which biomarkers to progress. A roadmap is provided for biomarker development from discovery to implementation and used to summarise the current status of proposed biomarkers for epidemiological studies. Most potential biomarkers remain at the discovery stage and for some there is sufficient evidence that further development is not warranted. One biomarker identified in the final stages of development and as a priority for further research is radiation specific mRNA transcript profiles.

Keywords: Molecular epidemiology; Biomarkers; Ionizing radiation; Effects; Exposure; Individual sensitivity

1 Introduction

It is well known that exposure to IR[1] increases the risk of cancer and, at higher doses, diseases such as cardiovascular diseases and cataracts [1,2]. However, there are important unanswered questions that need addressing to increase our understanding of the impact of low dose (below 100 mSv)/dose rate exposure (0.1 mSv min^{-1}) [3]. These include: Do tissues differ in their radiosensitivity? Is the dose response relationship linear at low doses/dose rates? How does inter-individual susceptibility impact on risks of cancer and other diseases? Are multiple exposures separated in time additive? How does radiation quality impact on radiosensitivity? Do internal emitters show the same dose response as external radiation?

Recent epidemiology studies have highlighted the importance of this research area. For instance increased risks of leukaemia and solid tumours were seen among ≥ ~ 300,000 nuclear industry workers exposed to very low dose rate (typically <10 mGy per year) and cumulative doses <100 mSv [4,5]. Increased risks of leukaemia and brain tumours were also reported in paediatric patients following doses of 30–50 mGy from CT scans [6,7] and an excess risk of childhood leukaemia following bone marrow doses of a few mGy of natural background gamma radiation [8]. Questions arise, however, about the impact of even lower doses where the power of classical epidemiological studies is limited and it is not currently possible to identify whether a subset of individuals within these large cohorts have an increased risk.

Molecular epidemiology studies could increase our understanding of the effects of low dose/dose rate radiation exposures on health. In 2012, potential biomarkers for use in such studies were reviewed as part of the European DoReMi (Low Dose Research towards Multidisciplinary Integration) project (http://www.doremi-noe.net) [9]. This review of radiation biomarkers of exposure, susceptibility, late effects and persistent effects and discussion of logistical and ethical aspects of large studies and the relevance of biomarkers for assessing the cellular and physiological effects of low dose IR exposure concluded that although there are many potential biomarkers none were sufficiently validated for use in large radiation epidemiology studies.

The present review aims to update the Pernot et al. paper, focusing on recent technical advances and studies carried out on existing or new biomarkers after low dose exposures. Based on this evaluation the goal is to identify those biomarkers that should be prioritised to help epidemiology studies address the outstanding questions of the impact of low dose/dose rate exposures. Biomarkers for cardiovascular effects have recently been reviewed as part of the DoReMi initiative [10] and are not covered here. This paper assesses which biomarkers and bioassays are ready to be used for evaluating individual radiosensitivity and enhancing our understanding of the shape of radiation dose-response curves for different health outcomes and are discussed in the context of epidemiological study designs and availability of biological samples. For the first time, a road-map is proposed for biomarker development for radiation epidemiology studies and the present status of radiation biomarkers in different stages of development and validation assessed in this context.

2 General epidemiological and biomarker considerations

The Pernot paper [9] provided a definition and classification of biomarkers, and summarised the characteristics of a good biomarker which is not revisited in detail here. The choice of biomarkers depends on the objective of the study and is also dictated by the epidemiological study design: case-control studies of diseases with a long latency period (such as cancer) will generally not be able to use biomarkers of exposure as the exposure will have occurred decades before biological sampling is done; cohort studies in which subjects are followed-up for decades, on the contrary, may permit biomarkers of exposure and biomarkers of effects to be examined in the very few studies where repeated collection of biological material from the same individuals over time is possible. Thus the issue of the time range over which an endpoint is persistant is essential in considering how informative it may be as a biomarker in epidemiological studies.

Ideally, radiation epidemiology biomarkers should be specific to radiation and independent of other environmental exposures such as tobacco or other treatments. Such a biomarker would simplify analysis and help to substantiate radiation causality. However, biomarkers often lack specificity and may reflect exposure to other environmental agents or chronic conditions such as inflammation. Of course, they may still be informative in predicting the development of radiation-induced disease if such exposures are, for example, additive or interactive. Such biomarkers could be useful provided information on potential modifying/confounding factors can be collected in the epidemiological studies to allow an assessment of interactions. All biomarkers must be reproducible between laboratories and over time, biologically plausible, and practical to use in large studies in terms of the study design, cost and feasibility of sample collection and analysis and preferably make use of biological samples collected non-invasively (e.g. saliva, nails, hair follicles, urine).

In addition to measuring endpoints in biological samples exposed *in vivo*, responses to IR can be measured by irradiating biological samples *ex vivo* under defined experimental conditions. The approach has inherent limitations related to the unknown effect of time between exposure and the collection of biological samples being assayed. It also requires samples to be stored to ensure that biological activities assayed *ex vivo* reflect those *in vivo*. Despite these limitations *ex vivo* irradiations are useful in molecular epidemiological studies where a) repeated sampling before and during irradiation is not possible, for example to assess DRC using the formation and persistence of gamma H2AX (γH2AX) (see Section 3.2.2) and b) in retrospective studies where it is not possible to measure biological responses after an exposure that occurred many years ago.

Low dose radiation biology research has identified several approaches as promising avenues for the development of radiation epidemiology biomarkers (see for instance the recent commentary from National Council on Radioprotection and (measurements should read: Measurements)measurements [11]). Moving from a biomarker discovery stage to validation and application in a molecular epidemiological setting requires biospecimen focused research to identify possible pre-analytical variables (e.g. processing delay, storage conditions, storage time, freeze/thaw cycles) that might affect biomarker detection and sufficiently large numbers of suitable biological samples for biomarker validation. Indeed, there remains a need to increase the number and size of radiation epidemiology biorepositories and to identify populations where sampling is feasible and potentially informative. For instance, studies investigating occupational exposures might be particularly amenable for the collection of multiple biological samples for assay comparisons, as samples can be collected during routine medical surveillance, subject to appropriate ethics clearance and agreements [12,13]. In parallel with the collection of biological samples, collecting “metadata” (e.g., radiation dose and information on other factors which may influence biomarker results, such as age, sex, socioeconomic status, body mass index, and medication use) is essential for interpreting biomarker results. It can, therefore, be efficient to collect biological samples in subsets of cohorts for which a substantial amount of metadata is available, such as in the occupational setting [14,15] or in the medical therapeutic setting (see for example [16]) where data and samples can be obtained before, during and after treatment as part of routine clinical patient follow-up.

In radiation epidemiology, there is interest in biomarkers involving biological samples that do not require drawing blood. For instance saliva has potential as a biological sample for biomarker development and in particular biomarkers of exposure [17]. Such an approach is particularly attractive when a large number of samples are required. Finger/toe nails are also easy and cheap biological samples to obtain. Hogervorst et al. [18] reported their comparison of DNA isolated from toenail samples stored for >25 years from the Netherlands Cohort Study [19] versus other sources of DNA. Although nail DNA was considerably degraded, it could be genotyped for a limited set of SNPs. For genotyping using next generation sequencing where DNA degradation is less of an issue, nails may therefore be an attractive DNA source.

Pilot studies and the subsequent validation of biomarkers require access to suitable biospecimens. These may be unavailable in existing biobanks and require assessing the suitability of alternative biospecimens or collecting new samples. For instance, a recent proof-of-principle study showed that volatile organic compounds in exhaled breath could be used to estimate radiation exposure [20]. However as discussed below, validating such measurements for integration into molecular epidemiological studies requires further work to assess the feasibility of the approach and, as appropriate samples are not already biobanked, sample collection. For other studies of potential biomarkers it might be possible to use stored samples, but the SOPs used for the collection and processing of materials may not be suitable for all applications. The development and validation of SOPs increase reproducibility and are essential steps in collecting and exploiting biological samples. This is particularly the case in multicenter studies, where protocol variations often considered fairly mundane, such as methods for assessing DNA yield, can differ substantially and influence results [18]. Recent publications have explored approaches for improving biomarker reproducibility by identifying sources of variability and highlighting the importance of biospecimen focused research to optimise SOPs. For example it has been shown that processing delays and the number of

freeze/thaw cycles affects miRNA expression levels [21]; miRNA expression levels are also affected by the method used for their isolation and the platform used for analysis [22] and the choice of primer pairs for the qPCR assessment of mRNA transcript levels, which need to reflect alternative splicing, can significantly impact on results [23]. Clearly such considerations will impact on cost and a careful cost/benefit evaluation is needed to justify the use of any biomarker in a molecular epidemiology setting and any changes in operating procedures.

Molecular epidemiology poses special ethical issues that were discussed by Pernot et al. [9] and, in more detail, by Gallo ~~et al.,~~et al., who discussed the need for strengthening the reporting of molecular epidemiological studies and outlined the STROBE-ME recommendations [24]. Advances in laboratory techniques enable the extraction of DNA, RNA and proteins from many stored samples at a sufficient quality and quantity for use in many -omic approaches, opening up the possibility to investigate biobanked samples for endpoints that were not included in the original informed consent forms. This is particularly the case for genetic analyses where, due to technical improvements and reductions in costs, sequencing of whole genomes is feasible in large cohorts. Currently most studies do not give information on individual health risks to study participants. Indeed few biomarkers have been validated in prospective studies as being predictive for the likelihood of developing a disease. In coming years these issues will evolve as the understanding of risks associated with individual susceptibility, and the specificity and sensitivity of different endpoints to assess individual risk, become clearer.

3 Potential IR biomarkers

In this update we consider progress in IR biomarker development and validation in molecular epidemiology settings using the classifications of the Pernot et al. review [9] and, as in this review, due to the nature and biokinetics of the dose distribution, approaches for the biological estimation of dose for internal emitters have been considered separately.

3.1 Cytogenetic biomarkers

3.1.1 Cytogenetics biomarkers of low dose exposure

The DC assay remains the international biodosimetry “gold standard” for recent radiation exposures [25] and is the technique with which newer biodosimetric approaches are compared. The DC assay can detect exposure to 0.1 Gy if up to 1000 cells are analysed and, based on the distortion of the Poisson distribution of the number of aberrations/cell, differentiates between partial and whole body exposures or to high or low LET radiation [26]. The scoring of DC based on chromosomal morphology requires a high level of expertise, and time to analy~~z~~se large numbers of cells. Automated DC scoring systems have been developed (see for instance [27–31]) and international networks have attempted to harmonize manual and automated scoring approaches [32–37]. However, automated DC scoring detects only half of the giemsa-stained dicentrics and rejects many metaphases. DC scoring can be simplified with the application of TC-FISH, which simultaneously stains telomeres and centromeres. This technique allows the detection of certain configurations of dicentrics which is technically challenging with classical Giesma staining and allows increases after doses >100 mGy to be assessed by counting 1000 cells [38]. The impact of lower doses can be assessed by counting more cells (see Table 1).

Table 1 Summary table of cytogenetic biomarkers.

alt-text: Table 1

	Exposure Assessment		Lower dose limit	Specific for Radiation quality	Partial Body exposure	Individual radio-sensitivity	Age dependent radiosensitivity in low dose range	Labor intensive	Automation possible	Applicable for large scale studies
	Past	Current								
Dicentrics	No	Yes	50 mGy[a]	Yes	Yes	No[b]	Yes	Yes if not automated	Yes (described for Giemsa stained dicentrics or by using PC and TC fluorescent probes)	Yes
Translocations	Yes (at group level)	Yes	300 mGy	Yes	Yes	No**	No	Yes	No	Yes
Intra-chromosomal aberrations	Yes	Yes	150 mGy	Yes	No	No	No	Yes	No	Yes
Micronuclei	Yes (only group level)	Yes	Several tens of mSv	No	No	No	No	No	Yes	Yes

MN-RET	Possibly (chronic genetic instability)	Yes Time dependent	10 mGy	Works after external and/or internal exposure	Bone marrow has to be exposed	Not tested	Not tested	No	Yes	Yes
Telomere length	No	No	Not assessed	No	Not assessed	Possibly	Probably No	Not assessed	Yes	Yes

[a] Requires the evaluation of 10,000 cells to evaluate exposures to 50 mGy.

[b] Radiation sensitive syndromes such as ATM and NBS homozygote carriers do show increased levels.

The DC assay has a low background, a high comparability between the *in vivo* and *in vitro* dose response and a low inter-individual variability. It should, therefore, be able to assess age-related sensitivity. Technical advances permiting high through-put analysis [31] should allow investigations into the low dose CT exposures. The latter has been hampered, for example, by the high number of cells required to resolve a 1.5–2 fold increase in IR induced dicentrics in umbilical cord blood [39,40] and children under 5 years of age (Gomolka et al. unpublished data) compared to levels seen in blood from adults after high dose exposures.

The well-established and standardized CBMN assay in PBLs remains a significant biodosimetry tool for IR exposure and a potential alternative to the DC assay, as it requires less time for evaluation of the results and cytogenetic expertise [25,41,42]. It too can be improved using centromeric probes and used for retrospective dosimetry [43]. Nevertheless, it does not achieve the sensitivity and specificity of the DC assay.

MN-RET is also recognised as a sensitive biomarker of cytogenetic damage [44] that can be measured using fixed peripheral blood samples. The MN-RET assay shows a linear dose response (R^2 0.988) after X-rays and also internal exposure following the injection of the radioisotope ^{18}F-FDG (R^2 0.999) in the mouse [45]. This increase was significant at 25 mGy after X-ray whole body exposure and at 33 mGy after ^{18}F-FDG exposure, demonstrating for the first time that the induction of DNA damage to mouse bone marrow can be detected and quantified accurately following injection of ^{18}F-FDG.

The MN-RET assay has not been extensively assessed in humans [46,47]. Issues such as inter-individual variability with larger study numbers and identifying confounding factors, such as diet [48] and alcohol intake [49] need to be resolved to assess its use as a biomarker of exposure. Nevertheless, the approach is worth further investigation because it can assess levels of genetic damage in the bone marrow, a critical organ for leukaemia, following acute or chronic low dose external exposure or following internal contamination.

In summary, the DC assay remains the best biodosimetry biomarker even if less labour intensive methods such as CBMN and MN-RET show promise. One outstanding question to be resolved is whether radiation sensitivity shows an age dependency. Several bioassays such as γH2AX, micronuclei and clonal survival assay (see for instance [50–52]) and changes in chromosomal aberration types [39,40] have been explored but lack the sensitivity in the low dose range. Automation of the DC assay offers the possibility to assess effects at doses <100 mGy if up to 10,000 cells are analysed. This is, however, not an appropriate method for the assessment of past exposures and other methods such as the detection of stable translocations or micronuclei should be used.

3.1.2 Cytogenetic biomarkers of susceptibility and late effects

Cytogenetic endpoints (e.g. the G2 assay) are also of interest as biomarkers of susceptibility and late effects. Several groups showed increased chromosomal radiosensitivity in cancer patients in comparison with matched healthy controls. Chromosomal radiosensitivity is clearly an inherited phenotype demonstrated in several family and twin studies (reviewed in [53]). However, cancer patients radiosensitive in one cytogenetic assay were not necessarily radiosensitive in a different assay [54]. Also, studies of cytogenetic tests of cancer susceptibility did not investigate radiation specificity. Progess has been made in identifying genetic variants associated with cancer predisposition, which might be a better approach (see Section 3.3). Regarding use of cytogenetic assays as biomarkers of late effects, studies showed some potential to predict risk of radiotherapy side-effects but, in general, the results are equivocal.

3.1.3 Cytogenic biomarkers of persistent effects

Translocation frequency, as detected by the monochrome FISH technique, was found to be linearly related to individual red bone marrow dose from incorporated Sr-89/90 above 300 mGy >50 years after irradiation in the Techa River residents [55]. The FISH translocation assay is also informative for combined external gamma and internal doses from Sr-90, albeit with fairly large uncertainties [56]. A significant linear relationship between translocations and red bone marrow dose >300 mGy from past prolonged external gamma-radiation exposure was also found in studies of Mayak workers [57]. However establishment of the relationship between translocations and dose from internal alpha-particle exposure is more complicated since translocations may be part of complex aberrations with more than three different chromosomes involved, again at moderate to high doses [58–60]. Studies of Mayak workers also demonstrated an association between intra-chromosome aberration frequency and absorbed dose from internal alpha-particles in RBM [61–63]. Estimation of individual dose using these approaches has a substantial

uncertainty (90–100%), however the uncertainty for group dose estimates is lower (30–40%). Thus individual retrospective dose assessment is highly problematic [64].

The modified micronucleus-centromere test has also been used as an exposure biomarker [12]. In former uranium miners with high absorbed dose from Rn and its progeny to the lung, an increase was found in the number of centromere-free micronuclei (micronuclei containing only acentric fragments), but not in the overall frequency of micronucleus containing cells in peripheral blood lymphocytes, over a decade after the end of employment when compared to an unexposed control group [12]. Results of this study are in agreement with other studies of former uranium miners from Germany [65], suggesting that cytogenetic damage from alpha-radiation can persist for many years after exposure.

3.1.4 Telomere length and loss as biomarkers of susceptibility and exposure

TL varies between individuals [66] and decreases with age. Cells with short or damaged telomeres have increased genetic instability, which probably plays an important role in cancer etiology. Individuals with short versus long telomeres also have more radiation induced micronuclei [67]. Indeed, a strong correlation between TL and clinical radiosensitivity was demonstrated in many *in vitro* and *in vivo* studies (reviewed in [68]). For example a recent study found that while mean TL was not affected in peripheral leukocytes from 25 patients after radiation treatment (mean dose 52 Gy), there was a significant decrease in the proportion of cells with short telomeres [69]. Comparisons of TL in Hodgkin lymphoma survivors with and without cardiovascular disease indicated that survivors who develop cardiovascular disease post-radiotherapy have significant telomere shortening [70]. Thus, whilst TL, telomere maintenance, and telomere dysfunction may play a role in the prediction of individual radiosensitivity and in the long-term health risks following high dose IR exposure, studies need to examine the variation after low dose exposures and to identify all confounding factors.

3.1.5 Potential IR specific chromosomal rearrangement signatures in thyroid tumour tissue

Chromosomal rearrangements are common genetic alterations in PTC, the most common thyroid tumour. These include rearrangements involving the RET proto-oncogene, the so-called RET/PTC rearrangements, all of which lead to constitutive activation of the MAPK pathway. The frequency of the RET/PTC1 and RET/PTC3 rearrangements was initially associated with radiation exposure levels in a study of the atomic bomb survivors and some, but not all, studies of post-Chernobyl PTC [71,72]. However, similar frequencies of RET rearrangements have been observed in PTC without any history of radiation exposure [73] and RET/PTC3 rearrangements in radiation induced and sporadic PTCs from young patients, indicating a relation with age of PTC onset rather than with radiation exposure history [74–76].

Ricarte-Filho et al. reported a higher frequency of fusion oncogenes in post-Chernobyl radiation-induced PTCs (84.6%) compared to sporadic PTCs (33.3%) in young people from the Ukraine. These included rare TRK and BRAF rearrangements and two newly described fusion oncogenes ETV6-NTRK3 and AGK-BRAF [77]. However, these differences should be interpreted with caution as whilst the radiation-induced PTCs were examined using candidate gene assays and next-generation RNA sequencing, the sporadic PTCs in this study were only screened for known genetic alterations and not analysed by next-generation RNA sequencing. As discussed by Santoro and Carlomagno [78], the study by Ricarte-Filho and colleagues demonstrates that radiation exposure caused a selective increase of oncogenic driver events generated by gene rearrangements compared with point mutations. Leeman-Neill et al., [79] detected the ETV6/NTRK3 rearrangement in 14.5% of post-Chernobyl PTCs and 2% of sporadic PTCs from the general U.S. population. Moreover, they showed that ETV6/NTRK3 can be directly induced in thyroid cells *in vitro* after exposure to 1 Gy of ^{131}I or gamma-irradiation and thus may represent a novel mechanism of radiation-induced carcinogenesis. However, these results should also be interpreted with caution as the sporadic and post-Chernobyl PTCs were not matched. Such findings require further validation in independent tumour cohorts including appropriate matched PTCs from exposed and non-exposed patients. It should be noted that chromosomal copy number changes are also noted in thyroid tumours (see Section ~~3.8~~3.8) and that currently no radiation specific chromosomal signature has been described in other tumour tissues.

3.2 DNA and nucleotide pool damage biomarkers

IR can induce DNA lesions, which are potential biomarkers of exposure, directly or indirectly. For in-depth reviews on the possibility of identifying radiation specific lesions, the reader is referred to excellent recent publications [80,81]. Of the lesions produced, DNA strand breaks can be measured directly or by using surrogate endpoints such as γH2AX foci or assays such as the comet assay. These techniques and their use were reviewed in Pernot et al. [9] and only γH2AX is revisited here, since its measurement has been integrated into several molecular epidemiological studies (see Section ~~3.2.2~~3.2.2). The nucleotide pool is also a target of IR and indirectly of oxidative stress and recent progress on the use of 8-oxo-dG as a biomarker of individual sensitivity to radiation is discussed in Section ~~3.2.3~~3.2.3.

3.2.1 DNA lesions

The measurement of radiation-induced DNA lesions remains a challenge as (1) the yield of formation per dose unit is very low, with <1 modification per 1–10 million normal bases per gray; and (2) many of the lesions produced by IR directly or indirectly are similar to those generated by endogenous stress. Thus, as discussed by Ravanat et al. [80], even in the absence of radiation, oxidative DNA lesions, such as 8-oxo-dG are detected in cells at levels around one modification per million DNA bases. Based on this, exposure to about 40–50 Gy is needed to double the yield of 8-oxo-dG above the background level. Thus, variation in levels of DNA lesions identical to those produced by

endogenous oxidative stress after exposures to low doses may be difficult to interpret in terms of radiation exposure biomarkers. Future attention needs to focus on lesions that are predominantly produced by IR, and not, or at a lower level, by endogenous oxidative stress such as DNA lesions characteristic of densely ionizing events, such as micronuclei, DC and translocations.

In this context, recent data suggest that the decomposition of the initially produced radicals contributes to the generation of complex DNA lesions. One example is the cytosine adduct dCyd341 [80] and 2′,3′-dideoxynucleosides may represent potential biomarkers of low energy electrons [82]. However technical progress and confirmation that such adducts are formed *in vivo* is still needed before investigation in a molecular epidemiology setting.

In contrast to endogenous oxidative stress, some radiation-induced lesions are generated in clusters. The profile of lesion clustering varies with radiation quality: high LET IR produces greater lesion clustering due to the confined energy deposition [83]. Within this confined space, direct ionisations along the DNA backbone will generate DSBs that can be only 10–20 bp apart and reactive free radicals leading to strand cross linking. The detection and quantification of clustered lesions because of their very nature remains technically challenging but particularly interesting because the approach has the potential to be radiation specific.

The relative proximity of different lesions in clusters generates a challenging situation for the cellular DNA repair machinery with the repair of clustered lesions occurring with a slower time course compared to that of discrete DSBs and isolated lesions (see [83] for recent review). The formation and repair of complex lesions could have particularly marked consequences after low dose chronic exposures and could contribute to the non-linear patterns observed in cell survival at doses <1 Gy (see [83] for recent review). It is technically feasible to envisage a bioassay where, for instance, lymphocytes could be irradiated *ex vivo* with radiation of different qualities and the formation of clustered lesions followed with time using surrogate endpoints such as formation of DNA repair foci detected by immunological based techniques or the Comet assay. However, such bioassays require access to appropriate radiation platforms and would be limited by all the technical and sensitivity constraints that apply to such measurements.

3.2.2 γH2AX and DNA repair foci

DNA DSBs are signalled by the accumulation of phosphorylated proteins at the damage sites forming DNA repair foci. One prominent protein in this DDR is the histone H2AX which is phosphorylated on Ser-139 [84], generating γH2AX that can be detected and quantified using specific antibodies. γH2AX can also arise in the absence of radiation exposure following replication fork stalling/collapse at regions of single stranded DNA and other processes that directly or secondarily induce DSBs such as the repair of lesions including DNA adducts, crosslinks, and UV-induced photolesions [85]. However the staining patterns are often morphologically different from the punctuate staining observed after DSB induction (see [86] for review) and with appropriate care, for example by using non-replicating lymphocytes, the γH2AX signal can specifically monitor DSBs.

The formation and persistence of γH2AX with time after radiation exposure has the potential to be a sensitive biomarker of exposure. Indeed foci can be detected after radiation exposure to low doses (10 mGy) such as those received after a CT scan [52,87,88]. However, detailed evaluation of γH2AX as a biomarker of low dose exposure has revealed substantial difficulties, which are discussed below. A further, important limitation is that the γH2AX signal disappears rapidly after exposure. Nonetheless, γH2AX can be used as a biomarker of DRC and thus as a potential biomarker of susceptibility.

3.2.2.1 Assay design and validation A number of methodological and technical issues relating to the measurement and comparison of γH2AX in human populations have been highlighted in a recent systematic literature review of 68 studies published between 2005 and 2012, that reported γH2AX levels as biomarkers of either DNA damage or repair [86]. The sample size in most of the studies was generally low (mean number 38 ranging from 5 to 352) with only 7/68 having a study group of 10 or more individuals. Comparing the different systems to assess and quantify γH2AX foci numbers or fluorescence (automated microscopic analysis, FACS analysis, laser scanning cytometer), FACS based methods are emerging as a fast, reliable method t analyse a large number of cells, increasing the strength of the statistical analysis. In addition, they allow assessment of heterogeneous populations and discrimination of cells in different phases of the cell cycle. However, compared to microscopic analysis the quantification of both the number and size of foci/cell is not possible. Considerable technical variability has been observed between techniques. FACS analyses showed that the standard error of the mean often reached more than 20% [89] and, using laser scanning cytometer to quantitatively total fluorescence per cell from triplicate slides, the average CV was estimated to be 8.28% and 9.69% for endogenous and radiation induced γ-H2AX levels, respectively [89]. Data from automated microscopi analysis indicated a CV of <7.5% following radiation (1 Gy X-ray) while for endogenous damage it was 20% (Gomolka personal communication).

As discussed by Valdiglesias et al. [86] a critical measure for assay standardisation and an important consideration to allow the comparison of results between centres, is the availability of an expected range of reference values to validate protocols and scoring criteria. Whilst numbers of basal γH2AX foci detected in peripheral blood by microscopy have been documented, no reference values could be reported in studies using flow cytometry which could limit inter-laboratory comparisons. In addition, the number of events analysed differ largely depending on the methodology used, which in turn will impact on the statistical analysis and the quantitative comparisons between studies.

Another factor that impacts on basal γH2AX foci levels is the cell type. Indeed, whilst the γH2AX assay can be applied to most types of cell or tissue, differences in background levels are reported. The choice of tissues will also depend on the purpose of the study and what is feasible and ethical to collect from the study population. The method of blood collection and treatment of samples, as well as the factors discussed above relating to technique and protocols, impact on γH2AX quantification and can explain part of the variation seen between laboratories in γH2AX measurements [88,90,91]

3.2.2.2 Intra-and inter-individual variation in response There is limited published information on intr(this should read: intra-individual)a individual variability in terms of γH2AX foci levels. For example, the analysis of tw

independent repeated blood samples from 8 males of γH2AX background levels and radiation induced levels resulted in reliable measurements with no statistically significant differences [92]. The variability observed was about 12%, within the technica variability for the assay.

Substantial inter-individual variability has been reported using stimulated or non-stimulated lymphocytes [93–95], lymphoblastoid cell lines [16] as well as circulating lymphocytes from prostate and breast cancer patients after fractionated radiotherapy [95] (reviewed in [86,95]). Individual factors such as age, gender, ethnicity and life style have been shown to affect the level of endogenous γH2AX radiation induced foci and residual foci after DSB repair [86,93,94]. For example the induction of γ-H2AX at 0.5 h after *ex vivo* blood irradiation, and peak formation at 2 h were independent of age, gender and ethnicity but varied with race and alcohol use, which delayed the peak to 4 h [94].

3.2.2.3 Suitability for assessment of exposure and radiation quality

3.2.2.3.1 Suitability of the procedure Several comparisons of γH2AX measurements in the high dose range [34,96,97] and one in the low dose range [88] have been performed using blood samples irradiated *ex vivo* to assess this endpoir for biodosimetry purposes. Compared to other endpoints (dicentrics, micronuclei, gene expression), the γH2AX assay was the most rapid, providing results within 24 h. However, it showed considerable variability at both high and low doses (0.1 to 6.4 Gy although the findings indicated that, based on γH2AX levels in lymphocytes, the most severely exposed individuals within a cohort could be identified, enabling their prioritisation for accurate chromosome dosimetry [97]. If the levels of γH2AX are to be use for biodosimetry, calibration curves for residual damage at different time points after exposure, adjusted for cell type and age are urgently needed (see below). Despite these limitations, it has to be recognised that levels of γH2AX could provide a means triage after a radiation incident and simple, fast and inexpensive protocols have been developed that make use of finger-prick-sized blood volumes [98,99].

3.2.2.3.2 Suitability to estimate low dose exposures An inter-laboratory low dose range comparison [88] using *ex vivo* irradiated lymphocytes showed that blinded samples could be successfully ranked on the basis of exposure dos (from 10 to 100 mGy) by monitoring mean foci/cell and that doses as low as 10 mGy analysed 30 min post exposure could be distinguished from sham-irradiated control samples. However, while a low level of γH2AX in lymphocyte samples can indicate a lo dose exposure, it did not accurately reflect an individual's exposure dose in the low dose range (10–50 mGy) due to technical and inter-individual variability as discussed above.

In contrast to exposure to a uniform acute dose, accidental partial body irradiation to a high dose (but causing a low total body dose) may be assessed more efficiently using foci analyses than by assessing dicentric formation [95,100,101]. Among breast and prostate cancer patients receiving radiotherapy, Zahnreich et al., demonstrated that foci quantitation in peripheral leukocytes immediately after a single acute heterogeneous radiation exposure to a small radiation field in the body is far more sensitive to the absorbed equivalent whole-body dose than the analysis of unstable aberrations (dicentrics) [95].

3.2.2.3.3 Suitability for assessing radiation quality The spatial distribution of radiation-induced DNA lesions within the cell nucleus depends on radiation quality. As discussed above, heavy ions, alpha particles and also low energ (e.g. 29 kV) X-rays induce a complex DNA damage pattern with densely localised DNA lesions which are difficult to repair accurately. Considerable progress has been made in our understanding of the DNA repair proteins involved in the repair of differer DNA lesions by recording the cellular and spatial distribution of different DNA repair enzyme induced foci, the phosphorylation-dephosphorylation kinetics and the foci size distribution with time [102–107]. Additional information about the complexity of th DNA damage and the influence of radiation quality can also be gained by double labelling of proteins involved in different repair pathways, such as 53BP1 and γH2AX [106,108]. Whether such measurements can be used in a bioassay setting, discussed belov remains to be established, but they could potentially be informative in assessing radiation quality and possibly inter-individual variability in DRC.

3.2.2.4 γH2AX as a biomarker of susceptibility

In contrast to the difficulties of exploiting γH2AX analysis for assessing exposure, there is accumulating evidence that the persistence of γH2AX with time after irradiation can be used a a surrogate endpoint to assess DRC, thereby reflecting its use as a biomarker of susceptibility. Indeed a deficiency in DNA repair, and in particular DSB repair has been shown to correlate with radiation sensitivity in patients diagnosed with AT, NBS, Ligase syndrome (and other NHEJ deficiencies), Fanconi anaemia and a number of other syndromes (reviewed in [86,102]). Altered γH2AX foci formation and persistence with time has also been linked to tumour radiation sensitivity (for examples see [109]), sever acute and late radiation toxicity (reviewed in [101,110]) and the risk of second malignant neoplasms in childhood cancer survivors [16]. However contradictory results on the ability of foci analyses to detect DDR defects as a surrogate marker for geneti radiation sensitivity exist and not all DDR defects will be resolved by one biomarker. Radiosensitivity detected by clonogenic assays does not always correlate with detectable defects in γH2AX removal, particularly for the analysis of patients wit hypomorphic genetic changes [111]. This may be due to the presence of cells in different cell cycle phases in the test material resulting in a high non DSB specific background of γH2AX the use of inappropriate cell systems, e.g. lymphocytes in G0 state fo analysis of homologous recombination; the challenging radiation dose, which may not be high enough to resolve slight differences in repair efficiency; or the way in which the data are statistically analysed. Usually the mean or median number of fo remaining after a defined repair time, or time dependent repair kinetics is the endpoint assessed to monitor DRC. A novel approach proposes that the average number of foci based on the cell cycle distribution should be used [102]. The co-localisation ratio γH2AX to 53BP1 can also serve as a parameter for radiation sensitivity. It was shown to be 1 in a normal cell line but to deviate from this ratio in radiosensitive cell lines. According to Martin et al. even mild differences in repair defects, masked usin standard average foci calculations, can be distinguished using these strategies [102]. This needs validating in further cell lines or lymphocytes with known radiation sensitive phenotypes. Finally, the radiation exposure scheme may also be important. It possible that after a single acute exposure, less inter-individual variation in DNA repair is seen than after fractionated irradiation as used in radiotherapy protocols [112].

In summary, detailed analysis has suggested that γH2AX analysis has limitations in its utility as a biomarker to detect low dose exposure due to technical and inter-individual variability, by confounding effects caused by exposures other than radiation including disease states. However, the assay does have the potential to be suitable for assessment of DSB repair capacity, which is important for assessing the response to radiation exposure.

3.2.3 Extracellular 8-oxo-dG

Extracellular 8-oxo-7,8-dihydro-2′- deoxyguanosine (8-oxo-dG) in blood serum, urine and cell culture medium has been suggested to be a general biomarker of oxidative stress. The levels vary with life style factors, certain disease states and exposure to different stressors such as IR [113–115]. As previously discussed [9], extracellular 8-oxo-dG levels in blood and urine is not a specific biomarker of radiation exposure due to the impact of confounding factors. However extracellular 8-oxo-dG as a biomarker for individual sensitivity to radiation may be a more promising application both as a diagnostic tool for personalized radiotherapy and as tool to better understand the extent of radiation-response variations in the normal population. Used in a bioassay, changes in the extracellular levels of 8-oxo-dG in blood serum in response to *ex vivo* low dose radiation have been shown to correlate with acute or late healthy tissue adverse reactions [115–119]. The development of techniques for analysis of 8-oxo-dG in saliva is ongoing and can add to the usefullness of saliva as a non-invasive source for biomarker studies in radiation research [17]. Further studies are needed to validate that 8-oxo-dG can be used as a diagnostic tool for individual sensitivity as well as of the mechanisms linking oxidative stress response with individual sensitivity.

3.3 Biomarkers related to germline variants

The established genetic basis for radiosensitivity underpins research aimed at identifying germline variants as biomarkers of susceptibility to radiation-induced health effects [120]. An advantage of the approach is that, once identified, any test will not suffer from the poor reproducibility associated with functional assays measuring radiosensitivity. There is no need to assess biomarker precision, accuracy and cross laboratory reliability as the technology needed is available in clinical laboratories. Germline variant biomarker research is all about discovery − finding enough variants to develop a test to identify individuals who are radiation sensitive. A disadvantage is the need for very large collaborative studies to identify sufficient variants.

Supplementary Table 3.3.1 lists the currently known genes where rare mutations are associated with susceptibility to radiation-induced effects. Many are associated with the DDR and are either very likely or known to increase risk of radiation-induced cancers. However, the rarity of homozygous carriers of such gene mutations in the general population and the fact that such individuals are generally identified phenotypically limits their contribution to radiation sensitivity in population studies. Heterozygous carriage of gene mutations associated with certain DDR disorders can confer a subtle phenotype, including cancer predisposition, and there is mounting evidence from mouse and human studies that even a two-fold reduction in the levels of some proteins can confer a significant increase in cancer risk [121]. Indeed an increased risk of primary breast cancer was reported in *BRCA1/2* mutation carriers exposed to low dose diagnostic radiation under 30 years of age [122]. Whether *ATM* heterozygotes have an increased risk of radiation effects continues to be debated, but better technology and study design are improving the quality of studies. Women's Environmental, Cancer, and Radiation Epidemiology (WECARE) is a population-based study of cases with contralateral breast cancer and matched controls with unilateral breast cancer. WECARE showed women carrying rare missense variants in *ATM* may have an increase risk of IR-induced breast cancer [123].

Although studying mutation carriers is of interest, studies need to be very large to have sufficient statistical power because of the low prevalence of individual mutations. Therefore, research has tended over the past 10 years to focus on identifying the common genetic variants seen in >1% of the population (SNPs) for which there is growing evidence of functional impact. The quality of SNP biomarker discovery in the IR field has improved since the Pernot publication aided by the establishment of the Radiogenomics Consortium [124] and the development of reporting of guidelines for radiogenomic studies [120]. Supplementary Table 3.3.2 lists recent publications involving >1000 participants that investigated SNPs associated with IR late effects.

Regarding SNPs associated with susceptibility, the WECARE study investigated 152 SNPs in activators and downstream targets of *ATM* (*CHEK2, MRE11A, MDC1, NBN, RAD50, TP53BP1*) in relation to IR exposure and contralateral breast cancer risk. Carriers of a haplotype in *RAD50* treated with IR had a greater contralateral breast cancer risk than unexposed carriers [125].

There has also been a number of GWAS since the Pernot publication. Large cooperative GWAS outside the IR field highlight the potential to identify a large number of SNPs, with individual variants having small effect sizes but together accounting for a meaningful proportion of susceptibility for a trait/disease [126].

The radiogenomic GWAS carried out to date show the potential to identify SNPs associated with IR effects and susceptibility (Supplementary Table 3.3.2 and references therein). For example, a variant in *PRDM1* was associated with second malignancies in individuals who underwent radiotherapy for Hodgin's lymphoma as children [127]. Interestingly the gene encodes a protein involved in the immune response to viral infection. It was suggested that *PRDM1* might be a candidate tumour suppressor gene mediated by IR exposure, which raises the possibility that alterations in immune regulation contribute to increased risk [128]. The GWAS involved younger subjects where the effect size for genetic susceptibility should be stronger (less masked by environmental influences such as smoking) thus increasing statistical power and reducing the sample sizes required [128].

Genetic studies are identifying SNPs associated with IR late effects and susceptibility and more will emerge over the next five years. Future studies are likely to explore other types of genetic variation that might be important: CNV, insertions and deletions, mitochondrial DNA variants and germline methylation. It might also be useful to investigate whether the SNPs associated with other endpoints relevant for IR epidemiology (e.g. cancer susceptibility, cardiovascular disease and cataracts) are associated with IR susceptibility.

It is anticipated that a future germline variant epidemiology biomarker would be a polygenic risk score based on genotyping tens to a couple of hundred of genetic variants. A blood or saliva sample would be required and the results would be generated rapidly in laboratories that are accredited to carry out genetic testing. Biomarkers related to genetic variants have promise for use in identifying individual risks for late effects and susceptabiltiy in epidemiology studies. The challenge lies in designing studies with sufficient statistical power and identifying cohorts with the data and samples required.

3.4 Biomarkers related to induced mutations

Whilst it is well established that IR is a mutagenic agent, whether IR induced tumours have a specific mutation profile remains to be fully established. Mutational signatures have been identified using whole exome sequencing in mouse models of second malignant neoplasms mutational signatures [129] and new findings using whole genome sequencing have unraveled a specific pattern of mutations in IR associated human tumours [130]. Two signatures of somatic mutations were found irrespectively of the tumour type. Both extra genome-wide small deletions (1–100 base pairs), often with micro-homology at the junction, and a significant increase in balanced inversions were detected. These distinctive mutational signatures could explain the carcinogenic potential of IR.

As discussed in Section 3.1.4 one of the few examples of a possible IR associated genetic alteration is the gain of chromosomal band 7q11.23 in PTC in young patients exposed to ^{131}I from the Chernobyl fallout [131]. An unbiased genome wide survey of germline mutations induced in mice after parental exposure to IR was recently conducted [132], showing a significantly higher frequency of *de novo* CNVs and insertion/deletion events in offspring of exposed fathers, as well as over-representation of clustered mutations in the spectrum of induced de novo single nucleotide variants. Whether this finding can be translated into a signature specific to IR exposure suitable for use in molecular epidemiological studies to, for instance, classify tumours as IR induced will be a future challenge.

3.5 Biomarkers related to transcriptional and translational changes

3.5.1 Biomarkers related to changes in RNA levels

A substantial number of recent studies have reported gene expression signatures for IR exposure biodosimetry identified using different classification models like *k*-Nearest Neighbors, Nearest (Shrunken) Centroids or Random Forests (Supplementary Table 3.5.1.1), and validated the IR-responsiveness of such signatures with ever decreasing doses (Table 2). These studies have been made possible by many technological advances, including; customized qRT-PCR arrays [133] or multiplex qRT-PCR assays [134,135], which allow rapid PCR amplification of a significant number of genes. Other technologies include the Nanostring [134,136] and Chemical Ligation Dependent Probe Amplification [137,138] methods, with the added advantage that they do not require prior complementary DNA synthesis. These advances, which would allow targeted screening of small gene signatures (typically <100 genes) as opposed to genome-wide technologies, are appropriate for high-throughput screenings in the situation of a large-scale event [139]. The recent use of exon-level microarrays to evaluate genome-wide IR-induced gene expression and alternative splicing in different experimental models has revealed that a number of genes express transcript variants in response to IR [23,140–142] which could prove to be highly specific IR biomarkers (further discussed below).

Table 2 Gene expression studies investigating the transcriptional response to low-dose (<100 mGy) radiation exposure.

alt-text: Table 2

Dose (mGy)	Dose rate	Quality	Cell type	Organism	Time point (h)	Genes reported to be responsive to ≤100 mGy and validated by qRT-PCR	Reference
0, 100, 2500	Not reported	4.5 MeV protons	3D epidermis model	Human	4, 16 and 24	ADAMTS1, APOBEC3A, ATF3, BCAS3, CDK9, CYP1B1, GPRC5A, IGF2, PSCA, PTGS2, ROR1	[290]
0, 10, 25, 50, 75, 100	1.5–4 cGy/min	gamma-rays	lymphoblastoid cell lines	Human	4	ASCL3, CD164, GGH, LIPA, PAM, PPT1, SCAMP1, SSR1	[291]
0, 20, 100. 500, 1000, 2000, 4000	0.7 Gy/min, 0.0286 Gy/min for 20 and 100 mGy	gamma-rays	PB	Human	6, 24, 48	FDXR, PFKFB3	[147]
0, 10, 25,50	10 mGy/min	X-rays	PBMC	Human	24 and 48	Not reported	[292]
0, 100, 1000	0.5 Gy/min	X-rays	3D skin model	Human	5 min, 3, 8 and 24	Not reported	[293]
0, 50, 1000	3 cGy/min	X-rays	PB	Human	8	CCR4, GNG11, PF4, POLH	[294]
0, 5, 10, 20, 50, 75, 100	4.9 mGy/min	X-rays	PB	Human	2 and 24	C12orf5/TIGAR, CCNG1, DDB2, GADD45A, FDXR, MDM2, PHPT1	[148]

0, 5, 10, 25, 50, 100, 500	50 mGy/min	gamma-rays	CD4+ lymphocytes	Human	2.5, 5, 7.5 and 10	Not reported	[295]

PB: Peripheral blood; PBMC: Peripheral blood mononuclear cell.

Most, if not all, of such studies have identified very similar gene signatures, despite differences in the subjects, IR doses, dose rates, radiation qualities, cell/tissue types, sampling times and gene expression platforms used (Table 2). For instance, in a comparison of four studies using different IR sources and biological samples (gamma-rays on whole blood, X-rays on PBMCs, alpha particles on PBMCs and gamma rays on primary keratinocytes and fibroblasts) [23] 14 genes showed dose-dependent induction in all studies: *DDB2*, *POLH*, *MDM2*, *RPS27L*, *FDXR*, *CCNG1*, *TRIAP1*, *SESN1*, *FBXO22*, *PPM1D*, *ANKRA2*, *CDKN1A*, *TRIM22*, and *BBC3*. This demonstrates the robustness of these signatures and their suitability as IR-exposure biomarkers. Indeed, a recent NATO biodosimetry study showed that single genes as well as gene signatures could be used to estimate exposure of blood samples to IR doses of 0.1 ~~to~~ 6.4 Gy with a similar accuracy and sensitivity as established cytogenetic assays [137]. Importantly these signatures, which have mostly emerged from studies involving *ex vivo* irradiated blood samples, are also applicable for estimating exposures in radiation therapy patients [139,143,144]. Changes in expression levels of some of these genes up to 48 h after irradiation have been demonstrated. Although this is a short time period for epidemiological studies, it would be sufficient for use in a radiological accident. Moreover, since the transcriptional responses of individual genes display different kinetics, one can envisage that gene expression signatures could also be used to accurately predict not only the dose, but also the time since exposure (Macaeva and Quintens, unpublished data). In this respect, the use of a signature consisting of a number of different genes, each with their maximum response at a different time point offers a significant advantage over a single gene biodosimeter.

Several studies have shown dose-dependent changes in gene expression after exposure to doses as low as 5 mGy (Table 2) [135,145]. Exposure to 5–25 mGy mostly induced genes involved in metabolic processes and chromatin organization, while the p53-mediated pathways and DDR were mostly activated at doses of 25 mGy and above [145]. This is attributed to the fact that at low doses a DSB is not induced in every cell. A linear dose-response relation up to 1 Gy was seen for nine genes with *CCNG1, DDB2, FDXR, GADD45A,* and *PHPT1* being significantly differentially expressed at doses ≤ 100 mGy. Such results highlight that gene signatures may well be applicable for biodosimetry purposes in the low-dose range.

Amongst the genes that have been identified as radiation-responsive, *FDXR* has emerged as accurate and reliable for dose estimation in human blood samples [137,146–148]. FDXR, also known as adrenodoxin reductase, is a mitochondrial flavoprotein that transfers electrons from NADPH to mitochondrial cytochrome P450 enzymes (Fig~~ure~~. 1), mediating the function of ferredoxin [149]. It is involved in multiple processes mediated through p53 as well as ROS associated apoptosis [150,151]. Quantitatively, *FDXR* is one of the most radiation-responsive genes (e.g. up to 46–fold upregulation 24 h after 4 Gy irradiation in human blood), with relatively small inter-individual variability. This allows easy discrimination between high and low dose exposure [148] although a saturation dose effect occurs at doses >2 Gy. *FDXR* has been validated as a sensitive gene to assess dose in irradiated patients in two large scale studies involving nine labs [137,139] and found to be the best gene for dose assessment by four of the seven partner laboratories in a NATO led exercise [137]. A RENEB inter-laboratory comparison exercise also found it was the best gene for dose estimation for *ex vivo* irradiated blood from 12 donors and, for the first time, could distinguish blood samples of prostate cancer patients exposed to 0.009–0.017 Gy (first fraction only, partial body exposure) [139].

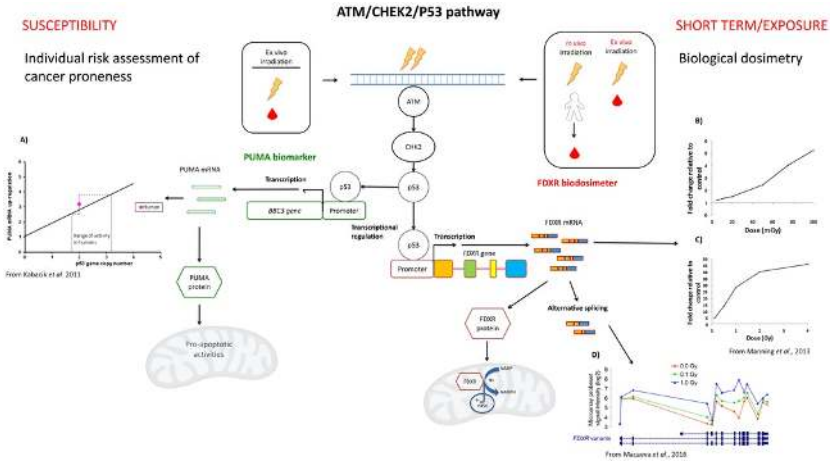

Fig. 1 PUMA and FDXR mRNAs are biomarkers of respectively cancer susceptibility and radiation exposure linked to the ATM/CHEK2/P53 pathway. (the quality of this figure is not very good . is it possible to replace it with the attached version.)

FDXR receives electrons from NADPH and initiates the electron-transport chain for cytochromes P450 within mitochondria. PUMA localizes to the mitochondria and is involved in the activation of apoptose. A) *Ex vivo* irradiation of whole blood (2 Gy) leads to early variations ir

PUMA mRNA (2 h post exposure) compared to control (0 Gy) samples that may potentially be used as susceptibility biomarker for cancer. The level of PUMA transcriptional up-regulation is associated with the activity level of the ATM/CHEK2/P53, an essential DDR pathway componen(the reference is missing . Please add after component (adapted from [134]).)t. B), and C) At low (10–100 mGy) and high (1–4 Gy) doses, *in vivo* and *ex vivo* irradiation of human blood or T-lymphocytes leads to a strong FDXR mRNA level increase 24 h after irradiation. FDXR has the potential to be used as a biodosimete(reference is missing . Please add after biodosimeter (adapted from [135]).)r. D) FDXR is one of the most extensively radiation-induced spliced genes in blood cells. The sensitivity of the radiation-induced signal is dependent on the exon targeted by the primers/probe combination(reference is missing. Please add after combination (adapted from [23]).).

alt-text: Fig. 1

One of the recurrent questions for the use of an endpoint as a low dose IR biomarker is its specificity. As the genes often found in IR signatures are responsive to DNA damage, smoking is a potential confounder of concern. Paul and Amundson showed that smoking did not affect the predictive performance of their 74-gene IR response signature in peripheral blood, and only one gene was differentially expressed between males and females [152]. Importantly, this signature classified 98% of the samples correctly according to dose (0, 0.1, 0.5, or 2 Gy), irrespective of gender and smoking. The effect of inflammatory stress has also been investigated. Whilst a panel of eight genes which, in combination with one phosphoprotein marker, could classify human peripheral blood samples with an accuracy of 88% depending on their IR exposure (0 or 2 Gy), inflammation status, or both [153] it remains to be established whether this will still be the case after lower exposure doses. A study in mice found that many genes would retain their potential utility as IR biomarkers regardless of whether the animals were treated with LPS or granulocyte-colony stimulating factor over a dose range of 0.2–0.6 Gy [144,154].

3.5.1.1 IR induced alternative splicing As discussed above, recent studies using exon-level microarrays to examine transcript changes have shown that a large number of genes, including *FDXR* (Fig. 1), express transcript variants i response to IR [23,140–142]. In the case of IR, this seems to result mostly from alternative promoter usage by p53 [140,142], although changes in RNA polymerase elongation [155] or interactions between the RNA polymerase and splicing factors [156,157 may also be involved, as has been shown for other genotoxicants, such as UV irradiation. However, the specificity of these alternative splicing events for exposure to IR compared to UV has yet to be established.

Importantly, it was demonstrated that gene and exon signatures from PBMCs were equally suitable for correctly (100% accuracy) classifying samples that were either not irradiated, irradiated with a low dose (0.1 Gy) or a high dose (1 Gy) of X-rays. As IR-induced differences in expression were more pronounced at the exon-level compared to genes, exon signatures may potentially be useful exposure markers for doses below 0.1 Gy [23]. IR-induced expression of transcript variants may explain differences in expression levels obtained by primer- or probe-based assays between different studies, which could result from the interrogation of more or less responsive exons. Therefore, when using such assays for biodosimetry purposes, it is important to target the correct combination of exons.

Further investigations into the transcriptional IR response of cells and tissues using, for instance, next-generation sequencing of transcriptomes, would be very valuable for identifying the exact sequence identity of IR-induced splice variants. Although not immediately applicable for biomarker purposes, the functional characterisation of these variants would significantly increase our understanding of the molecular mechanisms involved in the (low-dose) IR response.

3.5.1.2 Transcriptional biomarkers of susceptibility/late or persistent effects Although most of the work on transcriptional IR biomarkers has focused on finding biomarkers of IR exposure, several reports hav attempted to identify either pathways or gene signatures predictive of susceptibility and late health effects.

3.5.1.2.1 Transcriptional biomarkers of susceptibility The ATM/CHEK2/p53 pathway responds to DSBs, leading to cell cycle arrest and DNA repair through the transcription of genes including *CDKN1A* (*p21)* and *DDB2* (Fig. 1 Failure to undergo repair may result in enhanced apoptosis (e.g. via *BBC3* (PUMA)), permanent cell cycle arrest or senescence. Using mouse strains differing in copy numbers of *Atm*, *Trp53* (p53) and *Chek2,* IR-induced changes in transcription of the p5 target genes *Cdkn1a*, *Puma* and *Sesn*2 were strongly dependent on DDR pathway component copy number, with the best correlation being seen for *Puma* [139,163]. Atm/Chek2/p53 pathway activity as assessed by the *Puma* response correlated well with cance incidence in mice with differing *Trp53* gene copy number [158]. The mouse data was validated in humans by examining mitogen stimulated T-lymphocyte cultures from healthy donors, *ATM* mutation carriers and Li Fraumeni Syndrome patients followin irradiation [163]. *PUMA* upregulation after irradiation was observed, with the AT case having a very weak response and AT heterozygous carriers and LFS samples showing an intermediate response. Inter-individual variability in the activity of th ATM/CHEK2/p53 pathway was assessed in blood samples from the same 32 healthy donors irradiated *ex vivo* with 2 Gy. *PUMA* gene expression examined at 2 h was compared against the previously obtained mouse p53 copy number linear curve and wa found to be consistently over- or under-expressed in comparison to the mean for specific donors, suggesting natural variation between individuals (Badie personal communication). These data demonstrate that an integrative biological approach monitorin IR-induced changes in key p53 regulated genes in blood samples can provide a read-out of DDR pathway activity, with a potential link to susceptibility. P53-independent genes have also been proposed to predict susceptibility to IR. Forrester et al. identifie an 8-gene signature (*DDIT4L, DPT*, *FBN2*, *FST*, *GPRC5B*, *NOTCH3*, *PLCB1*, and *SGCG*) that could potentially predict fibrosis in patients prior to radiotherapy [159].

3.5.1.2.2 Transcriptional biomarkers of health effects There are few studies on the transcriptional changes linked to health effects. For instance $p16^{INK4A}$ may be a potential biomarker for the long-term health effects of childhoo radiotherapy in acute lymphoblastic leukaemia survivors [160]. $p16^{INK4A}$ is a marker of senescence and, although skin biopsies were taken on average 12 years after IR therapy, its expression was increased in irradiated skin compared to non-irradiated ski from about half of these patients.

Radiation workers (in medicine or industry) generally receive low doses of IR protracted over the duration of their work. In Mayak nuclear workers, who received higher doses in early years than most nuclear workers, associations were observed

between gene expression profiles in the peripheral blood and chronic non-cancer disease outcomes. Twelve mRNAs and nine miRNAs were significantly associated with six different diseases, particularly those related to atherosclerotic processes. These associations were gender- and dose-dependent and 26 potential confounders were considered including age at exposure, age at biosampling, demographic, social habits, data related to health status [161]. Other studies found long-term changes in gene expression in medical workers [162,163], and in thyroid cancer tissue from patients exposed to Chernobyl fallout [164,165]. Although the evidence from these studies remains preliminary and many from high dose exposures, they highlight the potential for gene expression signatures as biomarkers of late effects of IR exposure.

3.5.2 Biomarkers related to changes in protein levels

As discussed in [9], the identification of IR-associated protein biomarkers is challenging because of the time and dose-dependent variation in protein expression. A number of recent proteomic studies have investigated high dose IR induced alterations in the proteome of different bio-fluids including serum and urine with the aim of identifying biomarkers of exposure and IR sensitivity applicable to radiological emergencies For instance Chaze et al. [166], found an increased up-regulation of genes involved in the glycolysation in liver and increased serum cytokines, suggesting a systemic response to local irradiation of the skin to 20, 40, and 80 Gy gamma rays. Analysis of three proteins (Apol(please correct spelling should read: Apolipoprotein)ipprotein E, Factor X and Paththenate Kinase 4) in blood samples allowed discrimination up to 1 month following exposure to <2 Gy and >10 Gy in breast cancer patients, although differences at <2 Gy were subtle [167]. Similarly [168] found that changes in serum amyloid A (SAA) levels permitted a dose prediction model to discriminate ≤1 Gy from ≥2 Gy irradiated mice.

To investigate the mechanisms underlying individual IR sensitivity Skiöld et al. [169] compared the proteome profiles of leukocytes from *ex vivo* irradiated whole blood following 0, 1, or 150 mGy from normal responding and extremely radiosensitive patients. Proteomics analysis showed unique proteomic signatures separating the two groups at the basal level and after doses of 1 and 150 mGy. Pathway analysis suggested that the oxidative stress response, coagulation and acute phase response are hallmarks of IR sensitivity.

Although the literature after low dose IR remains limited these findings indicate that IR causes detectable changes in the bio-fluid profile at high doses and may differentiate radiosensitive individuals even at low doses. The main questions that needs addressing are; 1) whether the alterations in bio-fluid proteins are unique for IR exposure or whether they arise in more general physiological states such as inflammation; and 2) whether the low dose results are confirmed.

3.5.3 Correlations between proteins and mRNA and pathway mapping

The last two decades have seen an important emergence of technologies which allow high-throughput “omic” analyses. While originally analyzed separately, we have now reached the era of systems biology, in which these quantitative data-sets can be integrated. Studies investigating genome-wide correlations between mRNA and protein expression levels in different organisms found that mRNA levels predict only 30-sed separately, we have now reached the era of systems biology, in which these quantitative data-sets can be integrated. Studies investigating genome-wide correlations between mRNA and protein expression levels in different organisms found that mRNA levels predict only 30-50% of cellular protein levels [170], although higher correlations have also been observed [171], highlighting the importance of post-transcriptional regulatory processes and measurement noise that contribute to protein expression levels [172]. Indeed, correlations between mRNA and protein levels are significantly higher when considering genes that are differentially expressed after a treatment [173,174]. Also, removing experimental noise increases mRNA-protein expression correlations [170].

As discussed above, both transcriptomic and proteomic analyses have identified IR biomarkers, albeit mainly for exposure. However, mRNA and protein levels are rarely measured in the same study, nor have they been combined for the purpose of predicting unknown IR doses or as markers for IR sensitivity or late effects. A recent study analyzsed protein phosphorylation (H2AX, p53 and ATM) and gene expression of DDR genes (*ATF6*, *BAX*, *BBC3*, *DDB2*, *MDM2* and *TP53*) in T-lymphocytes from patients before and after (24 and 48 h) single-photon emission computed tomography myocardial perfusion imaging [175]. Patients received effective doses of 18.2 ± 10.6 mSv. Only a small number of these patients showed increased protein phosphorylation associated with increased expression of DDR genes (*BAX*, *DDB2*, *MDM2* and *TP53*). The other patients either had no change or decreased expression of DDR genes.

Another possible interaction between different molecular layers is that of the microRNome and proteome. Indeed, the function of microRNAs is to either degrade RNA messengers or prevent translation of their targets, thereby negatively influencing protein expression levels. Studies investigating correlations between microRNA and protein expression profiles in response to IR in different experimental models [176–178] found that moderate IR doses (200–500 mGy) affected expression of some microRNAs (see Section 3.6.23.6.2) while some of their predicted targets were oppositely regulated at the protein level.

In an important effort towards data integration following exposure of an *in vitro* 3-D human skin model to low IR doses [179], the temporal response (from 1 to 72 h) of dermal and epidermal layers to 100 mGy of X-rays was investigated using transcriptomic, proteomic, phosphoproteomic and metabolomic platforms. Besides cell cycle regulation and DNA damage signaling, mostly affected at the mRNA level, matrix regulation and oxidative stress response pathways were also modulated. The latter was predicted to be primarily regulated through the transcription factor SP1, which was shown to be activated (phosphorylated) in a dose-dependent way [179]. Such studies are in their infancy but demonstrate the power to identify pathways with expression changes modulated by low dose IR, which may represent potential IR biomarkers. They also highlight the dynamic nature of changes and the challenge of identifying a signature that is able to resolve both dose and time since exposure.

3.6 Biomarkers related to epigenomic modifications

3.6.1 IR induced protein post-translational modifications

The analysis of IR-induced changes in global proteome profiles highlights the broad range of alterations in the cellular processes that are regulated by PTMs including phosphorylation, acetylation, ubiquitination, and neddylation. The regulatory network of DRR is one of the best examples of a gene network regulated by PTMs which impact on protein activity and stability rapidly and independently of the changes in *de novo* protein synthesis [180,181] of which γH2AX is one of the most studied examples (Section ~~3.2.2~~3.2.2).

Recent advanced comparative proteomics methods applied in radiation biology [182] enable the analysis of PTM status after IR exposure. There are solid data describing the PTMs after high-dose exposure [183,184] but studies investigating PTMs after low-dose exposures are scarce, experimentally challenging and, as for high dose profiling, may be limited by the model system under investigation. For instance, the IR induced alterations in proteins found in a human skin model after IR doses (0.03, 0.1 and 2 Gy) were dispersed throughout the entire skin tissue with altered protein phosphorylation status being found in each layer with the majority corresponding to skin structural proteins (such as keratins and desmosomal proteins) involved in maintaining tissue integrity [185]. This suggests that the skin as a whole responds to IR and different epithelial layers may have different roles to maintain skin structural and genomic integrity following exposure to such a tissue stress.

Acetylation has mainly been studied in the context of transcriptional activity and as a regulator of chromatin accessibility through histone modification after high dose exposure to IR. For instance a hypoacetylation of H3K9, H3K56, H4K5, and H4K16 in lymphoblastoid cell lines is seen 15 min, 1 h and 24 h after exposure to 2 Gy and 10 Gy which may play a role in the increased cellular radiosensitivity [186]. The DDR is also regulated by the ubiquitination of key proteins [187]. High dose IR-induced DDR ubiquitination is as prevalent as phosphorylation and significantly more common than acetylation (see for instance [188]). It has been shown that protein ubiquitination is associated with the recruitment of DNA repair factors [189] and also the activation of key response proteins such as ATM. In addition to ubiquitin, it has been shown that the ubiquitin-like proteins such as small ubiquitin-like modifiers (SUMO), and neural precursor cell expressed developmentally down-regulated protein 8 (NEDD8) play essential roles in the cellular response to DNA damage [180]. However, such modifications have not been examined after low doses and it is not yet clear whether they would be good biomarkers of IR exposure or responses.

These examples illustrate how PTM studies may lead to identification of IR-specific-signatures to serve as possible biomarkers of exposure. Although the identification of PTMs is an anticipated goal in many clinical studies, the complex and sophisticated methodology necessary for a successful PTM analysis makes such studies challenging particularly in an epidemiological setting where sample collection with respect to exposure is often limited. Therefore, screening of potential IR-induced post-translational biomarkers with high confidence, reproducibility and accuracy using different biomaterials still remains a vision of the future.

3.6.2 Non-coding RNAs

ncRNAs are emerging as important biological molecules [190]. They are grouped broadly into two classes based on transcript size: small ncRNAs and lncRNAs. The small ncRNAs include miRNAs that are ~22 nucleotides long and are involved in the regulation of mRNAs following their transcription [191,192].

miRNA expression profiles are tissue-specific, can be modulated following exposure to both low and high LET irradiations and have potential as biomarkers of IR exposure [176,193–199] (Supplementary Table 3.6.2 and references therein). miRNA is more stable than mRNA, can be extracted from paraffin-embedded material [195], and is detectable in biological fluids after exposure to IR [196,197]. Although most studies to date involved blood samples, miRNAs can be measured in many biological fluids including saliva, tears, seminal fluid, breast milk and cerebrospinal fluid [200]. Most published data described the impact of high and moderate doses of IR and reported changes in a short time scale (minutes to hours) after exposure. There is a lack of data describing the effects of low doses and long-term effects (Supplementary Table 3.6.2).

LncRNAs also have developmental and tissue-specific expression patterns, and aberrant regulation in a variety of diseases, including cancer [190,201]. LncRNAs are mRNA-like transcripts ranging in length from 200 nucleotides to ~ 100 kilobases lacking significant open reading frames. These long polyadenylated RNAs do not code for proteins, but function directly as RNAs, recruiting chromatin modifiers to mediate transcriptional changes in processes ranging from X-inactivation (lncRNA XIST), imprinting (lncRNA H19) to genome-wide chromatin reprogramming (lncRNA HOTAIR) [202].

Evidence that IR exposure elicits dose- and time-dependent changes in the expression of ncRNAs that are influenced by the genetic background has come from studies monitoring the expression of 19 miRNAs and 3 lncRNAs [158] in stimulated human T lymphocytes obtained from two healthy donors and one patient with AT with the observation that FAS-AS1 lncRNA is up-regulated by IR exposure in an ATM-dependent fashion.

Exposure to low-dose IR also causes transiently elevated expression of the lncRNA *PARTICLE* [203]. PARTICLE is a tuner of cellular methylation following IR exposure. Significantly, the effect of PARTICLE in limiting the time and extent of the IR-induced increase in DNA methylation is more pronounced at lower than higher doses providing evidence of a non-linear effect. Increases in *PARTICLE* expression were noted in plasma samples from post-IR therapy patients [203]. Further experiments are needed to assess whether it may be a suitable biomarker of low dose exposures. Recently Macaeva *et al.,* 2016 suggested lncRNAs as potential IR biomarkers. For instance PAPPA-AS1, a lnc-RNA

transcribed from the opposite strand of the radiation-responsive gene PAPPA, is among the 20 best genes to distinguish between exposure to 0, 0.1 and 1.0 Gy [23].

In terms of future developments, apart from the need for more robust studies at low doses, an improvement in detection technologies and the development of dedicated biosensors is crucial. The future of ncRNA biomarkers is promising [204] and we are only at the very beginning of our understanding of their biological roles.

3.7 Other biomarkers

3.7.1 Biomarkers associated with RedOx imbalance

It is well accepted that oxidative stress, caused by the imbalance between the production of ROS or RNS species and their elimination by antioxidant defense systems, contributes to pathogenic mechanisms of several diseases. The markers of oxidative modifications associated with exposure to IR in experimental models and in humans were reviewed by Pernot et al. [9] and more recently by [81]. These include biomarkers of elevated ROS levels such as 8-oxo-dG (Section ~~3.2.3~~3.2.3), markers of antioxidants such as catalase expression, arising as a consequence of increased ROS, and markers of enzymes generating ROS. Studies linking IR exposure to the oxidation of biomolecules continue to emerge but few have investigated low-dose exposures. In mice with doses of 50–75 mGy, anti-oxidative effects were observed with increased expression and function of renal Nrf2 transcription factor and its target anti-oxidant enzymes SOD1, HO-1 or NQO-1 [205,206]. A study of cognitive defects in irradiated mice showed a decrease of total malondialdehyde-modified protein content in the hippocampus at 1 Gy [178] and the analysis of S-nitrosothiols, a post-translational modification of proteins, by mass spectrometry in brain, liver and plasma showed a decreased level after exposure to 100 mGy [207] suggesting that markers of redox imbalance might be associated indicative of cognitive defects. Persistent (40 week) increases in protein carbonylation in the cardiovascular system after a single heart dose to C57BL/6 mice were detected after exposure to 2 Gy but not after 0.2 Gy [176].

Thus whilst RedOx balance markers are important for the evaluation of IR induced effects and disease and are induced by high dose IR, further studies are needed at low-dose effects to assess their usefulness as indicators of damage or adaptive responses in molecular epidemiological studies.

3.7.2 Metabolites and metabolomics

Metabolomics has potential for the development of early biomarkers of exposure but the field is still in its infancy. Major obstacles in global metabolomics profiling are the identification of unknown compounds [208]; the sheer complexity of the metabolome, requiring advanced equipment and data processing tools [81]; and the lack of IR specificity of some metabolic biomarkers [209]. Nevertheless, this technology is powerful if used in defined conditions [81] especially if combined with other “omics” technologies [179] and is particularly amenable for use with urine samples. In addition, some changes can be detected before the onset of any clinical symptoms suggesting that they may be biomarkers for early disease onset [210].

Two technologies currently dominate metabolomics research: NMR spectroscopy and MS. Although NMR has many advantages (such as non-selectivity, lack of sampling bias and reproducibility), it is hampered by low sensitivity, requiring large amounts of sample. MS-based methods on the other hand are highly sensitive and incorporate upstream online analytical separation steps for metabolites, including LC, GC, or capillary electrophoresis. A broad spectrum of IR-induced metabolite alterations have been detected although many of the studies have used high dose treatments (Supplementary Table 3.7.1). Mitochondria are an established target of IR [211–214], and many metabolite biomarkers are directly or indirectly involved in mitochondrial metabolism.

3.7.2.1 Metabolite biomarkers in urine and serum Recent pre-clinical studies have shown the ability of metabolomic approaches to detect differences in urinary profiles related to dose, dose-rate and IR quality [215–218]. Gende specific differences were found in non-human primates [219] after doses > 6.5 Gy which persisted up to 7 days post-irradiation [220] and cancer patients undergoing total body irradiation [221]. Whether such differences exist after low dose exposure remains to be established.

Only a few IR metabolomics studies used serum samples but they employed high doses [222–224]. With improving technology, alterations were detected in rat serum 24 h after irradiation with 0.75 Gy gamma rays [225] and profiles after lower dose exposures are now technically feasible.

3.7.2.2 Metabolite biomarkers in cells and tissues Whilst metabolomic studies of body fluids reflect several different biological processes, only the study of cells and tissues will address tissue-specific IR exposure and effects.

Time- and dose-dependent changes were measurable in a full thickness human skin tissue model irradiated with 0.03 or 0.1 Gy and assessed at 3, 24 and 48 h [226]. While no changes in extracted metabolites were observed 3 h following irradiation with 0.03 or 0.1 Gy, by 48 h changes were seen at all doses. These metabolites have potential as biomarkers of radiation exposure. Analysis of the metabolites dysregulated at 48 h following low dose exposure identified pertubations in pathways involving DNA/RNA damage and repair, and lipid and energy metabolism.

Human B lymphoblastoid and fibroblasts cells were used to identify low dose responsive metabolites 10 h after exposure of 0.02, 0.1, and 1.0 Gy X-rays [227]. Measurable changes were seen at 0.1 Gy but not 0.02 Gy and there were marked

differences between the cell types. Li et al. investigated the effect of total body gamma IR (0.1, 0.5 and 3.0 Gy) on T cell activation and metabolism in irradiated male C57BL/6 mice at 4 hour, 1 week, and 2 week time points [228] The T cell receptor-activation induced metabolomics changes were altered in a radiation- dose- and time-dependent manner. Effects were seen at 0.5 Gy but not at lower doses, consistent with findings of a reduced percentage of naïve T cells reported in the A-bomb cohort [81,229]. It is not known whether these metabolomics changes contribute to the known immune cell stimulatory effect of low dose IR.

3.7.3 Biophysical markers

There is interest in the development of biodosimeters that measure physical or chemical changes in non-biological (e.g. cloth, glass, plastic etc) or biological (e.g. nails, teeth, hair) samples after IR exposure. The relatively stable chemical species generated are detectable by, for instance, EPR spectroscopy. A challenge for *ex vivo* EPR nail dosimetry is the overlap between the mechanically induced signals produced by cutting nails and the IR induced signals. This overlap can, to some extent, be overcome by spectral fitting (see for example [230]) and identifying stable IR-induced radical(s) [231]. To date this technology was used to estimate high dose localised exposures to the hands (>~~>~~10 Gy) after severe IR accidents (see [231] and references there in). EPR on tooth enamel was also used to measure exposure to accidental noble gas release from the Mayak Production Association, natural background IR (~0.7 ± 0.3 mGy annually to tooth enamel) and routinely released noble gases in Ozyorsk citizens (see for instance [232]). It has also been used in external dose reconstructions in Chernobyl accident recovery workers [233,234] and in people living near the contaminated Techa River [235]. In the latter studies, with relatively high doses, both EPR and FISH based dose estimates were comparable and agreed with estimates of external and ^{137}Cs-internal exposures calculated with the Techa River Dosimetry System. Alternative EPR test materials also show promise, e.g., glass from the touch screens of smart phones, but there are technical issues, such as the variability of samples from different smart phones and environmental conditions (see for example [236]) that must be resolved.

3.7.4 Circulating DNAs

ccfDNA is being explored as an early biomarker of cancer [237], and is of interest as a potential biomarker of IR exposure. Short DNA fragments, resulting from IR induced DSBs or released from cells as a result of physiological processes, can be extracted from blood and detected using qPCR techniques. After *in vivo* irradiation, atomic force microscopy can characterise the size distribution of individual DNA fragments extracted from blood, with high LET neutrons producing shorter DNA fragments than low LET electrons. This may be of interest for the development of exposure biomarkers which identify radiation quality. Two studies recently reported ccfDNA levels in populations exposed to low IR levels. Borghini et al. [238] found no correlation between recorded lifetime dose and ccf-DNA or mtDNA fragments in a subset of 15 interventional cardiologists. However, ccf-DNA and mtDNA fragments tended to be significantly increased in interventional cardiologists exposed to higher doses (≥52 mSv, median lifetime cumulative dose) compared to lower levels and controls. In contrast Korzeneva et al. [239] found decreased plasma ccfDNA concentrations in individuals exposed to low-dose gamma-neutron or tritium beta-radiation. Thus whilst the quantification of ccfDNA represents an attractive minimally invasive approach for developing IR biomarkers, it is too early to comment further on its potential.

3.7.5 DNA repair capacity measurements as biomarkers of susceptibility

There is substantial evidence for inter-individual differences in DNA repair capacity (reviewed in [240]). This ability can be assessed directly using γH2AX or other DNA repair foci measurements (see Section ~~3.2.2~~3.2.2) or indirectly by measuring mutagen sensitivity and both approaches have been used in a number of epidemiological studies. Assays in whole cells address the complexicity of the DNA damage response that is affected by genetic, epigenetic and transcriptomic modulators. However such assays do not provide direct mechanistic information about the genotoxic lesions or the modulated pathways.

Numerous methods have been developed for measuring DNA repair capacity directly which, as reviewed by Nagel et al. [240], have strengths and weaknesses. Some have the advantage of measuring repair of genomic DNA in intact cells but are often labour intensive and show large inter-laboratory variation, thus limiting their application in large-scale epidemiological studies. The widely used comet assay is associated with large inter-laboratory variation (see for example [241]), limiting its usefulness in multi-centre studies, particularly after low dose IR exposures. A comet assay chip has been developed [242–244] with a 100-fold higher throughput compared to the traditional assay but it requires validation in inter-laboratory comparisons.

Single cell network profiling is another potential approach to quantitatively measure DNA repair capacity in biological samples by characterizing signaling responses at the single cell level using multi-parametric flow cytometry following exposure to a DNA damaging agent. This approach has identified functional DDR readouts in both NHEJ and HR pathways and has been explored for predicting clinical outcome in oncology settings (see for instance [245] and references therein). Further experiments are needed to establish the specificity and sensitivity of such assays in terms of responses to low doses of IR.

Host cell reactivation assays offer a powerful way to measure DNA repair capacity in living cells and have been used in molecular epidemiological studies, mainly investigating the repair of UV or benzo[a]pyrene diol epoxide induced DNA lesions [246]. However this assay generally cannot assess the repair of lesions that do not block the progression of RNA polymerases and is limited further by the need for separate assays to measure individual DRC pathways, or at more than one dose of DNA damage. A recently developed multiplexed fluorescence based flow cytometric host cell reactivation assay addresses certain of these limitations by using different reporter plasmids to

measure the repair of several doses of multiple types of DNA damage in a single assay [240]. A next generation sequencing based assay was also developed that detects rare transcriptional mutagenesis events due to lesion bypass by the RNA polymerase, providing two tools for exploring relationships between global DNA repair capacity and disease susceptibility.

Functional DNA repair signatures can also be measured using protein extracts from cells or tissues making use of DNA substrates containing defined lesions. Using such an approach the BER, MMR, direct reversal by MGMT, NER, NHEJ, cross link repair and HR pathways were assessed in a number of epidemiological settings (see [240] and references there in). In addition an excision/synthesis repair assay using a biochip carrying plasmids containing different types of DNA damage typically repaired by different repair pathways assays has been developed and tested using a variety of human samples. This assays allows an individual's DNA repair phenotype towards a panel of lesions repaired by distinct repair pathways to be determined (see for instance [247]). One major limitation of both the HCR and biochip assays for assessing DNA repair capacity for IR induced lesions is the need for repair substrates carrying lesions representative of those found after both high and low LET irradiation and in particular clustered lesions.

Another outstanding question with respect to assessing DNA repair capacity is the choice of the most suitable biological material. Variation between tissues has not been extensively explored but for large scale prospective molecular epidemiological studies the choice of tissue is often limited to viable cells isolated from blood samples. However, the possibility of generating cells representative of various human tissues by differentiating induced pluripotent stem cells from skin fibroblasts that can be obtained from a single biopsy may become a reality in the near future and opens up the possibility for large prospective epidemiological studies investigating DRC in target tissues. Clearly such an approach will require ethical reflection and logistical considerations that to date have not been instigated at the European level.

3.7.6 Mitochondria as biomarkers for low dose IR exposure and effects

MtDNA is vulnerable to damage because, in comparison with nuclear DNA it lacks protective histones, has a high exon to intron ratio, has inefficient DNA repair machinery [211] and is located close to the mitochondrial respiratory transport chain, the most important cellular source of ROS [248–251]. Changes in mitochondrial function and number after exposure of cells or tissues to high IR doses have been found [252–254]. A sensitive measure of mtDNA damage is the accumulation of the common deletion generated during the processing of DNA damage within a 4977 base pair region flanked by two 13 base pair repeats [255,256]. This accumulation has been proposed as a sensitive marker for the evaluation of low dose IR-induced effects. For instance, Schilling-Tóth and coworkers have demonstrated an increase in common deletion levels 72 h after exposure to doses of 0.1 Gy in both primary and immortalized fibroblast cell lines [257]. This is however unlikely to be specific to IR as the common deletion is often observed in diseases, such as the Kearns-Sayre syndrome, that involve a premature aging process [258,259]. Despite this limitation, because of the stability of this change, it may represent a longer term exposure marker that warrants assessing in pilot studies.

3.8 Biomarkers of internal exposures

Internal contaminations by radionuclides can occur in a number of occupational, medical and environmental settings and require the use of specific biomarkers to provide a qualitative indication or a quantative assessment of absorbed dose to a specific organ or tissue. Following intake, the distribution throughout the body depends on the biokinetics and speciation of the radionuclide. The irradiation is protracted during the retention period of the radionuclide and its relative biological efficiency depends on the type of IR emitted. In addition, some radionuclides, for instance U and other heavy metals, have potential for chemical and even physical (e.g. in case of particle inhalation) toxicity.

3.8.1 Current techniques for the biological determination of exposure/intake of internal emitters

Incorporated radionuclides emitting penetrating IR (X-rays, γ or energetic β) can be monitored directly using external detectors. However this approach is not feasible for radionuclides emitting only (or mainly) α or low energy β radiation. Intakes of U, Pu and ^{3}H, among others, are usually monitored by *in vitro* analysis involving excreta or other biological samples (urine, feces, nose blow, nasal smear, saliva, rarely blood or biopsy). Dedicated models combined with a scenario of exposure (e.g. time and route of intake, physico-chemical form of the incorporated radionuclide) are needed to evaluate intake and dose. The precision of the assessed dose is strongly affected by the uncertainty of the exposure conditions and the biokinetics of the radionuclide. For most radionuclides and for medium to low levels of exposure, radioactivity measurements in urine and blood are representative of a recent exposure only, over a period depending on the decay scheme and the biokinetics of the radionuclide.

In recent years, epidemiological studies of occupational exposure to radionuclides have begun to benefit from detailed internal dosimetry protocols to evaluate annual organ doses from bioassay monitoring data and information on the conditions of exposure (see Supplementary Table 3.8.1 and references therein). There is clear potential to improve, by integrating biomarkers of exposure to radionuclides in epidemiological studies, the characterization of the shape of the dose-response for cancers [260,261], but also non-cancer diseases [262,263], and to improve the knowledge of the effects of internal contamination [264] and of radiation quality.

3.8.2 New biomarkers of exposure to internal emitters

The techniques currently being used for monitoring incorporated radionuclides present inherent limitations which results in substantial uncertainties in internal dose estimates. New biomarkers are thus needed that would

have longer persistence, and provide information about the chemical speciation or the isotopic form of the incorporated radionuclide, tissue-specific information on radionuclide presence, quantity, or associated biological damage.

Non-targeted approaches such as metabolomics, lipidomics and proteomics have provided promising preliminary results for new biomarkers of exposure to radionuclides, as demonstrated by recent studies of uranium (lowest estimated calculated dose of 0.15 mGy in the kidney), ^{137}Cs (lowest average cumulated dose of 4mGy) and ^{90}Sr (lowest average cumulated dose of 1 Gy) contamination in rats [216,217,265–267]. Translocations, complex chromosomal rearrangements and micronuclei in peripheral blood lymphocytes (see above) are also potentially useful to estimate cumulated red bone marrow doses resulting from protracted mixed exposure to internal and external IR following moderate to high doses, although further work is needed for complete validation [12,56,59,60,268]. Gender specific mRNA and miRNA gene expression patterns to internal ^{239}Pu exposure have been deciphered in former Mayak workers [269,270], which are promising markers of internal alpha-particle exposure and warrant further validation.

3.8.3 New biomarkers of effects

Compared to biomarkers of low LET IR, biomarkers of effects of internal emitters are more likely to be specific to the “target tissues or organs” of each radionuclide. To date potential biomarkers of IR-induced thyroid disease identified are those associated with ^{131}I exposure [131,271].

^{131}I associated gene expressions in thyroid tumours have been investigated, however, no common alterations were found (summarised in Supplementary Table 3.8.3 and references there in). Major limitations of these transcriptomic studies might be the small sample sizes. Another approach for the elucidation of ^{131}I specific molecular fingerprints was an integrative data analysis combining genomic copy number data with mRNA and protein expression levels. Using such an approach on PTC from young thyroid cancer patients exposed to ^{131}I from the Chernobyl fall-out, an apparent IR-specific DNA copy number gain on chromosomal band 7q11 and over-expression of CLIP2 mRNA and protein were found [131,271]. A ^{131}I dose-response relationship for the CLIP2 IR marker in two Ukrainian PTC cohorts for young patients with age at operation less than 20 years and age at exposure less than 5 years has highlighted the potential importance of this biomarker in low-dose radiation research [272]; no dose-response has been seen, however, in a separate cohort in Belarus (Grellier et al., in preparation).

Because of the chemical toxicity of certain radionuclides (e.g.: U, Be, Np, Ag, Pb, Tc, Se, B, Cd) biomarkers of toxic effects which are poorly covered by most epidemiological databases (e.g., kidney or brain toxicity) [273,274] can be of interest to discriminate between chemical and radiological effects. For instance, given that the kidney is the most susceptible human organ to the chemical toxicity of U, the use of specific and sensitive biomarkers of nephrotoxity such as Kim-1, β2-microglobuline, tubular enzymes or osteopontin [275–277] are promising.

Biomarkers of adverse biological effects have been investigated in several animal models. For instance specific cardiovascular markers associated with cardiovascular effects resulting from ^{137}Cs exposure [278,279] and kidney and brain markers after uranium exposure (total calculated dose of 0.15 mGy on the kidney) have been identified [280–283]. Biomarkers of adaptive response were observed in certain conditions of chronic exposure of mice to ^{137}Cs (absorbed dose 5~~ to 150~~-150 mGy) where inflammatory cytokines decrease [279] or chronic exposure of rats to uranium where renal glutathione levels increase dose-dependently (absorbed dose to the kidney 0.15 mGy to 5mGy) [284].

4 Discussion

4.1 Past and ~~F~~future

Pernot et al. [9] reviewed biomarkers for low dose IR epidemiological studies, and defined different classes (exposure, susceptibility, late effects, and persistent effects). The review raised awareness of the criteria required for a biomarker to be useful and helped focus research on the approaches and technologies needed to promote the discovery and validation of suitable biomarkers. It facilitated interactions between basic scientists studying the responses to IR exposure, those engaged in biomarker discovery and epidemiologists. The review also exposed the limitations inherent to using biomarkers including the need for sufficient sensitivity and specificity, and practicality for use in large scale studies using biological samples that can be collected in a logistically and ethically acceptable way.

Gaining a mechanistic understanding of the biochemical processes induced by low doses of IR is an important complement to the molecular epidemiological estimates of risk of low dose IR exposure as originally discussed in the HLEG report [285], and more recently the HPA’s independent Advisory Group on ionizing radiation report on Human Radiosensitivity [286] and the NCRP scientific commentary on Health Effects of low doses of radiation: perspectives on integrating radiation bioogy and epidemiology [11]. The extensive information gathered through the different “IR induced biomarker projects” and the rapid development of bioinformatics/system biology should provide the tools to identify the mechanisms underlying the cellular processes induced in response to low dose IR. Understanding the nature of the primary cellular targets, which are broader than just DNA, may help to evaluate the long-term impact on transformation, senescence or other endpoints relevant for the onset of health effects and would also identify novel targets for approaches for amelioration or protection from long-term health effects. Along with the relevance for IR protection research, a basic understanding of the mechanisms of action of low dose IR and their possible impact on human health could be important for low dose risk estimates from other stressors/pollutants in our envirnonment.

Since the Pernot et al. publication [9], there has been progress in the identification of potential new biomarkers of exposure and late effects related to advances in metabolomics and transcriptomics. The robust findings from *in*

vitro and *in vivo* animal models using much lower doses than previously used (below 1 Gy) highlight these advances. The increasing number of potential new biomarkers underpins the need to establish guidelines for the processes underlying biomarker validation, and a key aspect of this updated review is the development of a roadmap (Fig. 2). It is crucial that informed and carefully evaluated decisions are made on when to progress or drop a biomarker with issues of reproducibility, sensitivity and specificity being critical to the decision. Based on this roadmap the development status of proposed biomarkers for IR epidemiological studies have been reviewed (Table 3). One biomarker that has moved to the final stages of development is IR specific mRNA transcript profiles. However, most potential biomarkers have not reached these stages and for some there is sufficient evidence that further development for use in the low dose IR exposure field is not warranted.

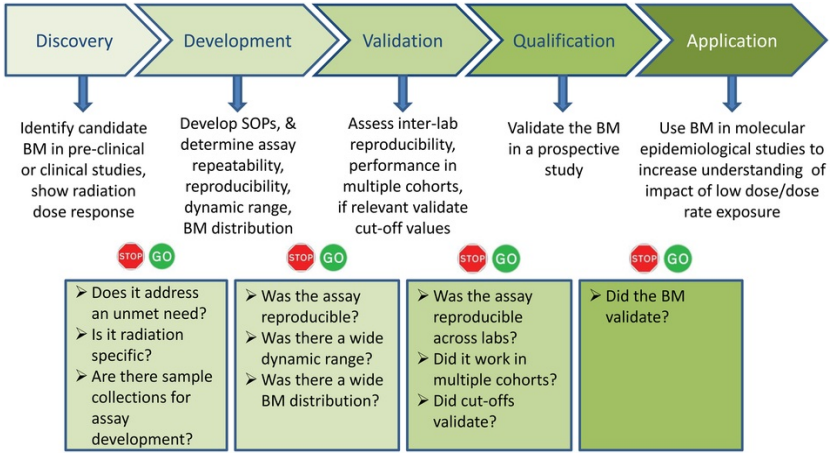

Fig. 2 Roadmap for Developing Biomarkers of Radiation Exposure, Susceptibility, Late Effects or Persistent Effects.

alt-text: Fig. 2

Table 3 Development status of radiation biomarkers.

alt-text: Table 3

Field	Endpoint	BM of low dose exposure	BM of response	
			Early effects	Late effects
Cytogenetics				
	Improved quantification of CBMN assay	GO-application	GO-application	GO-application
	Chromosome aberrations analysis using PCC + fish	GO-application	GO-application	GO-application
	RET/PTC1 and RET/PTC3 rearrangements in PTC	STOP not radiation specific	STOP not radiation specific	STOP not radiation specific
	EVT6/NTRK3 rearrangements in PTC		GO-qualification	GO-qualification
	Analysis of Micronucleated reticulocytes (mouse studies to date)	GO- development	GO- development	GO- development
DNA/nucleotide pool lesions				
	(this should be gammaH2AX - the symbol needs correcting please)gH2AX	STOP interlab variability-poor dosimetry	GO-validation	
	extracellular 8 oxoG	NO	GO-qualification	GO-qualification
	CtDNA	GO-validation	GO-validation	GO-validation

Germline variants/radiation induced mutations				
	Multigene signatures	GO-discovery	GO-discovery	GO-discovery
	Radiation specific mutation profile	GO-discovery	GO-discovery	GO-discovery
Gene amplification, transcriptional and expression profiling				
	Multigene signatures	GO-development	GO-development	GO-development
	FDXR	GO-validation	GO-validation	GO-validation
	CLIP2 amplification and protein expression	GO-validation	GO-validation	GO-validation
	DDR activation		GO-discovery	NO
Novel and/or emerging ideas				
	Redox balance	GO-discovery	GO-discovery	GO-discovery
	Metabolite biomarkers in urine, serum or saliva	GO-discovery	GO-discovery	GO-discovery
	Radiation induced post-translational modifications	GO-discovery	GO-discovery	NO
	Radiation specific DNA lesions	GO-discovery	NO	NO
	Telomere length	NO	GO-development	GO-development
	miRNAs	GO-discovery	GO-discovery	GO-discovery
	LnRNAs eg PAPPA-AS1 and PARTICLE	GO-discovery	GO-discovery	GO-discovery
	DRC assays	GO-development	GO-development	GO-development
	Accumulation of common deletion in mitochrondria	GO-discovery	GO-discovery	GO-discovery

4.2 Can biomarkers in molecular epidemiological studies provide insight into the outstanding questions relating to low dose exposures; strengths and limitations?

A goal of current research in the low dose IR field is to define the shape of the dose response for induced health effects. The integration of biomarkers into molecular epidemiology studies remains in its infancy but has the potential to address this goal. Possible strategies for addressing this for cardiovascular diseases [10], and the effects of uranium exposure [287] were recently reviewed by the DOREMI consortium.

These strategies cover not only the collection and timing of appropriate biological samples but also issues common to all epidemiological studies, such as study size, statistical power, potential biases (such as confounding, selection and recall) and random error. Typical confounders and potential effect modifiers include age, gender, ethnicity, smoking status, other environmental, occupational or medical exposures, and iodine deficiency (for IR induced thyroid cancer). If adequate control for confounding factors is not possible, even the best powered molecular epidemiological study may lead to incorrect inferences. Therefore, efforts to collect good quality data on potential confounders in molecular epidemiological studies are critical and are major components in the proposed future studies to investigate cardiovascular effects and uranium exposure [10,288,289]. Uncertainties and bias in estimating IR dose and inaccuracies in outcome determination will also affect study findings.

To date, and since the publication of the Pernot et al. review [9], molecular epidemiological studies of low dose IR continue to be small and suffer from low statistical power. The need for large studies is recognised but hampered by the logistics and costs of obtaining and biobanking several thousand samples from appropriate cohorts. Biological samples were collected from only a small proportion of ~~of~~ the many large epidemiological studies of low dose IR so far conducted – often only at a single time point and from a small proportion of individuals. Also, if the association between the biomarker and endpoint is weak, then the additional information that it provides will be limited and not cost-effective.

One over-arching consideration that impacts on study design and the potential for the collection of biological samples and thus limits the choice of biomarkers is the issue of time since exposure. Prospective studies have the potential to be highly informative and to provide distinct information in comparison with retrospective studies: biological samples and dosimetric information can be collected before the onset of disease and longitudinal sample collection and measurements may be possible. However, decades are needed in order to obtain results on long term effects. Retrospective studies are quicker but most of the biomarkers of exposure reviewed here currently lack sensitivity, specificity and persistence for use decades after exposure as illustrated in Fig. 3. Retrospective studies may be suitable, however, for the assessment of individual susceptibility and persistent effects, and for these a case-control study design would reduce the number of samples required. As discussed in Pernot et al. [9], bioassays can be particularly useful in this setting to assess biomarkers of susceptibility. It is recognised, however, that certain gene and protein expression profiles could vary with age that can complicate the interpretation of data.

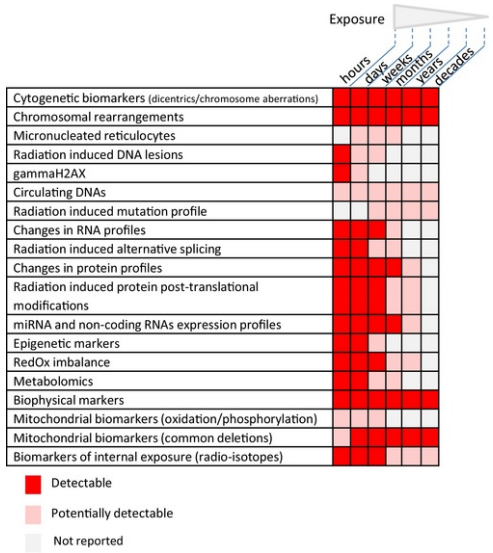

Fig. 3 Biomarker detectability with time.

This heatmap representation allows the selection of an appropriate biomarker with respect to time after exposure. Biomarkers were classified as easily detectable or potentially detectable with modern technology and assuming the availability of appropriate biological samples. It has to be noted that the majority of these biomarkers have yet to be validated using the proposed roadmap as a biomarker of low dose radiation exposure in human studies.

alt-text: Fig. 3

Thus, whilst the use of biomarkers in molecular epidemiological studies has the potential to provide mechanistic insight and increase the power of studies to answer key radiation protection questions, the choice of the most appropriate biomarkers in different study designs needs to be carefully evaluated.

5 Conclusions

Biomarkers for IR epidemiology studies must be sensitive to and specific for IR exposure, and need to be applicable to large numbers of biological samples that can be conveniently and ethically collected. These requirements continue to limit the number of candidates that are suitable for assessing low dose IR exposure, susceptibility, or effects. Technological and analytical developments and cost reductions are advancing the development of potential biomarkers but challenges remain. Whilst innovative ideas and increased mechanistic understanding of the responses to low dose and LDR IR are aiding biomarker discovery, a key issue is the decision of which biomarkers to progress. As seen in this review, most potential biomarkers remain at the discovery stage. For some, however, there is now sufficient evidence that further development is not warranted. Only one biomarker was identified in the final stages of development and as a priority for further research, radiation specific mRNA transcript profiles.

Robust validation is essential and a roadmap is provided to facilitate the progression from biomarker discovery to implementation. Use and periodic updating of this roadmap should allow the most informative biomarkers to be incorporated into IR epidemiology studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mrrev.2017.01.001.

References

[1] UNSCEAR, Summary of ~~low-dose radiation effects on h~~Low-dose Radiation Effects on Health, 2010, United Nations; New York http://www.unscear.org/docs/reports/2010/UNSCEAR_2010_Report_M.pdf.

[2] UNSCEAR, *Effects of Ionising Radiation* **Volume I**, 2006, United Nations; New York.

[3] S. Salomaa, D. Averbeck, A. Ottolenghi, L. Sabatier, S. Bouffler, M. Atkinson and J.-R. Jourdain, European low-dose radiation risk research strategy: future of research on biological effects at low doses, *Radiat. Prot. Dosimet.* **164**, 2015, 38–41, http://dx.doi.org/10.1093/rpd/ncu350.

[4] K. Leuraud, D.B. Richardson, E. Cardis, R.D. Daniels, M. Gillies, J.A. O'Hagan, G.B. Hamra, R. Haylock, D. Laurier, M. Moissonnier, M.K. Schubauer-Berigan, I. Thierry-Chef and A. Kesminiene, Ionising radiation and risk of death from leukaemia and lymphoma in radiation-monitored workers (INWORKS): an international cohort study, *Lancet Haematol.* **2**, 2015, e276–e281, http://dx.doi.org/10.1016/S2352-3026(15)00094-0.

[5] D.B. Richardson, E. Cardis, R.D. Daniels, M. Gillies, J.A. O'Hagan, G.B. Hamra, R. Haylock, D. Laurier, K. Leuraud, M. Moissonnier, M.K. Schubauer-Berigan, I. Thierry-Chef and A. Kesminiene, Risk of cancer from occupational exposure to ionising radiation: retrospective cohort study of workers in France, the United Kingdom, and the United States (INWORKS), *BMJ* **351**, 2015, h5359, http://dx.doi.org/10.1136/bmj.h5359.

[6] M.S. Pearce, J.A. Salotti, M.P. Little, K. McHugh, C. Lee, K.P. Kim, N.L. Howe, C.M. Ronckers, P. Rajaraman, A.W. Craft, L. Parker and A. Berrington de González, Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study, *Lancet* 2012, http://dx.doi.org/10.1016/s0140-6736(12)60815-0.

[7] J.D. Mathews, A.V. Forsythe, Z. Brady, M.W. Butler, S.K. Goergen, G.B. Byrnes, G.G. Giles, A.B. Wallace, P.R. Anderson, T.A. Guiver, P. McGale, T.M. Cain, J.G. Dowty, A.C. Bickerstaffe and S.C. Darby, Cancer risk in 680 000 people exposed to computed tomography scans in childhood or adolescence: data linkage study of 11 million Australians, *BMJ* **346**, 2013, f2360.

[8] G.M. Kendall, M.P. Little, R. Wakeford, K.J. Bunch, J.C.H. Miles, T.J. Vincent, J.R. Meara and M.F.G. Murphy, A record-based case-control study of natural background radiation and the incidence of childhood leukaemia and other cancers in Great Britain during 1980~~-~~–2006, *Leukemia* **27**, 2013, 3–9, http://dx.doi.org/10.1038/leu.2012.151.

[9] E. Pernot, J. Hall, S. Baatout, M.A. Benotmane, E. Blanchardon, S. Bouffler, H. El Saghire, M. Gomolka, A. Guertler, M. Harms-Ringdahl, P. Jeggo, M. Kreuzer, D. Laurier, C. Lindholm, R. Mkacher, R. Quintens, K. Rothkamm, L. Sabatier, S. Tapio, F. de Vathaire and E. Cardis, Ionizing radiation biomarkers for potential use in epidemiological studies, *Mutat. Res.* **751**, 2012, 258–286, http://dx.doi.org/10.1016/j.mrrev.2012.05.003.

[10] M. Kreuzer, A. Auvinen, E. Cardis, J. Hall, J.-R. Jourdain, D. Laurier, M.P. Little, A. Peters, K. Raj, N.S. Russell, S. Tapio, W. Zhang and M. Gomolka, Low-dose ionising radiation and cardiovascular diseases-Strategies for molecular epidemiological studies in Europe, *Mutat. Res. Rev. Mutat. Res.* **764**, 2015, 90–100, http://dx.doi.org/10.1016/j.mrrev.2015.03.002.

[11] NCRP, Health effects of low doses of radiation: perspectives on integrating radiation biology and epidemiology, *National Council for Radiation Protection and ~~m~~Measurements (NCRP)* 2015.

[12] F. Zölzer, Z. Hon, Z. Freitinger Skalická, R. Havránková, L. Navrátil, J. Rosina and J. Skopek, Persistence of genetic damage in lymphocytes from former uranium miners, *Cytogenet. Genome Res.* **136**, 2012, 288–294, http://dx.doi.org/10.1159/000338084.

[13] R.M. Takhauov, A.B. Karpov, E.N. Albach, M.V. Khalyuzova, M.B. Freidin, N.V. Litviakov, A.E. Sazonov, D.S. Isubakova, M.A. Bolshakov, S.A. Mezheritskiy, E.B. Mironova, J.V. Semenova, G.B. Nekrasov, A.S.

Izosimov, A.A. Gagarin, R.V. Brendakov, D.E. Maksimov and Y.D. Ermolaev, The bank of biological samples representing individuals exposed to long-term ionizing radiation at various doses, *Biopreservation Biobanking, Biobank*. **13**, 2015, 72–78, http://dx.doi.org/10.1089/bio.2014.0035.

[14] D. Laurier, I. Guseva Canu, S. Baatout, J.-M. Bertho, E. Blanchardon, S. Bouffler, E. Cardis, M. Gomolka, J. Hall, A. Kesminiene, M. Kreuzer and E. Rage, DoReMi workshop on multidisciplinary approaches to evaluating cancer risks associated with low-dose internal contamination, *Radioprotection* **47**, 2012, 119–148, http://dx.doi.org/10.1051/radiopro/2011162.

[15] O. Laurent, M. Gomolka, R. Haylock, A. Giussani, W. Atkinson, D. Bingham, S. Baatout, L. Tomasek, E. Cardis, J. Hall, E. Blanchardon and D. Laurier, DoReMi – Low Dose Research towards Multidisciplinary low dose research towards multidisciplinary integration, *D5.17: Report for an integrated (biology-dosimetry-epidemiology) research project on occupational Uranium eIntegrated (biology-dosimetry-epidemiology) Research Project on Occupational Uranium Exposure. Task 5.8: Concerted Uranium Research in Europe (CURE)* 2015, Final Report(Final Report).

[16] N. Haddy, L. Tartier, S. Koscielny, E. Adjadj, C. Rubino, L. Brugieres, H. Pacquement, I. Diallo, F. de Vathaire, D. Averbeck, J. Hall and S. Benhamou, Repair of ionizing radiation-induced DNA damage and risk of second cancer in childhood cancer survivors, *Carcinogenesis* **35**, 2014, 1745–1749, http://dx.doi.org/10.1093/carcin/bgu077.

[17] E. Pernot, E. Cardis and C. Badie, Usefulness of saliva samples for biomarker studies in radiation research, *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **23**, 2014, 2673–2680, http://dx.doi.org/10.1158/1055-9965.EPI-14-0588.

[18] J.G.F. Hogervorst, R.W.L. Godschalk, P.A. van den Brandt, M.P. Weijenberg, B.A.J. Verhage, L. Jonkers, J. Goessens, C.C.J.M. Simons, J.R. Vermeesch, F.J. van Schooten and L.J. Schouten, DNA from nails for genetic analyses in large-scale epidemiologic studies, *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **23**, 2014, 2703–2712, http://dx.doi.org/10.1158/1055-9965.EPI-14-0552.

[19] P.A. van den Brandt, R.A. Goldbohm, P. van't Veer, A. Volovics, R.J. Hermus and F. Sturmans, A large-scale prospective cohort study on diet and cancer in The Netherlands, *J. Clin. Epidemiol.* **43**, 1990, 285–295.

[20] M. Phillips, R.N. Cataneo, A. Chaturvedi, P.D. Kaplan, M. Libardoni, M. Mundada, U. Patel, K.D. Thrall and X. Zhang, Breath biomarkers of whole-body gamma irradiation in the Göttingen minipig, *Health Phys.* **108**, 2015, 538–546, http://dx.doi.org/10.1097/HP.0000000000000272.

[21] H. Zhao, J. Shen, Q. Hu, W. Davis, L. Medico, D. Wang, L. Yan, Y. Guo, B. Liu, M. Qin, M. Nesline, Q. Zhu, S. Yao, C.B. Ambrosone and S. Liu, Effects of preanalytic variables on circulating microRNAs in whole blood, *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **23**, 2014, 2643–2648, http://dx.doi.org/10.1158/1055-9965.EPI-14-0550.

[22] S.I. Daniels, F.C.M. Sillé, A. Goldbaum, B. Yee, E.F. Key, L. Zhang, M.T. Smith and R. Thomas, Improving power to detect changes in blood miRNA expression by accounting for sources of variability in experimental designs, *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **23**, 2014, 2658–2666, http://dx.doi.org/10.1158/1055-9965.EPI-14-0623.

[23] E. Macaeva, Y. Saeys, K. Tabury, A. Janssen, A. Michaux, M.A. Benotmane, W.H. De Vos, S. Baatout and R. Quintens, Radiation-induced alternative transcription and splicing events and their applicability to practical biodosimetry, *Sci. Rep.* **6**, 2016, 19251, http://dx.doi.org/10.1038/srep19251.

[24] V. Gallo, M. Egger, V. McCormack, P.B. Farmer, J.P.A. Ioannidis, M. Kirsch-Volders, G. Matullo, D.H. Phillips, B. Schoket, U. Stromberg, R. Vermeulen, C. Wild, M. Porta, P. Vineis and STROBE Statement, STrengthening the reporting of OBservational studies in epidemiology-Molecular epidemiology (STROBE-ME): an extension of the STROBE statement, *PLoS Med.* **8**, 2011, e1001117, http://dx.doi.org/10.1371/journal.pmed.1001117.

[25] IAEA, Cytogenetic Dosimetry Applications in Preparedness for and Response to Radiation Emergencies, 2011, International Atomic Energy Agency; Vienna, (accessed September 22, 2015) www-pub.iaea.org/MTCD/./PDF/EPR-Biodosimetry%202011_web.pdf.

[26] E. Schmid, L. Hieber, U. Heinzmann, H. Roos and A.M. Kellerer, Analysis of chromosome aberrations in human peripheral lymphocytes induced by in vitro alpha-particle irradiation, *Radiat. Environ. Biophys.* **35**, 1996, 179–184.

[27] A. Vaurijoux, E. Gregoire, S. Roch-Lefevre, P. Voisin, C. Martin, P. Voisin, L. Roy and G. Gruel, Detection of partial-body exposure to ionizing radiation by the automatic detection of dicentrics, *Radiat. Res.* **178**, 2012, 357–364.

[28] L.A. Beaton, C. Ferrarotto, B.C. Kutzner, J.P. McNamee, P.V. Bellier and R.C. Wilkins, Analysis of chromosome damage for biodosimetry using imaging flow cytometry, *Mutat. Res.* **756**, 2013, 192–195, http://dx.doi.org/10.1016/j.mrgentox.2013.04.002.

[29] G. Gruel, E. Gregoire, S. Lecas, C. Martin, S. Roch-Lefevre, A. Vaurijoux, P. Voisin, P. Voisin and J.F. Barquinero, Biological dosimetry by automated dicentric scoring in a simulated emergency, *Radiat. Res.* **179**, 2013, 557–569, http://dx.doi.org/10.1667/rr3196.1.

[30] A. De Amicis, S. De Sanctis, S. Di Cristofaro, V. Franchini, E. Regalbuto, G. Mammana and F. Lista, Dose estimation using dicentric chromosome assay and cytokinesis block micronucleus assay: comparison between manual and automated scoring in triage mode, *Health Phys.* **106**, 2014, 787–797, http://dx.doi.org/10.1097/hp.0000000000000097.

[31] G. Garty, A.W. Bigelow, M. Repin, H.C. Turner, D. Bian, A.S. Balajee, O.V. Lyulko, M. Taveras, Y.L. Yao and D.J. Brenner, An automated imaging system for radiation biodosimetry, *Microsc. Res. Technol.* **78**, 2015, 587–598, http://dx.doi.org/10.1002/jemt.22512.

[32] M. Di Giorgio, J.F. Barquinero, M.B. Vallerga, A. Radl, M.R. Taja, A. Seoane, J. De Luca, M.S. Oliveira, P. Valdivia, O.G. Lima, A. Lamadrid, J.G. Mesa, I.R. Aguilera, T.M. Cardoso, Y.C. Carvajal, C.A. Maldonado, M.E Espinoza, W. Martinez-Lopez, L. Mendez-Acuna, M.V. Di Tomaso, L. Roy, C. Lindholm, H. Romm, I. Guclu and D.C. Lloyd, Biological dosimetry intercomparison exercise: an evaluation of triage and routine mode results by robust methods, *Radiat. Res.* **175**, 2011, 638–649, http://dx.doi.org/10.1667/rr2425.1.

[33] C. Beinke, S. Barnard, H. Boulay-Greene, A. De Amicis, S. De Sanctis, F. Herodin, A. Jones, U. Kulka, F. Lista, D. Lloyd, P. Martigne, J. Moquet, U. Oestreicher, H. Romm, K. Rothkamm, M. Valente, V. Meineke, H. Braselmann and M. Abend, Laboratory intercomparison of the dicentric chromosome analysis assay, *Radiat. Res.* **180**, 2013, 129–137, http://dx.doi.org/10.1667/rr3235.1.

[34] E.A. Ainsbury, J. Al-Hafidh, A. Bajinskis, S. Barnard, J.F. Barquinero, C. Beinke, V. de Gelder, E. Gregoire, A. Jaworska, C. Lindholm, D. Lloyd, J. Moquet, R. Nylund, U. Oestreicher, S. Roch-Lefevre, K. Rothkamm, H. Romm, H. Scherthan, S. Sommer, H. Thierens, C. Vandevoorde, A. Vral and A. Wojcik, Inter- and intra-laboratory comparison of a multibiodosimetric approach to triage in a simulated, large scale radiation emergency, *Int. J. Radiat. Biol.* **90**, 2014, 193–202, http://dx.doi.org/10.3109/09553002.2014.868616.

[35] A. Jaworska, E.A. Ainsbury, P. Fattibene, C. Lindholm, U. Oestreicher, K. Rothkamm, H. Romm, H. Thierens, F. Trompier, P. Voisin, A. Vral, C. Woda and A. Wojcik, Operational guidance for radiation emergency response organisations in Europe for using biodosimetric tools developed in EU MULTIBIODOSE project, *Radiat. Prot. Dosim.* **164**, 2015, 165–169, http://dx.doi.org/10.1093/rpd/ncu294.

[36] U. Kulka, L. Ainsbury, M. Atkinson, S. Barnard, R. Smith, J.F. Barquinero, L. Barrios, C. Bassinet, C. Beinke, A. Cucu, F. Darroudi, P. Fattibene, E. Bortolin, S.D. Monaca, O. Gil, E. Gregoire, V. Hadjidekova, S. Haghdoost, V. Hatzi, W. Hempel, R. Herranz, A. Jaworska, C. Lindholm, K. Lumniczky, R. M'Kacher, S. Mortl, A. Montoro, J. Moquet, M. Moreno, M. Noditi, A. Ogbazghi, U. Oestreicher, F. Palitti, G. Pantelias, I. Popescu, M.J. Prieto, S. Roch-Lefevre, U. Roessler, H. Romm, K. Rothkamm, L. Sabatier, N. Sebastia, S. Sommer, G. Terzoudi, A. Testa, H. Thierens, F. Trompier, I. Turai, C. Vandevoorde, P. Vaz, P. Voisin, A. Vral, F Ugletveit, A. Wieser, C. Woda and A. Wojcik, Realising the European network of biodosimetry: RENEB-status quo, *Radiat. Prot. Dosim.* **164**, 2015, 42–45, http://dx.doi.org/10.1093/rpd/ncu266.

[37] R.C. Wilkins, L.A. Beaton-Green, S. Lachapelle, B.C. Kutzner, C. Ferrarotto, V. Chauhan, L. Marro, G.K. Livingston, H. Boulay Greene and F.N. Flegal, Evaluation of the annual Canadian biodosimetry network intercomparisons, *Int. J. Radiat. Biol.* **91**, 2015, 443–451, http://dx.doi.org/10.3109/09553002.2015.1012305.

[38] R. M'Kacher, E.E. Maalouf, M. Ricoul, L. Heidingsfelder, E. Laplagne, C. Cuceu, W.M. Hempel, B. Colicchio, A. Dieterlen and L. Sabatier, New tool for biological dosimetry: reevaluation and automation of the gold standard method following telomere and centromere staining, *Mutat. Res.* **770**, 2014, 45–53, http://dx.doi.org/10.1016/j.mrfmmm.2014.09.007.

[39] D.C. Lloyd and E.J. Reeder, Chromosome aberrations in ~~in~~ vitro irradiated lymphocytes from human cord blood, *Experientia* **35**, 1979, 176–177.

[40] M.V. Bakhmutsky, M.C. Joiner, T.B. Jones and J.D. Tucker, Differences in cytogenetic sensitivity to ionizing radiation in newborns and adults, *Radiat. Res.* **181**, 2014, 605–616, http://dx.doi.org/10.1667/rr13598.1.

[41] A. Vral, M. Fenech and H. Thierens, The micronucleus assay as a biological dosimeter of in vivo ionising radiation exposure, *Mutagenesis* **26**, 2011, 11–17, http://dx.doi.org/10.1093/mutage/geq078.

[42] ISO, Radiological ~~protection – Performance criteria for laboratories using the cytokinesis block micronucleus (CBMN) assay in peripheral blood lymphocytes for biological d~~Protection – Performance Criteria for Laboratories Using the Cytokinesis Block Micronucleus (CBMN) Assay in Peripheral Blood Lymphocytes for Biological Dosimetry, 2014, International Organization of Standardization; Geneva.

[43] F. Zölzer, Z. Hon, Z.F. Skalická, R. Havránková, L. Navrátil, J. Rosina and J. Škopek, Micronuclei in lymphocytes from radon spa personnel in the Czech Republic, *Int. Arch. Occup. Environ. Health.* **86**, 2013, 629–633

http://dx.doi.org/10.1007/s00420-012-0795-z.

[44] S.D. Dertinger, D.K. Torous, M. Hayashi and J.T. MacGregor, Flow cytometric scoring of micronucleated erythrocytes: an efficient platform for assessing in vivo cytogenetic damage, *Mutagenesis* **26**, 2011, 139–145, http://dx.doi.org/10.1093/mutage/geq055.

[45] G. Manning, K. Taylor, P. Finnon, J.A. Lemon, D.R. Boreham and C. Badie, Quantifying murine bone marrow and blood radiation dose response following (18)F-FDG PET with DNA damage biomarkers, *Mutat. Res.* **770**, 2014, 29–36, http://dx.doi.org/10.1016/j.mrfmmm.2014.09.002.

[46] S.D. Dertinger, Y. Chen, R.K. Miller, K.J. Brewer, T. Smudzin, D.K. Torous, N.E. Hall, K.A. Olvany, F.G. Murante and C.R. Tometsko, Micronucleated CD71-positive reticulocytes: a blood-based endpoint of cytogenetic damage in humans, *Mutat. Res.* **542**, 2003, 77–87.

[47] S.D. Dertinger, K. Camphausen, J.T. Macgregor, M.E. Bishop, D.K. Torous, S. Avlasevich, S. Cairns, C.R. Tometsko, C. Menard, T. Muanza, Y. Chen, R.K. Miller, K. Cederbrant, K. Sandelin, I. Pontén and G. Bolcsfoldi, Three-color labeling method for flow cytometric measurement of cytogenetic damage in rodent and human blood, *Environ. Mol. Mutagen.* **44**, 2004, 427–435, http://dx.doi.org/10.1002/em.20075.

[48] N. Kotova, C. Frostne, L. Abramsson-Zetterberg, E. Tareke, R. Bergman, S. Haghdoost, B. Paulsson, M. Törnqvist, D. Segerbäck, D. Jenssen and J. Grawé, Differences in micronucleus frequency and acrylamide adduct levels with hemoglobin between vegetarians and non-vegetarians, *Eur. J. Nutr.* **54**, 2015, 1181–1190, http://dx.doi.org/10.1007/s00394-014-0796-7.

[49] N. Kotova, D. Vare, N. Schultz, D. Gradecka Meesters, M. Stepnik, J. Grawé, T. Helleday and D. Jenssen, Genotoxicity of alcohol is linked to DNA replication-associated damage and homologous recombination repair, *Carcinogenesis* **34**, 2013, 325–330, http://dx.doi.org/10.1093/carcin/bgs340.

[50] A.P. Waugh, D.M. Beare, C.F. Arlett, M.H. Green and J. Cole, Comparative human cellular radiosensitivity: IV. The increased sensitivity of human neonatal cord blood lymphocytes to gamma-irradiation compared with lymphocytes from children and adults, *Int. J. Radiat. Biol.* **59**, 1991, 767–776.

[51] D.N. Floyd and A.M. Cassoni, Intrinsic radiosensitivity of adult and cord blood lymphocytes as determined by the micronucleus assay, *Eur. J. Cancer.* **30a**, 1994, 615–620.

[52] C. Vandevoorde, C. Franck, K. Bacher, L. Breysem, M.H. Smet, C. Ernst, A. De Backer, K. Van De Moortele, P. Smeets and H. Thierens, gamma-H2AX foci as in vivo effect biomarker in children emphasize the importance to minimize x-ray doses in paediatric CT imaging, *Eur. Radiol.* **25**, 2015, 800–811, http://dx.doi.org/10.1007/s00330-014-3463-8.

[53] C.M. West and G.C. Barnett, Genetics and genomics of radiotherapy toxicity: towards prediction, *Genome Med.* **3**, 2011, 52, http://dx.doi.org/10.1186/gm268.

[54] D. Scott, J.B. Barber, A.R. Spreadborough, W. Burrill and S.A. Roberts, Increased chromosomal radiosensitivity in breast cancer patients: a comparison of two assays, *Int. J. Radiat. Biol.* **75**, 1999, 1–10.

[55] A.V. Vozilova, N.B. Shagina, M.O. Degteva, A.A. Edwards, E.A. Ainsbury, J.E. Moquet, P. Hone, D.C. Lloyd, J.N. Fomina and F. Darroudi, Preliminary FISH-based assessment of external dose for residents exposed on the Techa River, *Radiat. Res.* **177**, 2012, 84–91.

[56] E.A. Ainsbury, J. Moquet, K. Rothkamm, F. Darroudi, A. Vozilova, M. Degteva, T.V. Azizova, D.C. Lloyd and J. Harrison, What radiation dose does the FISH translocation assay measure in cases of incorporated radionuclides for the Southern Urals populations?, *Radiat. Prot. Dosimet.* **159**, 2014, 26–33, http://dx.doi.org/10.1093/rpd/ncu118.

[57] L.E. Burak, Y. Kodama, M. Nakano, K. Ohtaki, M. Itoh, N.D. Okladnikova, E.K. Vasilenko, J.B. Cologne and N. Nakamura, FISH examination of lymphocytes from Mayak workers for assessment of translocation induction rate under chronic radiation exposures, *Int. J. Radiat. Biol.* **77**, 2001, 901–908, http://dx.doi.org/10.1080/09553000110063386.

[58] M.P. Hande, T.V. Azizova, L.E. Burak, V.F. Khokhryakov, C.R. Geard and D.J. Brenner, Complex chromosome aberrations persist in individuals many years after occupational exposure to densely ionizing radiation: an mFISH study, *Genes. Chromosomes Cancer* **44**, 2005, 1–9, http://dx.doi.org/10.1002/gcc.20217.

[59] N.V. Sotnik, S.V. Osovets, H. Scherthan and T.V. Azizova, mFISH analysis of chromosome aberrations in workers occupationally exposed to mixed radiation, *Radiat Env. Biophys.* **53**, 2014, 347–354, http://dx.doi.org/10.1007/s00411-014-0536-7.

[60] G.B. Curwen, N.V. Sotnik, K.K. Cadwell, T.V. Azizova, M.A. Hill and E.J. Tawn, Chromosome aberrations in workers with exposure to α-particle radiation from internal deposits of plutonium: expectations from in vitro studies and comparisons with workers with predominantly external γ-radiation exposure, *Radiat. Environ. Biophys.* **54**, 2015, 195–206, http://dx.doi.org/10.1007/s00411-015-0585-6.

[61] D.J. Brenner, N. Okladnikova, P. Hande, L. Burak, C.R. Geard and T. Azizova, Biomarkers specific to densely-ionising (high LET) radiations, *Radiat. Prot. Dosim.* **97**, 2001, 69–73.

[62] M.P. Hande, T.V. Azizova, C.R. Geard, L.E. Burak, C.R. Mitchell, V.F. Khokhryakov, E.K. Vasilenko and D.J. Brenner, Past exposure to densely ionizing radiation leaves a unique permanent signature in the genome, *Am. J. Hum. Genet.* **72**, 2003, 1162–1170, http://dx.doi.org/10.1086/375041.

[63] N.V. Sotnik, T.V. Azizova, F. Darroudi, E.A. Ainsbury, J.E. Moquet, J. Fomina, D.C. Lloyd, P.A. Hone and A.A. Edwards, Verification by the FISH translocation assay of historic doses to Mayak workers from external gamma radiation, *Radiat. Environ. Biophys.* 2015, http://dx.doi.org/10.1007/s00411-015-0614-5.

[64] S.V. Osovets, N.V. Sotnik, V. Meineke, H. Scherthan, H. Dorr and T.V. Azizova, Threshold limits for biological indication of prolonged radiation exposure using mFISH, *Health Phys.* **106**, 2014, 677–681, http://dx.doi.org/10.1097/hp.0000000000000057.

[65] A. Kryscio, W.U. Ulrich Müller, A. Wojcik, N. Kotschy, S. Grobelny and C. Streffer, A cytogenetic analysis of the long-term effect of uranium mining on peripheral lymphocytes using the micronucleus-centromere assay, *Int. J. Radiat. Biol.* **77**, 2001, 1087–1093, http://dx.doi.org/10.1080/09553000110070289.

[66] E. Gilson and A. Londono-Vallejo, Telomere length profiles in humans: all ends are not equal, *ABBV Cell Cycle* **6**, 2007, 2486–2494.

[67] M. Castella, S. Puerto, A. Creus, R. Marcos and J. Surralles, Telomere length modulates human radiation sensitivity in vitro, *Toxicol. Lett.* **172**, 2007, 29–36, http://dx.doi.org/10.1016/j.toxlet.2007.05.012.

[68] G. Shim, M. Ricoul, W.M. Hempel, E.I. Azzam and L. Sabatier, Crosstalk between telomere maintenance and radiation effects: ~~A key player in the process of radiation-induced carcinogenesisMutat Res Rev Mutat Res~~a key player in the process of radiation-induced carcinogenesis, *Mutat. Res. Rev. Mutat. Res.* 2014, http://dx.doi.org/10.1016/j.mrrev.2014.01.001.

[69] T. Maeda, K. Nakamura, K. Atsumi, M. Hirakawa, Y. Ueda and N. Makino, Radiation-associated changes in the length of telomeres in peripheral leukocytes from inpatients with cancer, *Int. J. Radiat. Biol.* **89**, 2013, 106–109, http://dx.doi.org/10.3109/09553002.2013.734945.

[70] R. M'Kacher, T. Girinsky, B. Colicchio, M. Ricoul, A. Dieterlen, E. Jeandidier, L. Heidingsfelder, C. Cuceu, G. Shim, M. Frenzel, A. Lenain, L. Morat, J. Bourhis, W.M. Hempel, S. Koscielny, J.F. Paul, P. Carde and L. Sabatier, Telomere shortening: a new prognostic factor for cardiovascular disease post-radiation exposure, *Radiat. Prot. Dosim.* **164**, 2015, 134–137, http://dx.doi.org/10.1093/rpd/ncu296.

[71] K. Hamatani, H. Eguchi, R. Ito, M. Mukai, K. Takahashi, M. Taga, K. Imai, J. Cologne, M. Soda, K. Arihiro, M. Fujihara, K. Abe, T. Hayashi, M. Nakashima, I. Sekine, W. Yasui, Y. Hayashi and K. Nakachi, RET/PTC rearrangements preferentially occurred in papillary thyroid cancer among atomic bomb survivors exposed to high radiation dose, *Cancer Res.* **68**, 2008, 7176–7182, http://dx.doi.org/10.1158/0008-5472.can-08-0293.

[72] R.J. Leeman-Neill, A.V. Brenner, M.P. Little, T.I. Bogdanova, M. Hatch, L.Y. Zurnadzy, K. Mabuchi, M.D. Tronko and Y.E. Nikiforov, RET/PTC and PAX8/PPARgamma chromosomal rearrangements in post-Chernobyl thyroid cancer and their association with iodine-131 radiation dose and other characteristics, *Cancer* **119**, 2013, 1792–1799, http://dx.doi.org/10.1002/cncr.27893.

[73] C. Romei and R. Elisei, RET/PTC ~~Translocations and Clinico-Pathological Features in Human Papillary Thyroid C~~translocations and clinico-Pathological features in human papillary thyroid carcinoma, *Front. Endocrinol. Lausanne* **3** (54), 2012, http://dx.doi.org/10.3389/fendo.2012.00054.

[74] Y.E. Nikiforov, J.M. Rowland, K.E. Bove, H. Monforte-Munoz and J.A. Fagin, Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children, *Cancer Res.* **57**, 1997, 1690–1694.

[75] R.M. Tuttle, Y. Lukes, L. Onstad, E. Lushnikov, A. Abrosimov, V. Troshin, A. Tsyb, S. Davis, K.J. Kopecky and G. Francis, ret/PTC activation is not associated with individual radiation dose estimates in a pilot study of neoplastic thyroid nodules arising in Russian children and adults exposed to Chernobyl fallout, *Thyroid* **18**, 2008, 839–846, http://dx.doi.org/10.1089/thy.2008.0072.

[76] S. Suzuki, Three-years ~~results and future scope of the Fukushima Thyroid Ultrasound Examination a~~Results and Future Scope of the Fukushima Thyroid Ultrasound Examination After the Fukushima NPP ~~a~~Accident, 2014 http://www.fmu.ac.jp/radiationhealth/symposium201409/media/9_S2_Suzuki_FINAL(0909).pdf.

[77] J.C. Ricarte-Filho, S. Li, M.E. Garcia-Rendueles, C. Montero-Conde, F. Voza, J.A. Knauf, A. Heguy, A. Viale, T. Bogdanova, G.A. Thomas, C.E. Mason and J.A. Fagin, Identification of kinase fusion oncogenes in post-Chernobyl radiation-induced thyroid cancers, *J. Clin. Invest.* **123**, 2013, 4935–4944, http://dx.doi.org/10.1172/jci69766.

[78] M. Santoro and F. Carlomagno, Oncogenic rearrangements driving ionizing radiation-associated human cancer, *J. Clin. Invest.* **123**, 2013, 4566–4568, http://dx.doi.org/10.1172/jci72725.

[79] R.J. Leeman-Neill, L.M. Kelly, P. Liu, A.V. Brenner, M.P. Little, T.I. Bogdanova, V.N. Evdokimova, M. Hatch, L.Y. Zurnadzy, M.N. Nikiforova, N.J. Yue, M. Zhang, K. Mabuchi, M.D. Tronko and Y.E. Nikiforov, ETV6-NTRK3 is a common chromosomal rearrangement in radiation-associated thyroid cancer, *Cancer* **120**, 2014, 799–807, http://dx.doi.org/10.1002/cncr.28484.

[80] J.-L. Ravanat, J. Breton, T. Douki, D. Gasparutto, A. Grand, W. Rachidi and S. Sauvaigo, Radiation-mediated formation of complex damage to DNA: a chemical aspect overview, *Br. J. Radiol.* **87**, 2014, 20130715, http://dx.doi.org/10.1259/bjr.20130715.

[81] J.A. Reisz, N. Bansal, J. Qian, W. Zhao and C.M. Furdui, Effects of ionizing radiation on biological molecules-mechanisms of damage and emerging methods of detection, *Antioxid. Redox Signal.* **21**, 2014, 260–292, http://dx.doi.org/10.1089/ars.2013.5489.

[82] G.S. Madugundu, Y. Park, L. Sanche and J.R. Wagner, Radiation-induced formation of 2′,3′-dideoxyribonucleosides in DNA: a potential signature of low-energy electrons, *J. Am. Chem. Soc.* **134**, 2012, 17366–17368, http://dx.doi.org/10.1021/ja306810w.

[83] S. Moore, F.K.T. Stanley and A.A. Goodarzi, The repair of environmentally relevant DNA double strand breaks caused by high linear energy transfer irradiation-no simple task, *DNA Repair* **17**, 2014, 64–73, http://dx.doi.org/10.1016/j.dnarep.2014.01.014.

[84] E.P. Rogakou, D.R. Pilch, A.H. Orr, V.S. Ivanova and W.M. Bonner, DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139, *J. Biol. Chem.* **273**, 1998, 5858–5868.

[85] Y. Ibuki and T. Toyooka, Evaluation of chemical phototoxicity, focusing on phosphorylated histone H2AX, *J. Radiat. Res.* **56**, 2015, 220–228, http://dx.doi.org/10.1093/jrr/rru105.

[86] V. Valdiglesias, S. Giunta, M. Fenech, M. Neri and S. Bonassi, gammaH2AX as a marker of DNA double strand breaks and genomic instability in human population studies, *Mutat. Res.* **753**, 2013, 24–40, http://dx.doi.org/10.1016/j.mrrev.2013.02.001.

[87] B.M. Halm, A.A. Franke, J.F. Lai, H.C. Turner, D.J. Brenner, V.M. Zohrabian and R. DiMauro, gamma-H2AX foci are increased in lymphocytes in vivo in young children 1 h after very low-dose X-irradiation: a pilot study, *Pediatr. Radiol.* **44**, 2014, 1310–1317, http://dx.doi.org/10.1007/s00247-014-2983-3.

[88] C. Vandevoorde, M. Gomolka, U. Roessler, D. Samaga, C. Lindholm, M. Fernet, J. Hall, E. Pernot, H. El-Saghire, S. Baatout, A. Kesminiene and H. Thierens, EPI-CT: in vitro assessment of the applicability of the gamma-H2AX-foci assay as cellular biomarker for exposure in a multicentre study of children in diagnostic radiology, *Int. J. Radiat. Biol.* 2015, 1–11, http://dx.doi.org/10.3109/09553002.2015.1047987.

[89] A. Andrievski and R.C. Wilkins, The response of gamma-H2AX in human lymphocytes and lymphocytes subsets measured in whole blood cultures, *Int. J. Radiat. Biol.* **85**, 2009, 369–376, http://dx.doi.org/10.1080/09553000902781147.

[90] R.W. Godschalk, C. Ersson, M. Stepnik, M. Ferlinska, J. Palus, J.P. Teixeira, S. Costa, G.D. Jones, J.A. Higgins, J. Kain, L. Moller, L. Forchhammer, S. Loft, Y. Lorenzo, A.R. Collins, F.J. van Schooten, B. Laffon, V. Valdiglesias, M. Cooke, V. Mistry, M. Karbaschi, D.H. Phillips, O. Sozeri, M.N. Routledge, K. Nelson-Smith, P. Riso, M. Porrini, A. Lopez de Cerain, A. Azqueta, G. Matullo, A. Allione and P. Moller, Variation of DNA damage levels in peripheral blood mononuclear cells isolated in different laboratories, *Mutagenesis* **29**, 2014, 241–249, http://dx.doi.org/10.1093/mutage/geu012.

[91] M. Wojewodzka, S. Sommer, M. Kruszewski, K. Sikorska, M. Lewicki, H. Lisowska, A. Wegierek-Ciuk, M. Kowalska and A. Lankoff, Defining ~~Blood Processing Parameters for Optimal Detection of gamma-H2AX Foci: A Small Blood Volume M~~blood processing parameters for optimal detection of gamma-H2AX foci: a small blood volume method, *Radiat. Res.* **184**, 2015, 95–104, http://dx.doi.org/10.1667/rr13897.1.

[92] J. Heydenreich, C. Otto, F. Mayer and A. Carlsohn, Reliability of a ~~Fully Automated Interpretation of gamma – H2AX Foci in Lymphocytes of Moderately Trained Subjects under Resting C~~fully automated interpretation of gamma – H2AX foci in lymphocytes of moderately trained subjects under resting conditions, *J. Nutr. Metab.* **2014**, 2014, 478324, http://dx.doi.org/10.1155/2014/478324.

[93] Y. He, Y. Gong, J. Lin, D.W. Chang, J. Gu, J.A. Roth and X. Wu, Ionizing radiation-induced gamma-H2AX activity in whole blood culture and the risk of lung cancer, *Cancer Epidemiol. Biomark. Prev.* **22**, 2013, 443–451 http://dx.doi.org/10.1158/1055-9965.epi-12-0794.

[94] P.M. Sharma, B. Ponnaiya, M. Taveras, I. Shuryak, H. Turner and D.J. Brenner, High throughput measurement of gammaH2AX DSB repair kinetics in a healthy human population, *PLoS One* **10**, 2015, e0121083, http://dx.doi.org/10.1371/journal.pone.0121083.

[95] S. Zahnreich, A. Ebersberger, B. Kaina and H. Schmidberger, Biodosimetry ~~Based on gamma-H2AX Quantification and Cytogenetics after Partial- and Total-Body Irradiation during Fractionated R~~based on gamma-H2AX quantification and cytogenetics after partial- and total-Body irradiation during fractionated radiotherapy, *Radiat. Res.* **183**, 2015, 432–446, http://dx.doi.org/10.1667/rr13911.1.

[96] K. Rothkamm, C. Beinke, H. Romm, C. Badie, Y. Balagurunathan, S. Barnard, N. Bernard, H. Boulay-Greene, M. Brengues, A. De Amicis, S. De Sanctis, R. Greither, F. Herodin, A. Jones, S. Kabacik, T. Knie, U. Kulka, F. Lista, P. Martigne, A. Missel, J. Moquet, U. Oestreicher, A. Peinnequin, T. Poyot, U. Roessler, H. Scherthan, B. Terbrueggen, H. Thierens, M. Valente, A. Vral, F. Zenhausern, V. Meineke, H. Braselmann an M. Abend, Comparison of established and emerging biodosimetry assays, *Radiat. Res.* **180**, 2013, 111–119, http://dx.doi.org/10.1667/RR3231.1.

[97] S. Barnard, E.A. Ainsbury, J. Al-hafidh, V. Hadjidekova, R. Hristova, C. Lindholm, O. Monteiro Gil, J. Moquet, M. Moreno, U. Rossler, H. Thierens, C. Vandevoorde, A. Vral, M. Wojewodzka and K. Rothkamm, The first gamma-H2AX biodosimetry intercomparison exercise of the developing European biodosimetry network RENEB, *Radiat. Prot. Dosim.* **164**, 2015, 265–270, http://dx.doi.org/10.1093/rpd/ncu259.

[98] J. Moquet, S. Barnard and K. Rothkamm, Gamma-H2AX biodosimetry for use in large scale radiation incidents: comparison of a rapid 96 well lyse/fix protocol with a routine method, *PeerJ* ~~.~~**2**, 2014, e282, http://dx.doi.org/10.7717/peerj.282.

[99] D. Heylmann and B. Kaina, The γH2AX DNA damage assay from a drop of blood, *Sci. Rep.* **6**, 2016, 22682, http://dx.doi.org/10.1038/srep22682.

[100] S. Horn, S. Barnard and K. Rothkamm, Gamma-H2AX-based dose estimation for whole and partial body radiation exposure, *PLoS One* **6**, 2011, e25113, http://dx.doi.org/10.1371/journal.pone.0025113.

[101] K. Rothkamm, S. Barnard, J. Moquet, M. Ellender, Z. Rana and S. Burdak-Rothkamm, DNA damage foci: ~~M~~meaning and significance, *Env. Mol. Mutagen.* **56**, 2015, 491–504, http://dx.doi.org/10.1002/em.21944.

[102] O.A. Martin, A. Ivashkevich, S. Choo, L. Woodbine, P.A. Jeggo, R.F. Martin and P. Lobachevsky, Statistical analysis of kinetics, distribution and co-localisation of DNA repair foci in irradiated cells: cell cycle effect and implications for prediction of radiosensitivity, *DNA Repair Amst.* **12**, 2013, 844–855, http://dx.doi.org/10.1016/j.dnarep.2013.07.002.

[103] V. Vandersickel, P. Beukes, B. Van Bockstaele, J. Depuydt, A. Vral and J. Slabbert, Induction and disappearance of gammaH2AX foci and formation of micronuclei after exposure of human lymphocytes to (6)(0)Co gamma-rays and p(66)+ Be(40) neutrons, *Int. J. Radiat. Biol.* **90**, 2014, 149–158, http://dx.doi.org/10.3109/09553002.2014.860252.

[104] F. Antonelli, A. Campa, G. Esposito, P. Giardullo, M. Belli, V. Dini, S. Meschini, G. Simone, E. Sorrentino, S. Gerardi, G.A. Cirrone and M.A. Tabocchini, Induction and ~~Repair of DNA DSB as Revealed by H2AX Phosphorylation Foci in Human Fibroblasts Exposed to Low- and High-LET Radiation: Relationship with Early and Delayed Reproductive Cell D~~repair of DNA DSB as revealed by H2AX phosphorylation foci in human fibroblasts exposed to low- and high-LET radiation: relationship with early and delayed reproductive cell death, *Radiat. Res.* **183**, 2015, 417–431, http://dx.doi.org/10.1667/rr13855.1.

[105] S. Horn, D. Brady and K. Prise, Alpha particles induce pan-nuclear phosphorylation of H2AX in primary human lymphocytes mediated through ATM, *Biochim. Biophys. Acta.* **1853**, 2015, 2199–2206, http://dx.doi.org/10.1016/j.bbamcr.2015.06.010.

[106] Y. Lorat, C.U. Brunner, S. Schanz, B. Jakob, G. Taucher-Scholz and C.E. Rube, Nanoscale analysis of clustered DNA damage after high-LET irradiation by quantitative electron microscopy-the heavy burden to repair, *DNA Repair Amst.* **28**, 2015, 93–106, http://dx.doi.org/10.1016/j.dnarep.2015.01.007.

[107] K. Sato, M. Nishikino, T. Kawachi, T. Shimokawa, T. Imai, T. Teshima, H. Nishimura and M. Kando, A laser-plasma-produced soft X-ray laser at 89 eV generates DNA double-strand breaks in human cancer cells, *J Radiat. Res.* **56**, 2015, 633–638, http://dx.doi.org/10.1093/jrr/rrv015.

[108] S. Schanz, N. Schuler, Y. Lorat, L. Fan, L. Kaestner, G. Wennemuth, C. Rube and C.E. Rube, Accumulation of DNA damage in complex normal tissues after protracted low-dose radiation, *DNA Repair Amst.* **11**, 2012, 823–832, http://dx.doi.org/10.1016/j.dnarep.2012.07.005.

[109] H. Willers, L. Gheorghiu, Q. Liu, J.A. Efstathiou, L.J. Wirth, M. Krause and C. von Neubeck, DNA ~~Damage Response Assessments in Human Tumor Samples Provide Functional Biomarkers of R~~damage response assessments in human tumor samples provide functional biomarkers of radiosensitivity, *Semin. Radiat. Oncol.* **25**, 2015, 237–250, http://dx.doi.org/10.1016/j.semradonc.2015.05.007.

[110] S. Pouliliou and M.I. Koukourakis, Gamma histone 2AX (gamma-H2AX)as a predictive tool in radiation oncology, *Biomarkers* **19**, 2014, 167–180, http://dx.doi.org/10.3109/1354750x.2014.898099.

[111] L. Woodbine, S. Grigoriadou, A.A. Goodarzi, E. Riballo, C. Tape, A.W. Oliver, M.C. van Zelm, M.S. Buckland, E.G. Davies, L.H. Pearl and P.A. Jeggo, An Artemis polymorphic variant reduces Artemis activity and confers cellular radiosensitivity, *DNA Repair* **9**, 2010, 1003–1010, http://dx.doi.org/10.1016/j.dnarep.2010.07.001.

[112] C.E. Rube, A. Fricke, J. Wendorf, A. Stutzel, M. Kuhne, M.F. Ong, P. Lipp and C. Rube, Accumulation of DNA double-strand breaks in normal tissues after fractionated irradiation, *Int. J. Radiat. Oncol. Biol. Phys.* **76**, 2010, 1206–1213, http://dx.doi.org/10.1016/j.ijrobp.2009.10.009.

[113] S. Haghdoost, S. Czene, I. Näslund, S. Skog and M. Harms-Ringdahl, Extracellular 8-oxo-dG as a sensitive parameter for oxidative stress in vivo and in vitro, *Free Radic. Res.* **39**, 2005, 153–162, http://dx.doi.org/10.1080/10715760500043132.

[114] S. Haghdoost, Y. Maruyama, R. Pecoits-Filho, O. Heimburger, A. Seeberger, B. Anderstam, M.E. Suliman, S. Czene, B. Lindholm, P. Stenvinkel and M. Harms-Ringdahl, Elevated serum 8-oxo-dG in hemodialysis patients: a marker of systemic inflammation?, *Antioxid. Redox Signal.* **8**, 2006, 2169–2173, http://dx.doi.org/10.1089/ars.2006.8.2169.

[115] K. Datta, S. Suman and A.J. Fornace, Radiation persistently promoted oxidative stress, activated mTOR via PI3 K/Akt, and downregulated autophagy pathway in mouse intestine, *Int. J. Biochem. Cell Biol.* **57**, 2014 167–176, http://dx.doi.org/10.1016/j.biocel.2014.10.022.

[116] S. Skiöld, I. Naslund, K. Brehwens, A. Andersson, P. Wersall, E. Lidbrink, M. Harms-Ringdahl, A. Wojcik and S. Haghdoost, Radiation-induced stress response in peripheral blood of breast cancer patients differs between patients with severe acute skin reactions and patients with no side effects to radiotherapy, *Mutat. Res.* **756**, 2013, 152–157, http://dx.doi.org/10.1016/j.mrgentox.2013.04.014.

[117] D. Danielsson, K. Brehwens, M. Halle, M. Marczyk, A. Sollazzo, J. Polanska, E. Munck-Wikland, A. Wojcik and S. Haghdoost, Influence of genetic background and oxidative stress response on risk of mandibular osteoradionecrosis after radiotherapy of head and neck cancer, *Head Neck* 2014, http://dx.doi.org/10.1002/hed.23903.

[118] S. Haghdoost, P. Svoboda, I. Näslund, M. Harms-Ringdahl, A. Tilikides and S. Skog, Can 8-oxo-dG be used as a predictor for individual radiosensitivity?, *Int. J. Radiat. Oncol. Biol. Phys.* **50**, 2001, 405–410.

[119] K. Roszkowski and R. Olinski, Urinary 8-oxoguanine as a predictor of survival in patients undergoing radiotherapy, *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **21**, 2012, 629–634, http://dx.doi.org/10.1158/1055-9965.EPI-11-0981.

[120] S.L. Kerns, C.M.L. West, C.N. Andreassen, G.C. Barnett, S.M. Bentzen, N.G. Burnet, A. Dekker, D. De Ruysscher, A. Dunning, M. Parliament, C. Talbot, A. Vega and B.S. Rosenstein, Radiogenomics: the search for genetic predictors of radiotherapy response, *Future Oncol. Lond. Engl.* **10**, 2014, 2391–2406, http://dx.doi.org/10.2217/fon.14.173.

[121] P. Jeggo, The role of the DNA damage response mechanisms after low-dose radiation exposure and a consideration of potentially sensitive individuals, *Radiat. Res.* **174**, 2010, 825–832, http://dx.doi.org/10.1667/RR1844.1.

[122] A. Pijpe, N. Andrieu, D.F. Easton, A. Kesminiene, E. Cardis, C. Noguès, M. Gauthier-Villars, C. Lasset, J.-P. Fricker, S. Peock, D. Frost, D.G. Evans, R.A. Eeles, J. Paterson, P. Manders, C.J. van Asperen, M.G.E.M. Ausems, H. Meijers-Heijboer, I. Thierry-Chef, M. Hauptmann, D. Goldgar, M.A. Rookus and F.E. van Leeuwen, Exposure to diagnostic radiation and risk of breast cancer among carriers of BRCA1/2 mutations: retrospective cohort study (GENE-RAD-RISK), *BMJ* **345**, 2012, e5660.

[123] J.L. Bernstein, R.W. Haile, M. Stovall, J.D. Boice, R.E. Shore, B. Langholz, D.C. Thomas, L. Bernstein, C.F. Lynch, J.H. Olsen, K.E. Malone, L. Mellemkjaer, A.-L. Borresen-Dale, B.S. Rosenstein, S.N. Teraoka, A.T. Diep, S.A. Smith, M. Capanu, A.S. Reiner, X. Liang, R.A. Gatti and P. Concannon, WECARE Study Collaborative Group, Radiation exposure, the ATM Gene, and contralateral breast cancer in the women's environmental cancer and radiation epidemiology study, *J. Natl. Cancer Inst.* **102**, 2010, 475–483, http://dx.doi.org/10.1093/jnci/djq055.

[124] C. West, B.S. Rosenstein, J. Alsner, D. Azria, G. Barnett, A. Begg, S. Bentzen, N. Burnet, J. Chang-Claude, E. Chuang, C. Coles, K. De Ruyck, D. De Ruysscher, A. Dunning, R. Elliott, L. Fachal, J. Hall, K. Haustermans, C. Herskind, T. Hoelscher, T. Imai, M. Iwakawa, D. Jones, C. Kulich, EQUAL-ESTRO, J.-H. Langendijk, P. O'Neils, M. Ozsahin, M. Parliament, A. Polanski, B. Rosenstein, D. Seminara, P. Symonds, C. Talbot, H. Thierens, A. Vega, C. West and J. Yarnold, Establishment of a ~~Radiogenomics C~~radiogenomics consortium, *Int. J. Radiat. Oncol. Biol. Phys.* **76**, 2010, 1295–1296, http://dx.doi.org/10.1016/j.ijrobp.2009.12.017.

[125] J.D. Brooks, S.N. Teraoka, A.S. Reiner, J.M. Satagopan, L. Bernstein, D.C. Thomas, M. Capanu, M. Stovall, S.A. Smith, S. Wei, R.E. Shore, J.D. Boice, C.F. Lynch, L. Mellemkjaer, K.E. Malone, X. Liang, Wecare Study Collaborative Group, R.W. Haile, P. Concannon and J.L. Bernstein, Variants in activators and downstream targets of ATM, radiation exposure, and contralateral breast cancer risk in the WECARE study, *Hum. Mutat.* **33**, 2012, 158–164, http://dx.doi.org/10.1002/humu.21604.

[126] N. Mavaddat, P.D.P. Pharoah, K. Michailidou, J. Tyrer, M.N. Brook, M.K. Bolla, Q. Wang, J. Dennis, A.M. Dunning, M. Shah, R. Luben, J. Brown, S.E. Bojesen, B.G. Nordestgaard, S.F. Nielsen, H. Flyger, K. Czene,

H. Darabi, M. Eriksson, J. Peto, I. Dos-Santos-Silva, F. Dudbridge, N. Johnson, M.K. Schmidt, A. Broeks, S. Verhoef, E.J. Rutgers, A. Swerdlow, A. Ashworth, N. Orr, M.J. Schoemaker, J. Figueroa, S.J. Chanock, L. Brinton, J. Lissowska, F.J. Couch, J.E. Olson, C. Vachon, V.S. Pankratz, D. Lambrechts, H. Wildiers, C. Van Ongeval, E. van Limbergen, V. Kristensen, G. Grenaker Alnæs, S. Nord, A.-L. Borresen-Dale, H. Nevanlinna T.A. Muranen, K. Aittomäki, C. Blomqvist, J. Chang-Claude, A. Rudolph, P. Seibold, D. Flesch-Janys, P.A. Fasching, L. Haeberle, A.B. Ekici, M.W. Beckmann, B. Burwinkel, F. Marme, A. Schneeweiss, C. Sohn, A. Trentham-Dietz, P. Newcomb, L. Titus, K.M. Egan, D.J. Hunter, S. Lindstrom, R.M. Tamimi, P. Kraft, N. Rahman, C. Turnbull, A. Renwick, S. Seal, J. Li, J. Liu, K. Humphreys, J. Benitez, M. Pilar Zamora, J.I. Arias Perez, P. Menéndez, A. Jakubowska, J. Lubinski, K. Jaworska-Bieniek, K. Durda, N.V. Bogdanova, N.N. Antonenkova, T. Dörk, H. Anton-Culver, S.L. Neuhausen, A. Ziogas, L. Bernstein, P. Devilee, R.A.E.M. Tollenaar C. Seynaeve, C.J. van Asperen, A. Cox, S.S. Cross, M.W.R. Reed, E. Khusnutdinova, M. Bermisheva, D. Prokofyeva, Z. Takhirova, A. Meindl, R.K. Schmutzler, C. Sutter, R. Yang, P. Schürmann, M. Bremer, H. Christiansen, T.-W. Park-Simon, P. Hillemanns, P. Guénel, T. Truong, F. Menegaux, M. Sanchez, P. Radice, P. Peterlongo, S. Manoukian, V. Pensotti, J.L. Hopper, H. Tsimiklis, C. Apicella, M.C. Southey, H. Brauch, T. Brüning, Y.-D. Ko, A.J. Sigurdson, M.M. Doody, U. Hamann, D. Torres, H.-U. Ulmer, A. Försti, E.J. Sawyer, I. Tomlinson, M.J. Kerin, N. Miller, I.L. Andrulis, J.A. Knight, G. Glendon, A. Marie Mulligan, G. Chenevix-Trench, R. Balleine, G.G. Giles, R.L. Milne, C. McLean, A. Lindblom, S. Margolin, C.A. Haiman, B.E. Henderson, F. Schumacher, L. Le Marchand, U. Eilber, S. Wang-Gohrke, M.J. Hooning, A. Hollestelle, A.M.W. van den Ouweland, L.B. Koppert, J. Carpenter, C. Clarke, R. Scott, A. Mannermaa, V. Kataja, V.-M. Kosma, J.M. Hartikainen, H. Brenner, V. Arndt, C. Stegmaier, A. Karina Dieffenbach, R. Winqvist, K. Pylkäs, A. Jukkola Vuorinen, M. Grip, K. Offit, J. Vijai, M. Robson, R. Rau-Murthy, M. Dwek, R. Swann, K. Annie Perkins, M.S. Goldberg, F. Labrèche, M. Dumont, D.M. Eccles, W.J. Tapper, S. Rafiq, E.M. John, A.S. Whittemore, S. Slager, D. Yannoukakos, A.E. Toland, S. Yao, W. Zheng, S.L. Halverson, A. González-Neira, G. Pita, M. Rosario Alonso, N. Álvarez, D. Herrero, D.C. Tessier, D. Vincent, F. Bacot, C. Luccarini, C. Baynes, S. Ahmed, M. Maranian, C.S. Healey, J. Simard, P. Hall, D.F. Easton and M. Garcia-Closas, Prediction of breast cancer risk based on profiling with common genetic variants, *J. Natl. Cancer Inst.* **107**, 2015, http://dx.doi.org/10.1093/jnci/djv036.

[127] T. Best, D. Li, A.D. Skol, T. Kirchhoff, S.A. Jackson, Y. Yasui, S. Bhatia, L.C. Strong, S.M. Domchek, K.L. Nathanson, O.I. Olopade, R.S. Huang, T.M. Mack, D.V. Conti, K. Offit, W. Cozen, L.L. Robison and K. Onel, Variants at 6q21 implicate PRDM1 in the etiology of therapy-induced second malignancies after Hodgkin's lymphoma, *Nat. Med.* **17**, 2011, 941–943, http://dx.doi.org/10.1038/nm.2407.

[128] L.M. Morton and S.J. Chanock, A step toward slaying the hydra of second cancers, *Nat. Med.* **17**, 2011, 924–925, http://dx.doi.org/10.1038/nm.2428.

[129] A.L. Sherborne, P.R. Davidson, K. Yu, A.O. Nakamura, M. Rashid and J.L. Nakamura, Mutational ~~Analysis of Ionizing Radiation Induced N~~analysis of ionizing radiation induced neoplasms, *Cell Rep.* **12**, 2015, 1915–1926, http://dx.doi.org/10.1016/j.celrep.2015.08.015.

[130] S. Behjati, et al., Mutational signatures of ionizing radiation in second malignancies, *Nat. Commun.* 2016, in press (n.d.).

[131] J. Hess, G. Thomas, H. Braselmann, V. Bauer, T. Bogdanova, J. Wienberg, H. Zitzelsberger and K. Unger, Gain of chromosome band 7q11 in papillary thyroid carcinomas of young patients is associated with exposure to low-dose irradiation, *Proc. Natl. Acad. Sci. U. S. A.* **108**, 2011, 9595–9600, http://dx.doi.org/10.1073/pnas.1017137108.

[132] A.B. Adewoye, S.J. Lindsay, Y.E. Dubrova and M.E. Hurles, The genome-wide effects of ionizing radiation on mutation induction in the mammalian germline, *Nat. Commun.* **6**, 2015, 6684, http://dx.doi.org/10.1038/ncomms7684.

[133] V. Chauhan, M. Howland and R. Wilkins, Identification of gene-based responses in human blood cells exposed to alpha particle radiation, *BMC Med. Genom.* **7** (43), 2014, http://dx.doi.org/10.1186/1755-8794-7-43.

[134] S. Kabacik, A. Mackay, N. Tamber, G. Manning, P. Finnon, F. Paillier, A. Ashworth, S. Bouffler and C. Badie, Gene expression following ionising radiation: identification of biomarkers for dose estimation and prediction of individual response, *Int. J. Radiat. Biol.* **87**, 2011, 115–129, http://dx.doi.org/10.3109/09553002.2010.519424.

[135] G. Manning, S. Kabacik, P. Finnon, S. Bouffler and C. Badie, High and low dose responses of transcriptional biomarkers in ex vivo X-irradiated human blood, *Int. J. Radiat. Biol.* **89**, 2013, 512–522, http://dx.doi.org/10.3109/09553002.2013.769694.

[136] G. Manning, S. Kabacik, P. Finnon, F. Paillier, S. Bouffler and C. Badie, Assessing a new gene expression analysis technique for radiation biodosimetry applications, *Radiat. Meas.* **46**, 2011, 1014–1018, http://dx.doi.org/10.1016/j.radmeas.2011.05.019.

[137] C. Badie, S. Kabacik, Y. Balagurunathan, N. Bernard, M. Brengues, G. Faggioni, R. Greither, F. Lista, A. Peinnequin, T. Poyot, F. Herodin, A. Missel, B. Terbrueggen, F. Zenhausern, K. Rothkamm, V. Meineke, H. Braselmann, C. Beinke and M. Abend, Laboratory intercomparison of gene expression assays, *Radiat. Res.* **180**, 2013, 138–148, http://dx.doi.org/10.1667/RR3236.1.

[138] J. Lucas, H.K. Dressman, S. Suchindran, M. Nakamura, N.J. Chao, H. Himburg, K. Minor, G. Phillips, J. Ross, M. Abedi, R. Terbrueggen and J.P. Chute, A translatable predictor of human radiation exposure, *PLoS One* **9**, 2014, e107897, http://dx.doi.org/10.1371/journal.pone.0107897.

[139] M. Abend, C. Badie, R. Quintens, R. Kriehuber, G. Manning, E. Macaeva, M. Njima, D. Oskamp, S. Strunz, S. Moertl, S. Doucha-Senf, S. Dahlke, J. Menzel and M. Port, Examining ~~Radiation-Induced In Vivo and In Vitro Gene Expression Changes of the Peripheral Blood in Different Laboratories for Biodosimetry Purposes: First RENEB Gene Expression S~~radiation-Induced in vivo and In vitro gene expression changes of the peripheral blood in different laboratories for biodosimetry purposes: first RENEB gene expression study, *Radiat. Res.* **185**, 2016, 109–123, http://dx.doi.org/10.1667/RR14221.1.

[140] C.N. Sprung, J. Li, D. Hovan, M.J. McKay and H.B. Forrester, Alternative transcript initiation and splicing as a response to DNA damage, *PLoS One* **6**, 2011, e25758, http://dx.doi.org/10.1371/journal.pone.0025758.

[141] H.B. Forrester, J. Li, D. Hovan, A.N. Ivashkevich and C.N. Sprung, DNA repair genes: alternative transcription and gene expression at the exon level in response to the DNA damaging agent, ionizing radiation, *PLoS One* **7**, 2012, e53358, http://dx.doi.org/10.1371/journal.pone.0053358.

[142] R. Quintens, T. Verreet, A. Janssen, M. Neefs, L. Leysen, A. Michaux, M. Verslegers, N. Samari, G. Pani, J. Verheyde, S. Baatout and M.A. Benotmane, Identification of novel radiation-induced p53-dependent transcripts extensively regulated during mouse brain development, *Biol. Open* **4**, 2015, 331–344, http://dx.doi.org/10.1242/bio.20149969.

[143] S. Paul, C.A. Barker, H.C. Turner, A. McLane, S.L. Wolden and S.A. Amundson, Prediction of in vivo radiation dose status in radiotherapy patients using ex vivo and in vivo gene expression signatures, *Radiat. Res.* **175**, 2011, 257–265, http://dx.doi.org/10.1667/RR2420.1.

[144] J. Lucas, H.K. Dressman, S. Suchindran, M. Nakamura, N.J. Chao, H. Himburg, K. Minor, G. Phillips, J. Ross, M. Abedi, R. Terbrueggen and J.P. Chute, A translatable predictor of human radiation exposure, *PLoS One* **9**, 2014, e107897, http://dx.doi.org/10.1371/journal.pone.0107897.

[145] I. Nosel, A. Vaurijoux, J.-F. Barquinero and G. Gruel, Characterization of gene expression profiles at low and very low doses of ionizing radiation, *DNA Repair* **12**, 2013, 508–517, http://dx.doi.org/10.1016/j.dnarep.2013.04.021.

[146] K.-Y. Jen and V.G. Cheung, Transcriptional response of lymphoblastoid cells to ionizing radiation, *Genome Res.* **13**, 2003, 2092–2100, http://dx.doi.org/10.1101/gr.1240103.

[147] K. Knops, S. Boldt, O. Wolkenhauer and R. Kriehuber, Gene expression in low- and high-dose-irradiated human peripheral blood lymphocytes: possible applications for biodosimetry, *Radiat. Res.* **178**, 2012, 304–312.

[148] G. Manning, S. Kabacik, P. Finnon, S. Bouffler and C. Badie, High and low dose responses of transcriptional biomarkers in ex vivo X-irradiated human blood, *Int. J. Radiat. Biol.* **89**, 2013, 512–522, http://dx.doi.org/10.3109/09553002.2013.769694.

[149] Y. Imamichi, T. Mizutani, Y. Ju, T. Matsumura, S. Kawabe, M. Kanno, T. Yazawa and K. Miyamoto, Transcriptional regulation of human ferredoxin reductase through an intronic enhancer in steroidogenic cells, *Biochim. Biophys. Acta* **1839**, 2014, 33–42, http://dx.doi.org/10.1016/j.bbagrm.2013.11.005.

[150] G. Liu and X. Chen, The ferredoxin reductase gene is regulated by the p53 family and sensitizes cells to oxidative stress-induced apoptosis, *Oncogene* **21**, 2002, 7195–7204, http://dx.doi.org/10.1038/sj.onc.1205862.

[151] P.M. Hwang, F. Bunz, J. Yu, C. Rago, T.A. Chan, M.P. Murphy, G.F. Kelso, R.A. Smith, K.W. Kinzler and B. Vogelstein, Ferredoxin reductase affects p53-dependent 5-fluorouracil-induced apoptosis in colorectal cancer cells, *Nat. Med.* **7**, 2001, 1111–1117, http://dx.doi.org/10.1038/nm1001-1111.

[152] S. Paul and S.A. Amundson, Gene expression signatures of radiation exposure in peripheral white blood cells of smokers and non-smokers, *Int. J. Radiat. Biol.* **87**, 2011, 791–801, http://dx.doi.org/10.3109/09553002.2011.568574.

[153] H. Budworth, A.M. Snijders, F. Marchetti, B. Mannion, S. Bhatnagar, E. Kwoh, Y. Tan, S.X. Wang, W.F. Blakely, M. Coleman, L. Peterson and A.J. Wyrobek, DNA repair and cell cycle biomarkers of radiation exposure and inflammation stress in human blood, *P~~loS One.~~LoS One* **7**, 2012, e48619, http://dx.doi.org/10.1371/journal.pone.0048619.

[154] J.D. Tucker, W.E. Grever, M.C. Joiner, A.A. Konski, R.A. Thomas, J.M. Smolinski, G.W. Divine and G.W. Auner, Gene expression-based detection of radiation exposure in mice after treatment with granulocyte colony-stimulating factor and lipopolysaccharide, *Radiat. Res.* **177**, 2012, 209–219.

[155] M.J. Muñoz, M.S. Pérez Santangelo, M.P. Paronetto, M. de la Mata, F. Pelisch, S. Boireau, K. Glover-Cutter, C. Ben-Dov, M. Blaustein, J.J. Lozano, G. Bird, D. Bentley, E. Bertrand and A.R. Kornblihtt, DNA damage regulates alternative splicing through inhibition of RNA polymerase II elongation, *Cell* **137**, 2009, 708–720, http://dx.doi.org/10.1016/j.cell.2009.03.010.

[156] M. Dutertre, G. Sanchez, M.-C. De Cian, J. Barbier, E. Dardenne, L. Gratadou, G. Dujardin, C. Le Jossic-Corcos, L. Corcos and D. Auboeuf, Cotranscriptional exon skipping in the genotoxic stress response, *Nat. Struct. Mol. Biol.* **17**, 2010, 1358–1366, http://dx.doi.org/10.1038/nsmb.1912.

[157] M.P. Paronetto, B. Miñana and J. Valcárcel, The Ewing sarcoma protein regulates DNA damage-induced alternative splicing, *Mol. Cell.* **43**, 2011, 353–368, http://dx.doi.org/10.1016/j.molcel.2011.05.035.

[158] S. Kabacik, G. Manning, C. Raffy, S. Bouffler and C. Badie, Time, dose and ataxia telangiectasia mutated (ATM) status dependency of coding and noncoding RNA expression after ionizing radiation exposure, Radiat, *Res* .**183**, 2015, 325–337, http://dx.doi.org/10.1667/RR13876.1.

[159] H.B. Forrester, J. Li, T. Leong, M.J. McKay and C.N. Sprung, Identification of a radiation sensitivity gene expression profile in primary fibroblasts derived from patients who developed radiotherapy-induced fibrosis, *Radiother. Oncol. J. Eur. Soc. Ther. Radiol. Oncol.* **111**, 2014, 186–193, http://dx.doi.org/10.1016/j.radonc.2014.03.007.

[160] S. Marcoux, O.N.L. Le, C. Langlois-Pelletier, C. Laverdière, A. Hatami, P. Robaey and C.M. Beauséjour, Expression of the senescence marker p16INK4a in skin biopsies of acute lymphoblastic leukemia survivors: a pilot study, *Radiat. Oncol. Lond. Engl.* **8**, 2013, 252, http://dx.doi.org/10.1186/1748-717x-8-252.

[161] M. Abend, T. Azizova, K. Müller, H. Dörr, S. Doucha-Senf, H. Kreppel, G. Rusinova, I. Glazkova, N. Vyazovskaya, K. Unger, H. Braselmann and V. Meineke, Association of radiation-induced genes with noncancer chronic diseases in Mayak workers occupationally exposed to prolonged radiation, *Radiat. Res.* **183**, 2015, 249–261, http://dx.doi.org/10.1667/RR13758.1.

[162] A.L. Fachin, S.S. Mello, P. Sandrin-Garcia, C.M. Junta, T. Ghilardi-Netto, E.A. Donadi, G.A. da, S. Passos and E.T. Sakamoto-Hojo, Gene expression profiles in radiation workers occupationally exposed to ionizing radiation, *J. Radiat. Res. (Tokyo)* **50**, 2009, 61–71.

[163] E. Morandi, C. Severini, D. Quercioli, S. Perdichizzi, M.G. Mascolo, W. Horn, M. Vaccari, M.C. Nucci, V. Lodi, F.S. Violante, C. Bolognesi, S. Grilli, P. Silingardi and A. Colacci, Gene expression changes in medical workers exposed to radiation, *Radiat. Res.* **172**, 2009, 500–508, http://dx.doi.org/10.1667/RR1545.1.

[164] M. Abend, R.M. Pfeiffer, C. Ruf, M. Hatch, T.I. Bogdanova, M.D. Tronko, J. Hartmann, V. Meineke, K. Mabuchi and A.V. Brenner, Iodine-131 dose-dependent gene expression: alterations in both normal and tumour thyroid tissues of post-Chernobyl thyroid cancers, *Br. J. Cancer* **109**, 2013, 2286–2294, http://dx.doi.org/10.1038/bjc.2013.574.

[165] G. Dom, M. Tarabichi, K. Unger, G. Thomas, M. Oczko-Wojciechowska, T. Bogdanova, B. Jarzab, J.E. Dumont, V. Detours and C. Maenhaut, A gene expression signature distinguishes normal tissues of sporadic and radiation-induced papillary thyroid carcinomas, *Br. J. Cancer* **107**, 2012, 994–1000, http://dx.doi.org/10.1038/bjc.2012.302.

[166] H.L. Chaze, Thibault Chambon Christophe, Haddad Iman, Vinh Joelle, Peyrat Jean-Philippe, Benderitter Marc and Guipaud Olivier, Serum Proteome Analysis for Profiling Predictive Protein Markers Associated with the Severity of Skin Lesions Induced by Ionizing Rproteome analysis for profiling predictive protein markers associated with the severity of skin lesions induced by ionizing radiation, *Proteomes* **1**, 2013, 40–69, http://dx.doi.org/10.3390/proteomes1020040.

[167] M. Deperas-Kaminska, A. Bajinskis, M. Marczyk, J. Polanska, P. Wersall, E. Lidbrink, E.A. Ainsbury, O. Guipaud, M. Benderitter, S. Haghdoost and A. Wojcik, Radiation-induced changes in levels of selected proteins in peripheral blood serum of breast cancer patients as a potential triage biodosimeter for large-scale radiological emergencies, *Health Phys.* **107**, 2014, 555–563, http://dx.doi.org/10.1097/hp.0000000000000158.

[168] M. Sproull, T. Kramp, A. Tandle, U. Shankavaram and K. Camphausen, Serum Amyloid A as a Biomarker for Radiation Eamyloid a as a biomarker for radiation exposure, *Radiat. Res.* **184**, 2015, 14–23, http://dx.doi.org/10.1667/rr13927.1.

[169] S. Skiöld, O. Azimzadeh, J. Merl-Pham, I. Naslund, P. Wersall, E. Lidbrink, S. Tapio, M. Harms-Ringdahl and S. Haghdoost, Unique proteomic signature for radiation sensitive patients; a comparative study

between normo-sensitive and radiation sensitive breast cancer patients, *Mutat. Res.* **776**, 2015, 128–135, http://dx.doi.org/10.1016/j.mrfmmm.2014.12.002.

[170] G. Csárdi, A. Franks, D.S. Choi, E.M. Airoldi and D.A. Drummond, Accounting for experimental noise reveals that mRNA levels, amplified by post-transcriptional processes, largely determine steady-state protein levels in yeast, *PLoS Genet.* **11**, 2015, e1005206, http://dx.doi.org/10.1371/journal.pgen.1005206.

[171] T. Maier, M. Güell and L. Serrano, Correlation of mRNA and protein in complex biological samples, *FEBS Lett.* **583**, 2009, 3966–3973, http://dx.doi.org/10.1016/j.febslet.2009.10.036.

[172] C. Vogel and E.M. Marcotte, Insights into the regulation of protein abundance from proteomic and transcriptomic analyses, *Nat. Rev. Genet.* **13**, 2012, 227–232, http://dx.doi.org/10.1038/nrg3185.

[173] D. Greenbaum, C. Colangelo, K. Williams and M. Gerstein, Comparing protein abundance and mRNA expression levels on a genomic scale, *Genom. Biol.* **4**, 2003, 117, http://dx.doi.org/10.1186/gb-2003-4-9-117.

[174] A. Koussounadis, S.P. Langdon, I.H. Um, D.J. Harrison and V.A. Smith, Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system, *Sci. Rep.* **5**, 2015, 10775, http://dx.doi.org/10.1038/srep10775.

[175] W.H. Lee, P. Nguyen, S. Hu, G. Liang, S.-G. Ong, L. Han, V. Sanchez-Freire, A.S. Lee, M. Vasanawala, G. Segall and J.C. Wu, Variable activation of the DNA damage response pathways in patients undergoing single-photon emission computed tomography myocardial perfusion imaging, *Circ. Cardiovasc. Imaging* **8**, 2015, e002851, http://dx.doi.org/10.1161/CIRCIMAGING.114.002851.

[176] Z. Barjaktarovic, N. Anastasov, O. Azimzadeh, A. Sriharshan, H. Sarioglu, M. Ueffing, H. Tammio, A. Hakanen, D. Leszczynski, M.J. Atkinson and S. Tapio, Integrative proteomic and microRNA analysis of primary human coronary artery endothelial cells exposed to low-dose gamma radiation, *Radiat. Environ. Biophys.* **52**, 2013, 87–98, http://dx.doi.org/10.1007/s00411-012-0439-4.

[177] S.J. Kempf, S. Buratovic, C. von Toerne, S. Moertl, B. Stenerlöw, S.M. Hauck, M.J. Atkinson, P. Eriksson and S. Tapio, Ionising radiation immediately impairs synaptic plasticity-associated cytoskeletal signalling pathways in HT22 cells and in mouse brain: an in vitro/in vivo comparison study, *PLoS One* **9**, 2014, e110464, http://dx.doi.org/10.1371/journal.pone.0110464.

[178] S.J. Kempf, A. Casciati, S. Buratovic, D. Janik, C. von Toerne, M. Ueffing, F. Neff, S. Moertl, B. Stenerlöw, A. Saran, M.J. Atkinson, P. Eriksson, S. Pazzaglia and S. Tapio, The cognitive defects of neonatally irradiated mice are accompanied by changed synaptic plasticity, adult neurogenesis and neuroinflammation, *Mol. Neurodegener.* **9**, 2014, 57, http://dx.doi.org/10.1186/1750-1326-9-57.

[179] S.C. Tilton, M.M. Matzke, M.B. Sowa, D.L. Stenoien, T.J. Weber, W.F. Morgan and K.M. Waters, Data integration reveals key homeostatic mechanisms following low dose radiation exposure, *Toxicol. Appl. Pharmacol.* **285**, 2015, 1–11, http://dx.doi.org/10.1016/j.taap.2015.01.019.

[180] J.S. Brown, N. Lukashchuk, M. Sczaniecka-Clift, S. Britton, C. le Sage, P. Calsou, P. Beli, Y. Galanty and S.P. Jackson, Neddylation promotes ubiquitylation and release of Ku from DNA-damage sites, *Cell Rep.* **11**, 2015, 704–714, http://dx.doi.org/10.1016/j.celrep.2015.03.058.

[181] C. Oberle and C. Blattner, Regulation of the DNA ~~Damage Response to DSBs by Post-Translational M~~damage response to DSBs by post-Translational modifications, *Curr. Genom.* **11**, 2010, 184–198, http://dx.doi.org/10.2174/138920210791110979.

[182] O. Azimzadeh, M.J. Atkinson and S. Tapio, Proteomics in radiation research: present status and future perspectives, *Radiat. Env. Biophys.* **53**, 2014, 31–38, http://dx.doi.org/10.1007/s00411-013-0495-4.

[183] P. Beli, N. Lukashchuk, S.A. Wagner, B.T. Weinert, J.V. Olsen, L. Baskcomb, M. Mann, S.P. Jackson and C. Choudhary, Proteomic investigations reveal a role for RNA processing factor THRAP3 in the DNA damage response, *Mol. Cell.* **46**, 2012, 212–225, http://dx.doi.org/10.1016/j.molcel.2012.01.026.

[184] L.K. Povlsen, P. Beli, S.A. Wagner, S.L. Poulsen, K.B. Sylvestersen, J.W. Poulsen, M.L. Nielsen, S. Bekker-Jensen, N. Mailand and C. Choudhary, Systems-wide analysis of ubiquitylation dynamics reveals a key role for PAF15 ubiquitylation in DNA-damage bypass, *Nat. Cell Biol.* **14**, 2012, 1089–1098, http://dx.doi.org/10.1038/ncb2579.

[185] F. Yang, K.M. Waters, B.J. Webb-Robertson, M.B. Sowa, C. von Neubeck, J.T. Aldrich, L.M. Markillie, R.M. Wirgau, M.A. Gritsenko, R. Zhao, D.G. Camp 2nd, R.D. Smith and D.L. Stenoien, Quantitative phosphoproteomics identifies filaggrin and other targets of ionizing radiation in a human skin model, *Exp. Dermatol.* **21**, 2012, 352–357, http://dx.doi.org/10.1111/j.1600-0625.2012.01470.x.

[186] B. Maroschik, A. Gurtler, A. Kramer, U. Rossler, M. Gomolka, S. Hornhardt, S. Mortl and A.A. Friedl, Radiation-induced alterations of histone post-translational modification levels in lymphoblastoid cell lines, *Radiat. Oncol.* **9**, 2014, 15, http://dx.doi.org/10.1186/1748-717x-9-15.

[187] P. Beli and S.P. Jackson, Ubiquitin regulates dissociation of DNA repair factors from chromatin, *Oncotarget* **6**, 2015, 14727–14728.

[188] A.E. Elia, A.P. Boardman, D.C. Wang, E.L. Huttlin, R.A. Everley, N. Dephoure, C. Zhou, I. Koren, S.P. Gygi and S.J. Elledge, Quantitative ~~Proteomic Atlas of Ubiquitination and Acetylation in the DNA Damage R~~proteomi atlas of ubiquitination and acetylation in the DNA damage response, *Mol. Cell* 2015, http://dx.doi.org/10.1016/j.molcel.2015.05.006.

[189] S.P. Jackson and D. Durocher, Regulation of DNA damage responses by ubiquitin and SUMO, *Mol. Cell.* **49**, 2013, 795–807, http://dx.doi.org/10.1016/j.molcel.2013.01.017.

[190] J.E. Wilusz, H. Sunwoo and D.L. Spector, Long noncoding RNAs: functional surprises from the RNA world, *Genes Dev.* **23**, 2009, 1494–1504, http://dx.doi.org/10.1101/gad.1800909.

[191] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, *Cell* **116**, 2004, 281–297.

[192] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, *Cell* **136**, 2009, 215–233, http://dx.doi.org/10.1016/j.cell.2009.01.002.

[193] U. Aypar, W.F. Morgan and J.E. Baulch, Radiation-induced epigenetic alterations after low and high LET irradiations, *Mutat. Res.* **707**, 2011, 24–33, http://dx.doi.org/10.1016/j.mrfmmm.2010.12.003.

[194] A. Kraemer, N. Anastasov, M. Angermeier, K. Winkler, M.J. Atkinson and S. Moertl, MicroRNA-mediated processes are essential for the cellular radiation response, *Radiat. Res.* **176**, 2011, 575–586.

[195] N. Anastasov, I. Hofig, I.G. Vasconcellos, K. Rappl, H. Braselmann, N. Ludyga, G. Auer, M. Aubele and M.J. Atkinson, Radiation resistance due to high expression of miR-21 and G2/M checkpoint arrest in breast cancer cells, *Radiat. Oncol.* **7**, 2012, 206, http://dx.doi.org/10.1186/1748-717x-7-206.

[196] I. Summerer, M. Niyazi, K. Unger, A. Pitea, V. Zangen, J. Hess, M.J. Atkinson, C. Belka, S. Moertl and H. Zitzelsberger, Changes in circulating microRNAs after radiochemotherapy in head and neck cancer patients, *Radiat. Oncol.* **8**, 2013, 296, http://dx.doi.org/10.1186/1748-717x-8-296.

[197] I. Summerer, K. Unger, H. Braselmann, L. Schuettrumpf, C. Maihoefer, P. Baumeister, T. Kirchner, M. Niyazi, E. Sage, H.M. Specht, G. Multhoff, S. Moertl, C. Belka and H. Zitzelsberger, Circulating microRNAs as prognostic therapy biomarkers in head and neck cancer patients, *Br. J. Cancer* ~~.~~**113**, 2015, 76–82, http://dx.doi.org/10.1038/bjc.2015.111.

[198] L. Beer, R. Seemann, R. Ristl, A. Ellinger, M.M. Kasiri, A. Mitterbauer, M. Zimmermann, C. Gabriel, M. Gyöngyösi, W. Klepetko, M. Mildner and H.J. Ankersmit, High dose ionizing radiation regulates micro RNA and gene expression changes in human peripheral blood mononuclear cells, *BMC Genom.* **15**, 2014, 814, http://dx.doi.org/10.1186/1471-2164-15-814.

[199] C.Z. Zhang and G. Peng, Non-coding RNAs: ~~An emerging player in DNA damage responseMutat. Res. –~~an emerging player in DNA damage response, *Mutat. Res. –* *Rev. Mutat. Res.* **763**, 2015, 202–211, http://dx.doi.org/10.1016/j.mrrev.2014.11.003.

[200] J.A. Weber, D.H. Baxter, S. Zhang, D.Y. Huang, K.H. Huang, M.J. Lee, D.J. Galas and K. Wang, The microRNA spectrum in 12 body fluids, *Clin. Chem.* **56**, 2010, 1733–1741, http://dx.doi.org/10.1373/clinchem.2010.147405.

[201] Y. Tay, L. Kats, L. Salmena, D. Weiss, S.M. Tan, U. Ala, F. Karreth, L. Poliseno, P. Provero, F. Di Cunto, J. Lieberman, I. Rigoutsos and P.P. Pandolfi, Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs, *Cell* **147**, 2016, 344–357, http://dx.doi.org/10.1016/j.cell.2011.09.029.

[202] T. Hung and H.Y. Chang, Long noncoding RNA in genome regulation: prospects and mechanisms, *RNA Biol.* **7**, 2010, 582–585.

[203] V.B. O'Leary, S.V. Ovsepian, L.G. Carrascosa, F.A. Buske, V. Radulovic, M. Niyazi, S. Moertl, M. Trau, M.J. Atkinson and N. Anastasov, PARTICLE, a ~~Triplex-Forming Long ncRNA, Regulates Locus-Specific Methylation in Response to Low-Dose I~~triplex-Forming long ncRNA, regulates locus-Specific methylation in response to low-Dose irradiation, *Cell Rep.* **11**, 2015, 474–485, http://dx.doi.org/10.1016/j.celrep.2015.03.043.

[204] M. Fernandez-Mercado, L. Manterola, E. Larrea, I. Goicoechea, M. Arestin, M. Armesto, D. Otaegui and C.H. Lawrie, The circulating transcriptome as a source of non-invasive cancer biomarkers: concepts and controversies of non-coding and coding RNA in body fluids, *J. Cell. Mol. Med.* 2015, http://dx.doi.org/10.1111/jcmm.12625.

[205] M. Shao, X. Lu, W. Cong, X. Xing, Y. Tan, Y. Li, X. Li, L. Jin, X. Wang, J. Dong, S. Jin, C. Zhang and L. Cai, Multiple low-dose radiation prevents type 2 diabetes-induced renal damage through attenuation of dyslipidemia and insulin resistance and subsequent renal inflammation and oxidative stress, *PLoS One* **9**, 2014, e92574, http://dx.doi.org/10.1371/journal.pone.0092574.

[206] C. Zhang, X. Xing, F. Zhang, M. Shao, S. Jin, H. Yang, G. Wang, J. Cui, L. Cai, W. Li and X. Lu, Low-dose radiation induces renal SOD1 expression and activity in type 1 diabetic mice, *Int. J. Radiat. Biol.* **90**, 2014,

224–230, http://dx.doi.org/10.3109/09553002.2014.877174.

[207] F. Nicolas, C. Wu, S. Bukhari, S.M. de Toledo, H. Li, M. Shibata and E.I. Azzam, S-Nitrosylation in ~~Organs of Mice Exposed to Low or High Doses of γ Rays: The Modulating Effect of Iodine Contrast Agent at a Low Radiation D~~organs of mice exposed to low or high doses of γ-Rays: the modulating effect of iodine contrast agent at a low radiation dose, *Proteomes* **3**, 2015, 56–73, http://dx.doi.org/10.3390/proteomes3020056.

[208] B.P. Bowen and T.R. Northen, Dealing with the unknown: metabolomics and metabolite atlases, *J. Am. Soc. Mass Spectrom.* **21**, 2010, 1471–1476, http://dx.doi.org/10.1016/j.jasms.2010.04.003.

[209] Y. Liu, R. Huang, L. Liu, J. Peng, B. Xiao, J. Yang, Z. Miao and H. Huang, Metabonomics study of urine from Sprague-Dawley rats exposed to Huang-yao-zi using (1)H NMR spectroscopy, *J. Pharm. Biomed. Anal.* **52**, 2010, 136–141, http://dx.doi.org/10.1016/j.jpba.2009.12.026.

[210] V.K. Singh, V.L. Newman, P.L. Romaine, M. Hauer-Jensen and H.B. Pollard, Use of biomarkers for assessing radiation injury and efficacy of countermeasures, *Expert Rev. Mol. Diagn.* **16**, 2016, 65–81, http://dx.doi.org/10.1586/14737159.2016.1121102.

[211] W.W. Kam and R.B. Banati, Effects of ionizing radiation on mitochondria, *Free Radic ~~Biol.~~ Biol. Med.* **65**, 2013, 607–619, http://dx.doi.org/10.1016/j.freeradbiomed.2013.07.024.

[212] O. Azimzadeh, H. Scherthan, H. Sarioglu, Z. Barjaktarovic, M. Conrad, A. Vogt, J. Calzada-Wack, F. Neff, M. Aubele, C. Buske, M.J. Atkinson and S. Tapio, Rapid proteomic remodeling of cardiac tissue caused by total body ionizing radiation, *Proteomics* **11**, 2011, 3299–3311, http://dx.doi.org/10.1002/pmic.201100178.

[213] Z. Barjaktarovic, D. Schmaltz, A. Shyla, O. Azimzadeh, S. Schulz, J. Haagen, W. Dorr, H. Sarioglu, A. Schafer, M.J. Atkinson, H. Zischka and S. Tapio, Radiation-induced signaling results in mitochondrial impairment in mouse heart at 4 weeks after exposure to X-rays, *PLoS One* **6**, 2011, e27811, http://dx.doi.org/10.1371/journal.pone.0027811.

[214] Z. Barjaktarovic, A. Shyla, O. Azimzadeh, S. Schulz, J. Haagen, W. Dörr, H. Sarioglu, M.J. Atkinson, H. Zischka and S. Tapio, Ionising radiation induces persistent alterations in the cardiac mitochondrial function of C57BL/6 mice 40 weeks after local heart exposure, *Radiother. Oncol. J. Eur. Soc. Ther. Radiol. Oncol.* **106**, 2013, 404–410, http://dx.doi.org/10.1016/j.radonc.2013.01.017.

[215] M. Goudarzi, T.D. Mak, C. Chen, L.B. Smilenov, D.J. Brenner and A.J. Fornace, The effect of low dose rate on metabolomic response to radiation in mice, *Radiat Env. Biophys.* **53**, 2014, 645–657, http://dx.doi.org/10.1007/s00411-014-0558-1.

[216] M. Goudarzi, W. Weber, T.D. Mak, J. Chung, M. Doyle-Eisele, D. Melo, D.J. Brenner, R.A. Guilmette and A.J. Fornace, Development of urinary biomarkers for internal exposure by cesium-137 using a metabolomics approach in mice, *Radiat. Res.* **181**, 2014, 54–64, http://dx.doi.org/10.1667/rr13479.1.

[217] M. Goudarzi, W.M. Weber, T.D. Mak, J. Chung, M. Doyle-Eisele, D.R. Melo, S.J. Strawn, D.J. Brenner, R.A. Guilmette and A.J. Fornace, Jr., A ~~Comprehensive Metabolomic Investigation in Urine of Mice Exposed to S~~comprehensive metabolomic investigation in urine of mice exposed to strontium-90, *Radiat. Res.* **183**, 2015, 665–674, http://dx.doi.org/10.1667/rr14011.1.

[218] E.C. Laiakis, D. Trani, B.H. Moon, S.J. Strawn and A.J. Fornace, Jr., Metabolomic profiling of urine samples from mice exposed to protons reveals radiation quality and dose specific differences, *Radiat. Res.* **183**, 2015, 382–390, http://dx.doi.org/10.1667/rr3967.1.

[219] C.H. Johnson, A.D. Patterson, K.W. Krausz, J.F. Kalinich, J.B. Tyburski, D.W. Kang, H. Luecke, F.J. Gonzalez, W.F. Blakely and J.R. Idle, Radiation metabolomics. 5. Identification of urinary biomarkers of ionizing radiation exposure in nonhuman primates by mass spectrometry-based metabolomics, *Radiat. Res.* **178**, 2012, 328–340.

[220] E.L. Pannkuk, E.C. Laiakis, S. Authier, K. Wong and A.J. Fornace, Jr., Global ~~Metabolomic Identification of Long-Term Dose-Dependent Urinary Biomarkers in Nonhuman Primates Exposed to Ionizing R~~metabolomic identification of long-Term dose-Dependent urinary biomarkers in nonhuman primates exposed to ionizing radiation, *Radiat. Res.* **184**, 2015, 121–133.

[221] E.C. Laiakis, T.D. Mak, S. Anizan, S.A. Amundson, C.A. Barker, S.L. Wolden, D.J. Brenner and A.J. Fornace, Jr., Development of a metabolomic radiation signature in urine from patients undergoing total body irradiation, *Radiat. Res.* **181**, 2014, 350–361, http://dx.doi.org/10.1667/rr13567.1.

[222] A.R. Khan, P. Rana, M.M. Devi, S. Chaturvedi, S. Javed, R.P. Tripathi and S. Khushu, Nuclear magnetic resonance spectroscopy-based metabonomic investigation of biochemical effects in serum of gamma-irradiated mice, *Int. J. Radiat. Biol.* **87**, 2011, 91–97, http://dx.doi.org/10.3109/09553002.2010.518211.

[223] E.C. Laiakis, K. Strassburg, R. Bogumil, S. Lai, R.J. Vreeken, T. Hankemeier, J. Langridge, R.S. Plumb, A.J. Fornace, Jr. and G. Astarita, Metabolic phenotyping reveals a lipid mediator response to ionizing

radiation, *J. Proteome Res.* **13**, 2014, 4143–4154, http://dx.doi.org/10.1021/pr5005295.

[224] M. Goudarzi, W.M. Weber, T.D. Mak, J. Chung, M. Doyle-Eisele, D.R. Melo, D.J. Brenner, R.A. Guilmette and A.J. Fornace Jr, Metabolomic and lipidomic analysis of serum from mice exposed to an internal emitter, cesium-137, using a shotgun LC-MS(E) approach, *J. Proteome Res.* **14**, 2015, 374–384, http://dx.doi.org/10.1021/pr500913n.

[225] H. Liu, Z. Wang, X. Zhang, Y. Qiao, S. Wu, F. Dong and Y. Chen, Selection of candidate radiation biomarkers in the serum of rats exposed to gamma-rays by GC/TOFMS-based metabolomics, *Radiat. Prot. Dosim.* **154**, 2013, 9–17, http://dx.doi.org/10.1093/rpd/ncs138.

[226] Z.P. Hu, Y.M. Kim, M.B. Sowa, R.J. Robinson, X. Gao, T.O. Metz, W.F. Morgan and Q. Zhang, Metabolomic response of human skin tissue to low dose ionizing radiation, *Mol. Biosyst.* **8**, 2012, 1979–1986, http://dx.doi.org/10.1039/c2mb25061f.

[227] N. Tsuyama, H. Mizuno, A. Katafuchi, Y. Abe, Y. Kurosu, M. Yoshida, K. Kamiya and A. Sakai, Identification of low-dose responsive metabolites in X-irradiated human B lymphoblastoid cells and fibroblasts, *J. Radiat. Res.* **56**, 2015, 46–58, http://dx.doi.org/10.1093/jrr/rru078.

[228] H.H. Li, Y.W. Wang, R. Chen, B. Zhou, J.D. Ashwell and A.J. Fornace Jr, Ionizing ~~Radiation Impairs T Cell Activation by Affecting Metabolic R~~radiation impairs t cell activation by affecting metabolic reprogramming, *Int. J. Biol. Sci.* **11**, 2015, 726–736, http://dx.doi.org/10.7150/ijbs.12009.

[229] Y. Kusunoki and T. Hayashi, Long-lasting alterations of the immune system by ionizing radiation exposure: implications for disease development among atomic bomb survivors, *Int. J. Radiat. Biol.* **84**, 2008, 1–14, http://dx.doi.org/10.1080/09553000701616106.

[230] X. He, S.G. Swarts, E. Demidenko, A.B. Flood, O. Grinberg, J. Gui, M. Mariani, S.D. Marsh, A.E. Ruuge, J.W. Sidabras, D. Tipikin, D.E. Wilcox and H.M. Swartz, Development and validation of an ex vivo electron paramagnetic resonance fingernail biodosimetric method, *Radiat. Prot. Dosimetry* **159**, 2014, 172–181, http://dx.doi.org/10.1093/rpd/ncu129.

[231] F. Trompier, A. Romanyukha, R. Reyes, H. Vezin, F. Queinnec and D. Gourier, State of the art in nail dosimetry: free radicals identification and reaction mechanisms, *Radiat. Environ. Biophys.* **53**, 2014, 291–303, http://dx.doi.org/10.1007/s00411-014-0512-2.

[232] A. Wieser, E. Vasilenko, E. Aladova, P. Fattibene, N. Semiochkina and M. Smetanin, Electron paramagnetic resonance measurements of absorbed dose in teeth from citizens of Ozyorsk, *Radiat. Environ. Biophys.* **53**, 2014, 321–333, http://dx.doi.org/10.1007/s00411-014-0527-8.

[233] V. Chumak, S.V. Sholom and L. Pasalskaya, Application of high precision EPR dosimetry for reconstruction of doses to Chernobyl populations, *Radiat. Prot. Dosim.* **84**, 1999, 515–520.

[234] V.V. Chumak, S.V. Sholom, E.V. Bakhanova, L.F. Pasalskaya and A.V. Musijachenko, High precision of EPR dosimetry as a reference tool for validation of other techniques, *Appl. Radiat. Isot.* **62**, 2005, 141–146.

[235] M.O. Degteva, N.B. Shagina, E.A. Shishkina, A.V. Vozilova, A.Y. Volchkova, M.I. Vorobiova, A. Wieser, P. Fattibene, S. Della Monaca, E. Ainsbury, J. Moquet, L.R. Anspaugh and B.A. Napier, Analysis of EPR and FISH studies of radiation doses in persons who lived in the upper reaches of the Techa River, *Radiat. Environ. Biophys.* 2015, http://dx.doi.org/10.1007/s00411-015-0611-8.

[236] P. Fattibene, F. Trompier, A. Wieser, M. Brai, B. Ciesielski, C. De Angelis, S. Della Monaca, T. Garcia, H. Gustafsson, E.O. Hole, M. Juniewicz, K. Krefft, A. Longo, P. Leveque, E. Lund, M. Marrale, B. Michalec, G. Mierzwińska, J.L. Rao, A.A. Romanyukha and H. Tuner, EPR dosimetry intercomparison using smart phone touch screen glass, *Radiat. Environ. Biophys.* **53**, 2014, 311–320, http://dx.doi.org/10.1007/s00411-014-0533-x.

[237] D. Pang, A.R. Thierry and A. Dritschilo, DNA studies using atomic force microscopy: capabilities for measurement of short DNA fragments, *Front. Mol. Biosci.* **2**, 2015, 1, http://dx.doi.org/10.3389/fmolb.2015.00001.

[238] A. Borghini, A. Mercuri, S. Turchi, M.R. Chiesa, E. Piccaluga and M.G. Andreassi, Increased circulating cell-free DNA levels and mtDNA fragments in interventional cardiologists occupationally exposed to low levels of ionizing radiation, *Environ. Mol. Mutagen.* **56**, 2015, 293–300, http://dx.doi.org/10.1002/em.21917.

[239] I.B. Korzeneva, S.V. Kostuyk, L.S. Ershova, A.N. Osipov, V.F. Zhuravleva, G.V. Pankratova, L.N. Porokhovnik and N.N. Veiko, Human circulating plasma DNA significantly decreases while lymphocyte DNA damage increases under chronic occupational exposure to low-dose gamma-neutron and tritium β-radiation, *Mutat. Res.* **779**, 2015, 1–15, http://dx.doi.org/10.1016/j.mrfmmm.2015.05.004.

[240] Z.D. Nagel, I.A. Chaim and L.D. Samson, Inter-individual variation in DNA repair capacity: a need for multi-pathway functional assays to promote translational DNA repair research, *DNA Repair* **19**, 2014, 199–213, http://dx.doi.org/10.1016/j.dnarep.2014.03.009.

[241] C. Ersson, P. Møller, L. Forchhammer, S. Loft, A. Azqueta, R.W.L. Godschalk, F.-J. van Schooten, G.D.D. Jones, J.A. Higgins, M.S. Cooke, V. Mistry, M. Karbaschi, D.H. Phillips, O. Sozeri, M.N. Routledge, K. Nelson Smith, P. Riso, M. Porrini, G. Matullo, A. Allione, M. Stepnik, M. Ferlińska, J.P. Teixeira, S. Costa, L.-A. Corcuera, A. López de Cerain, B. Laffon, V. Valdiglesias, A.R. Collins and L. Möller, An ECVAG inter-laboratory validation study of the comet assay: inter-laboratory and intra-laboratory variations of DNA strand breaks and FPG-sensitive sites in human mononuclear cells, *Mutagenesis* **28**, 2013, 279–286, http://dx.doi.org/10.1093/mutage/get001.

[242] D.K. Wood, D.M. Weingeist, S.N. Bhatia and B.P. Engelward, Single cell trapping and DNA damage analysis using microwell arrays, *Proc. Natl. Acad. Sci. U. S. A.* **107**, 2010, 10008–10013, http://dx.doi.org/10.1073/pnas.1004056107.

[243] J. Ge, S. Prasongtanakij, D.K. Wood, D.M. Weingeist, J. Fessler, P. Navasummrit, M. Ruchirawat and B.P. Engelward, CometChip: a high-throughput 96-well platform for measuring DNA damage in microarrayed human cells, *J. Vis. Exp.* 2014, e50607, http://dx.doi.org/10.3791/50607.

[244] J. Ge, D.N. Chow, J.L. Fessler, D.M. Weingeist, D.K. Wood and B.P. Engelward, Micropatterned comet assay enables high throughput and sensitive DNA damage quantification, *Mutagenesis* **30**, 2015, 11–19, http://dx.doi.org/10.1093/mutage/geu063.

[245] D.B. Rosen, L.Y. Leung, B. Louie, J.A. Cordeiro, A. Conroy, I. Shapira, S.Z. Fields, A. Cesano and R.E. Hawtin, Quantitative measurement of alterations in DNA damage repair (DDR) pathways using single cell network profiling (SCNP), *J. Transl. Med.* **12**, 2014, 184, http://dx.doi.org/10.1186/1479-5876-12-184.

[246] C. Li, L.-E. Wang and Q. Wei, DNA repair phenotype and cancer susceptibility-a mini review, *Int. J. Cancer J. Int. Cancer* **124**, 2009, 999–1007, http://dx.doi.org/10.1002/ijc.24126.

[247] A. Forestier, F. Sarrazy, S. Caillat, Y. Vandenbrouck and S. Sauvaigo, Functional DNA repair signature of cancer cell lines exposed to a set of cytotoxic anticancer drugs using a multiplexed enzymatic repair assay on biochip, *PLoS One* **7**, 2012, e51754, http://dx.doi.org/10.1371/journal.pone.0051754.

[248] F.M. Yakes and B. Van Houten, Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress, *Proc. Natl. Acad. Sci. U. S. A.* **94**, 1997, 514–519.

[249] J.F. Turrens, Mitochondrial formation of reactive oxygen species, *J. Physiol.* **552**, 2003, 335–344, http://dx.doi.org/10.1113/jphysiol.2003.049478.

[250] D.B. Zorov, M. Juhaszova and S.J. Sollott, Mitochondrial ROS-induced ROS release: an update and review, *Biochim. Biophys. Acta.* **1757**, 2006, 509–517, http://dx.doi.org/10.1016/j.bbabio.2006.04.029.

[251] M.W. van Gisbergen, A.M. Voets, M.H.W. Starmans, I.F.M. de Coo, R. Yadak, R.F. Hoffmann, P.C. Boutros, H.J.M. Smeets, L. Dubois and P. Lambin, How do changes in the mtDNA and mitochondrial dysfunction influence cancer and cancer therapy? Challenges, opportunities and models, *Mutat. Res. Rev. Mutat. Res.* **764**, 2015, 16–30, http://dx.doi.org/10.1016/j.mrrev.2015.01.001.

[252] G.J. Kim, K. Chandrasekaran and W.F. Morgan, Mitochondrial dysfunction, persistently elevated levels of reactive oxygen species and radiation-induced genomic instability: a review, *Mutagenesis* **21**, 2006, 361–367, http://dx.doi.org/10.1093/mutage/gel048.

[253] J.W. Kim and C.V. Dang, Cancer's molecular sweet tooth and the Warburg effect, *Cancer Res.* **66**, 2006, 8927–8930, http://dx.doi.org/10.1158/0008-5472.CAN-06-1501.

[254] S.M. Nugent, C.E. Mothersill, C. Seymour, B. McClean, F.M. Lyng and J.E. Murphy, Increased mitochondrial mass in cells with functionally compromised mitochondria after exposure to both direct gamma radiation and bystander factors, *Radiat. Res.* **168**, 2007, 134–142, http://dx.doi.org/10.1667/RR0769.1.

[255] J.M. Shoffner, M.T. Lott, A.S. Voljavec, S.A. Soueidan, D.A. Costigan and D.C. Wallace, Spontaneous Kearns-Sayre/chronic external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy, *Proc. Natl. Acad. Sci. U. S. A.* **86**, 1989, 7952–7956.

[256] G.A. Cortopassi, D. Shibata, N.W. Soong and N. Arnheim, A pattern of accumulation of a somatic deletion of mitochondrial DNA in aging human tissues, *Proc. Natl. Acad. Sci. U. S. A.* **89**, 1992, 7370–7374.

[257] B. Schilling-Tóth, N. Sándor, E. Kis, M. Kadhim, G. Sáfrány and H. Hegyesi, Analysis of the common deletions in the mitochondrial DNA is a sensitive biomarker detecting direct and non-targeted cellular effects

of low dose ionizing radiation, *Mutat. Res.* **716**, 2011, 33–39, http://dx.doi.org/10.1016/j.mrfmmm.2011.07.018.

[258] M. Obara-Moszynska, J. Maceluch, W. Bobkowski, A. Baszko, O. Jaremba, M.R. Krawczynski and M. Niedziela, A novel mitochondrial DNA deletion in a patient with Kearns-Sayre syndrome: a late-onset of the fatal cardiac conduction deficit and cardiomyopathy accompanying long-term rGH treatment, *BMC Pediatr.* **13**, 2013, 27, http://dx.doi.org/10.1186/1471-2431-13-27.

[259] H. Reichmann, F. Degoul, R. Gold, B. Meurers, U.P. Ketelsen, J. Hartmann, C. Marsac and P. Lestienne, Histological, enzymatic and mitochondrial DNA studies in patients with Kearns-Sayre syndrome and chroni progressive external ophthalmoplegia, *Eur. Neurol.* **31**, 1991, 108–113.

[260] J.D. Boice, Jr., S.S. Cohen, M.T. Mumma, E.D. Ellis, D.L. Cragle, K.F. Eckerman, P.W. Wallace, B. Chadda, J.S. Sonderman, L.D. Wiggs, B.S. Richter and R.W. Leggett, Mortality among mound workers exposed to polonium-210 and other sources of radiation, 1944–1979, *Radiat. Res.* **181**, 2014, 208–228, http://dx.doi.org/10.1667/RR13395.1.

[261] S. Zollner, M.E. Sokolnikov and M. Eidemuller, Beyond two-stage models for lung carcinogenesis in the Mayak workers: implications for plutonium risk, *PLoS One* **10**, 2015, e0126238, http://dx.doi.org/10.1371/journal.pone.0126238.

[262] T.V. Azizova, G.V. Zhuntova, R.G. Haylock, M.B. Moseeva, E.S. Grigoryeva, N. Hunter, M.V. Bannikova, Z.D. Belyaeva and E. Bragin, Chronic bronchitis in the cohort of Mayak workers first employed 1948–1958, *Radiat. Res.* **180**, 2013, 610–621, http://dx.doi.org/10.1667/RR13228.1.

[263] T.V. Azizova, E.S. Grigoryeva, R.G. Haylock, M.V. Pikulina and M.B. Moseeva, Ischemic heart disease incidence and mortality in an extended cohort of Mayak workers first employed in 1948–1982, *Br. J. Radiol.* 2015.

[264] J. Grellier, W. Atkinson, P. Bérard, K. Binks, I.G. Canu, R. Cockerel, M.T. Do, H. Engels, D. MacGregor, E. Samson, J. Figuerola, D. Bingham, A. Birchall, E. Blanchardon, R. Bull, C. Chailleton-de Vathaire, A. Foster, L. Holmstock, C. Hurtgen, M. Puncher, A.E. Riddell, I. Thierrry-Chef, M. Tirmarche, E. Cardis, Risk of lung cancer mortality in nuclear workers from to internal exposure to a particle emitting radionuclides, (in preparation)

[265] V. Malard, J.C. Gaillard, F. Berenguer, N. Sage and E. Quemeneur, Urine proteomic profiling of uranium nephrotoxicity, *Biochim. Biophys. Acta* **1794**, 2009, 882–891, http://dx.doi.org/10.1016/j.bbapap.2009.01.010.

[266] S. Grison, J.C. Martin, L. Grandcolas, N. Banzet, E. Blanchardon, E. Tourlonias, C. Defoort, G. Fave, R. Bott, I. Dublineau, P. Gourmelon and M. Souidi, The metabolomic approach identifies a biological signature of low-dose chronic exposure to cesium 137, *J. Radiat. Res.* **53**, 2012, 33–43.

[267] S. Grison, G. Fave, M. Maillot, L. Manens, O. Delissen, E. Blanchardon, N. Banzet, C. Defoort, R. Bott, I. Dublineau, J. Aigueperse, P. Gourmelon, J.C. Martin and M. Souidi, Metabolomics identifies a biological response to chronic low-dose natural uranium contamination in urine samples, *Metabolomics* **9**, 2013, 1168–1180, http://dx.doi.org/10.1007/s11306-013-0544-7.

[268] F. Zölzer, Z. Hon, Z.F. Skalická, R. Havránková, L. Navrátil, J. Rosina and J. Škopek, Micronuclei in lymphocytes from currently active uranium miners, *Radiat. Environ. Biophys.* **51**, 2012, 277–282, http://dx.doi.org/10.1007/s00411-012-0422-0.

[269] M. Abend, T. Azizova, K. Muller, H. Dorr, S. Senf, H. Kreppel, G. Rusinova, I. Glazkova, N. Vyazovskaya, D. Schmidl, K. Unger and V. Meineke, Gene expression analysis in Mayak workers with prolonged occupational radiation exposure, *Health Phys.* **106**, 2014, 664–676, http://dx.doi.org/10.1097/HP.0000000000000018.

[270] M. Abend, T. Azizova, K. Muller, H. Dorr, S. Senf, H. Kreppel, G. Rusinova, I. Glazkova, N. Vyazovskaya, K. Unger and V. Meineke, Independent validation of candidate genes identified after a whole genome screening on Mayak workers exposed to prolonged occupational radiation, *Radiat. Res.* **182**, 2014, 299–309, http://dx.doi.org/10.1667/RR13645.1.

[271] M. Selmansberger, A. Feuchtinger, L. Zurnadzhy, A. Michna, J.C. Kaiser, M. Abend, A. Brenner, T. Bogdanova, A. Walch, K. Unger, H. Zitzelsberger and J. Hess, CLIP2 as radiation biomarker in papillary thyroid carcinoma, *Oncogene* **34**, 2015, 3917–3925, http://dx.doi.org/10.1038/onc.2014.311.

[272] M. Selmansberger, J.C. Kaiser, J. Hess, D. Güthlin, I. Likhtarev, V. Shpak, M. Tronko, A. Brenner, M. Abend, M. Blettner, K. Unger, P. Jacob and H. Zitzelsberger, Dose-dependent expression of CLIP2 in post-Chernobyl papillary thyroid carcinomas, *Carcinogenesis* **36**, 2015, 748–756, http://dx.doi.org/10.1093/carcin/bgv043.

[273] Y. Gueguen and C. Rouas, Données nouvelles sur la néphrotoxicité de l'uranium [New data on uranium nephrotoxicity], *Radioprotection* **47**, 2012, 345–359, http://dx.doi.org/10.1051/radiopro/2012015.

[274] P. Lestaevel, B. Dhieux, O. Delissen, M. Benderitter and J. Aigueperse, Uranium modifies or not behavior and antioxidant status in the hippocampus of rats exposed since birth, *J. Toxicol. Sci.* **40**, 2015, 99–107, http://dx.doi.org/10.2131/jts.40.99.

[275] P. Kurttio, A. Auvinen, L. Salonen, H. Saha, J. Pekkanen, I. Makelainen, S.B. Vaisanen, I.M. Penttila and H. Komulainen, Renal effects of uranium in drinking water, *Environ. Health Perspect.* **110**, 2002, 337–342.

[276] O. Prat, E. Ansoborlo, N. Sage, D. Cavadore, J. Lecoix, P. Kurttio and E. Quemeneur, From cell to man: evaluation of osteopontin as a possible biomarker of uranium exposure, *Environ. Int.* **37**, 2011, 657–662, http://dx.doi.org/10.1016/j.envint.2011.01.004.

[277] M.L. Zamora, J.M. Zielinski, G.B. Moodie, R.A. Falcomer, W.C. Hunt and K. Capello, Uranium in drinking water: renal effects of long-term ingestion by an aboriginal community, *Arch. Environ. Occup. Health* **64**, 2009, 228–241, http://dx.doi.org/10.1080/19338240903241267.

[278] Y. Gueguen, P. Lestaevel, L. Grandcolas, C. Baudelin, S. Grison, J.R. Jourdain, P. Gourmelon and M. Souidi, Chronic contamination of rats with 137 cesium radionuclide: impact on the cardiovascular system, *Cardiovasc. Toxicol.* **8**, 2008, 33–40, http://dx.doi.org/10.1007/s12012-008-9013-3.

[279] C. Le Gallic, Y. Phalente, L. Manens, I. Dublineau, M. Benderitter, Y. Gueguen, S. Lehoux and T.G. Ebrahimian, Chronic ~~Internal Exposure to Low Dose 137Cs Induces Positive Impact on the Stability of Atherosclerotic Plaques by Reducing Inflammation in ApoE-/- M~~internal exposure to low dose 137Cs induces positive impact on the stability of atherosclerotic plaques by reducing inflammation in apoE-/- mice, *PLoS One* **10**, 2015, e0128539, http://dx.doi.org/10.1371/journal.pone.0128539.

[280] X. Arzuaga, M. Gehlhaus and J. Strong, Modes of action associated with uranium induced adverse effects in bone function and development, *Toxicol. Lett.* **236**, 2015, 123–130, http://dx.doi.org/10.1016/j.toxlet.2015.05.006.

[281] H. Bensoussan, L. Grancolas, B. Dhieux-Lestaevel, O. Delissen, C.M. Vacher, I. Dublineau, P. Voisin, P. Gourmelon, M. Taouis and P. Lestaevel, Heavy metal uranium affects the brain cholinergic system in rat following sub-chronic and chronic exposure, *Toxicology* **261**, 2009, 59–67, http://dx.doi.org/10.1016/j.tox.2009.04.054.

[282] C. Bussy, P. Lestaevel, B. Dhieux, C. Amourette, F. Paquet, P. Gourmelon and P. Houpert, Chronic ingestion of uranyl nitrate perturbs acetylcholinesterase activity and monoamine metabolism in male rat brain, *Neurotoxicology* **27**, 2006, 245–252, http://dx.doi.org/10.1016/j.neuro.2005.11.003.

[283] L. Vicente-Vicente, Y. Quiros, F. Perez-Barriocanal, J.M. Lopez-Novoa, F.J. Lopez-Hernandez and A.I. Morales, Nephrotoxicity of uranium: pathophysiological, diagnostic and therapeutic perspectives, *Toxicol. Sci.* **118**, 2010, 324–347, http://dx.doi.org/10.1093/toxsci/kfq178.

[284] C. Poisson, J. Stefani, L. Manens, O. Delissen, D. Suhard, C. Tessier, I. Dublineau and Y. Gueguen, Chronic uranium exposure dose-dependently induces glutathione in rats without any nephrotoxicity, *Free Radic. Res.* **48**, 2014, 1218–1231, http://dx.doi.org/10.3109/10715762.2014.945441.

[285] HLEG, Report of High Level and Expert Group on European Low Dose Risk Reseach, 2009 www.hleg.de.

[286] HPA, Human Radiosensitivity Report of the ~~i~~Independent Advisory Group on Ionising Radiation, 2013, Health Protection Agency (HPA); Childton, Didcot, UK.

[287] O. Laurent, M. Gomolka, R. Haylock, E. Blanchardon, A. Giussani, W. Atkinson, S. Baatout, D. Bingham, E. Cardis, J. Hall, L. Tomasek, S. Ancelet, C. Badie, G. Bethel, J.-M. Bertho, R. Bull, C. Challeton De Vathaire, R. Cockerill, E. Davesne, T. Ebrahimian, H. Engels, M. Gillies, J. Grellier, S. Grison, Y. Gueguen, S. Hornhardt, C. Ibanez, S. Kabacik, L. Kotik, M. Kreuzer, A.L. Le Bacq, J. Marsh, D. Nosske, J. O'Hagan, E Pernot, M. Puncher, E. Rage, T. Riddell, L. Roy, E. Samson, M. Souidi, M. Turner, S. Zhivin and D. Laurier, Concerted Uranium Research in Europe (CURE): toward a collaborative project integrating dosimetry, epidemiology and radiobiology to study the effects of occupational uranium exposure, *J. Radiol. Prot.* 2017, ~~in press~~(in press).

[288] O. Laurent, M. Gomolka, R. Haylock, E. Blanchardon, A. Giussani, W. Atkinson, S. Baatout, D. Bingham, E. Cardis, J. Hall, L. Tomasek, S. Ancelet, C. Badie, G. Bethel, J.-M. Bertho, S. Bouet, R. Bull, C. Challeton-de Vathaire, R. Cockerill, E. Davesne, T. Ebrahimian, H. Engels, M. Gillies, J. Grellier, S. Grison, Y. Gueguen, S. Hornhardt, C. Ibanez, S. Kabacik, L. Kotik, M. Kreuzer, A.L. Lebacq, J. Marsh, D. Nosske, J. O'Hagan, E. Pernot, M. Puncher, E. Rage, T. Riddell, L. Roy, E. Samson, M. Souidi, M.C. Turner, S. Zhivin and D. Laurier, Concerted Uranium Research in Europe (CURE): toward a collaborative project integrating

dosimetry, epidemiology and radiobiology to study the effects of occupational uranium exposure, *J. Radiol. Prot. Off. J. Soc. Radiol. Prot.* **36**, 2016, 319–345, http://dx.doi.org/10.1088/0952-4746/36/2/319.

[289] Y. Guéguen, L. Roy, S. Hornhardt, C. Badie, J. Hall, S. Baatout, E. Pernot, L. Tomasek, O. Laurent, T. Ebrahimian, C. Ibanez, S. Grison, S. Kabacik, D. Laurier and M. Gomolka, Biomarkers for uranium risk assessment for the development of the CURE (Concerted Uranium Research in Europe) molecular epidemiological protocol, *Radiat. Res.* 2016, ~~in press, n.d.~~(in press, n.d.).

[290] A. Mezentsev and S.A. Amundson, Global gene expression responses to low- or high-dose radiation in a human three-dimensional tissue model, *Radiat. Res.* **175**, 2011, 677–688, http://dx.doi.org/10.1667/RR2483.1.

[291] A.J. Wyrobek, C.F. Manohar, V.V. Krishnan, D.O. Nelson, M.R. Furtado, M.S. Bhattacharya, F. Marchetti and M.A. Coleman, Low dose radiation response curves, networks and pathways in human lymphoblastoid cells exposed from 1 to 10cGy of acute gamma radiation, *Mutat. Res.* **722**, 2011, 119–130, http://dx.doi.org/10.1016/j.mrgentox.2011.03.002.

[292] A. Riecke, C.G. Rufa, M. Cordes, J. Hartmann, V. Meineke and M. Abend, Gene expression comparisons performed for biodosimetry purposes on in vitro peripheral blood cellular subsets and irradiated individuals, *Radiat. Res.* **178**, 2012, 234–243.

[293] R. Yunis, H. Albrecht, K.M. Kalanetra, S. Wu and D.M. Rocke, Genomic characterization of a three-dimensional skin model following exposure to ionizing radiation, *J. Radiat. Res. (Tokyo)* **53**, 2012, 860–875, http://dx.doi.org/10.1093/jrr/rrs063.

[294] H. El-Saghire, H. Thierens, P. Monsieurs, A. Michaux, C. Vandevoorde and S. Baatout, Gene set enrichment analysis highlights different gene expression profiles in whole blood samples X-irradiated with low and high doses, *Int. J. Radiat. Biol.* **89**, 2013, 628–638, http://dx.doi.org/10.3109/09553002.2013.782448.

[295] I. Nosel, A. Vaurijoux, J.-F. Barquinero and G. Gruel, Characterization of gene expression profiles at low and very low doses of ionizing radiation, *DNA Repair* **12**, 2013, 508–517, http://dx.doi.org/10.1016/j.dnarep.2013.04.021.

Footnotes

1Abbreviations: ^{3}H: tritium; 8-oxodG: 8-oxo-7,8-dihydro-2′-deoxyguanosine; AT: Ataxia telangiectasia; ATM: Ataxia telangiectasia mutated; ATR: Ataxia telangiectasia and Rad3 related; BER; base excision repair; bp; base pair; CBMN: cytokinesis block micronuclei; ccfDNA: Cell-free circulating DNA; CNV: copy number variant; Cs: cesium; CT: computerized tomography; CURE: Concerted Action for an Integrated (biology-dosimetry epidemiology) Research project on Occupational Uranium; CV; coefficient of variation; CVD: cardiovascular diseases; DC: dicentric; DDR: DNA damage response; DRC: DNA repair capacity; DNA: deoxyribonucleic acid dNTP: deoxyribonucleotide triphosphate; DoReMi: European project towards Low Dose Research towards Multidisciplinary Integration; DSB: double strand break; EPI-CT: Epidemiological study to quantify risks for paediatric computerized tomography and to optimise doses; EPR: electron paramagnetic resonance; FDXR: Ferredoxin Reductase; GWAS: genome-wide association study; HO
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-: hydroxyl radicals; HPLC-–MS: Liquid chromatography–mass spectrometry; HCR: Host cell reactivation; HRR: homologous recombinational repair; HRS: hyper radiosensitivity; I: iodine; IR: ionizing radiation; IRR: increased radiation resistance; LDR: low dose rate; LET: linear energy transfer; LFS: Li-Fraumeni syndrome; lncRNA: long-non-coding RNA; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinase; miRNA: micro RNA; MN-RET: Micronucleated reticulocytes; MS: mass spectrometry; mRNA: messager RNA; mtDNA: mitochondrial DNA; NBS; Nijmegan Breakage Syndrome; ncRNA: non-coding RNA; NHEJ: non-homologous end joining; NLCS: Netherlands Cohort Study; NMR: nuclear magnetic resonance; OPERRA: Open Project for European Radiation Research; PBL: peripheral blood lymphocytes; PCC: Premature Condensed Chromosome; PTC: papillary thyroid carcinoma; Pu: plutonium; qRT-PCR:quantitative reverse-transcriptase PCR; RENEB: Realising the European Network of Dosimetry EU Coordination action; RNA: ribonucleic acid; ROS: reactive oxygen species; RT: reverse transcription; SNP: single nucleotide polymorphism; SOPs: standard operating procedures; Sr: strontium; STROBE-ME: STrengthening the Reporting of OBservational studies in Epidemiology–Molecular Epidemiology; TC-FISH: telomere/centromere-fluorescence in situ hybridization; TL: telemore length; U: uranium; UNSCEAR: United Nations Scientific Committee on Effects of Atomic Radiation.

Appendix A. Supplementary data

The following are Supplementary data to this article:

Multimedia Component 1

Queries and Answers

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