Abstracts of the 7th International MELODI Workshop

"Next Generation Radiation Protection Research"
The seventh International MELODI Workshop is being jointly organised by HMGU (Helmholtz Zentrum München, Germany) and the BfS (the Federal Office for Radiation Protection, Germany). The workshop will take place on 9-11 November 2015 in Munich at the HMGU/BfS campus.

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The Multidisciplinary European Low Dose Initiative (MELODI, (www.melodi-online.eu) is an European Platform dedicated to low dose radiation risk research. In 2010 MELODI was founded as a registered association with 15 members.

The purpose of MELODI is to:

- propose R&T priorities for Europe in its field of competence - EUROPE 2020 Strategy
- seek the views of stakeholders on the priorities for research, keep them informed on progress made, and contribute to the dissemination of knowledge
- interface with international partners like WHO and IAEA.

Based on the outcomes of the yearly MELODI workshops a Strategic Research Agenda (SRA) is being progressively developed. To assure an open and vivid discussion and development of the SRA the contribution from a large number of scientists and stakeholders is needed.

The 7th MELODI Workshop “Next Generation Radiation Protection Research” focusses on new technologies enabling improved health risk estimates after exposures to ionizing radiation with low doses. Major projects of the EURATOM framework programme 7 will be reviewed. Key topics of the Workshop include:

- low-dose risk
- factors affecting individual risk
- new biology
- radiation effects in stem cells
- infrastructures, education and training
- Cross-links to the following other radiation protection fields will be discussed:
  - dosimetry, represented by the platform EURADOS
  - radioecology, represented by the platform ALLIANCE
  - emergency preparedness, represented by NERIS
  - radiation protection for medical applications of ionizing radiation.

Invited and proffered papers will be presented in the oral sessions. Two poster sessions will allow for discussions of further aspects. Key messages of the contributions should include new results or technologies and implications for the SRA for low dose radiation risk research.

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Head of Scientific Programme Committee  Head of Organising Committee
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Low-Dose Radiation Research: Where are we?
DoReMi – Where are we?

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In 2009, the European High Level and Expert Group (HLEG) identified key policy and scientific questions to be addressed through a strategic research agenda for low dose radiation risk. This initiated the establishment of a European Research Platform, called MELODI (Multidisciplinary European Low Dose Research Initiative) and launching of the DoReMi Network of Excellence in the Euratom 7th Framework Programme in 2010. DoReMi acted as an operational tool for the sustained development of the MELODI platform during its early years. A long term Strategic Research Agenda for European low dose radiation risk research has been developed by MELODI. Strategic planning of DoReMi research activities has been carried out in close collaboration with MELODI. The DoReMi project will come to its end in December 2015. Thus it is time to look back and evaluate the achievements during the last six years.

DoReMi continued the initial work of HLEG by contributing to the development of the long-term SRA of MELODI, and by establishing the more detailed shorter-term DoReMi TRA. The research agendas provided by MELODI and DoReMi have helped to identify priorities for low dose risk research not only by the organisations involved but also in national, European and global contexts. The planned enhancement of the DoReMi network through the calls for partners with new expertise has resulted in the inclusion of 24 new beneficiaries in DoReMi. This has enhanced the competence of the consortium in several key areas, by integrating research experts in biomarker identification, immunological/inflammatory pathways, and the effects of chronic low dose exposures, cataractogenesis, vascular effects, stem cells, epigenetics, novel mechanisms of genome reorganisation, as well as retrospective dosimetry. The three DoReMi competitive calls attracted proposals from 89 different organisations in 25 countries (including 21 European MS). A total of 27 new RTD tasks have been added to the project portfolio via the calls. The RTD approaches have been closely coordinated through discussions on needs for research infrastructures and analytical platforms, as well as targeted stimulation of training and education of next-generation researchers at the European level.

The DoReMi research program has focused on three areas identified by the HLEG as most promising for addressing/resolving key policy questions: (1) the shape of dose response curve for cancer, (2) individual susceptibilities, and (3) non-cancer effects. The first program has two overarching aims: (i) to improve knowledge of the dose dependency of cancers induced by low doses/dose rates in humans and the processes that drive the development of such cancers, and (ii) to improve low dose/dose-rate risk projection models through improved integration of the knowledge of these biological processes. The second program aims to determine the contribution of individual variations in sensitivity to modifying the risk of developing cancer following exposure to low doses/dose rates. The influences of inter-individual differences are addressed at three levels, population studies, animal models and in vitro models. The third program focuses on vascular effects, lens opacities and cognitive effects. While some epidemiological studies indicate that such effects could arise as late effects of low dose irradiation or contamination, there is almost a
complete lack of knowledge on the mechanisms contributing to these effects at low doses/dose-rates.

The DoReMi research program has brought to light the existence of non-linear dose and dose-rate dependent responses, the involvement of non-targeted and immunological responses, changes in gene expression and epigenetic profiles, relevant biomarkers for exposure, disease, and regulation of individual radiation sensitivity and advances in the dose dependency and mechanisms of cancer and non-cancers as well as in low dose health risk evaluation models and epidemiological risk assessments. It has been sustained by advanced programs for education and training and the development of radiation research infrastructures. The Transitional Research Agenda developed by DoReMi provides recommendations on the most promising research lines likely to improve knowledge on the mechanisms of low dose effects and the evaluation of low dose health risks during the coming next years.

The availability of suitable infrastructures for performing low dose risk research is specifically addressed by DoReMi. Experimental radiation research is highly dependent on the availability of appropriate radiation sources that are reliable, capable of delivering a range of radiations, robust and accurate. Low dose research also needs access to well defined epidemiological cohorts, reliable databases and biobanks and as well as to the appropriate platforms for analysis. After the initial mapping of infrastructures and their availability, DoReMi has provided access to several new infrastructures that will enhance the European capabilities in addressing scientific questions relevant for low dose risk.

DoReMi has also contributed to the integration and coordination of education and training resources within the radiation risk research community to provide high-level training for research scientists and a career structure that will attract and retain top-level graduates within this research discipline. The organisation of the short 2-3 weeks training courses sponsored by DoReMi has been highly successful. By now, around 500 students have been introduced to new areas of research on low-dose radiation risk. Furthermore, a Bologna-compliant MSc course of study in radiation biology has been launched recently at the Technical University of Munich.

Dissemination of information on ongoing low dose risk research to the general public, the scientific community, policy makers and stakeholders is an important part of DoReMi networking activity. More information on DoReMi activities can be found at the DoReMi website (http://www.doremi-noe.net/). The publicly accessible part of the site contains general information on scientific aspects of low dose radiation research as well as aspects of training and education activities and infrastructures. The website is seen as an important tool for internal and external communication. The website is promoting interdisciplinary interaction, increasing European integration of research and facilitating the spreading of knowledge and enhancing visibility outside of DoReMi. Important key documents such as the DoReMi TRA, as well as links to the MELODI website, other platforms and EURATOM RTD activities can be found on the website. Input from the wider scientific community is actively encouraged via the website to capture new ideas and foster the development of new research strategies within DoReMi and MELODI.

Although much is known about the quantitative effects of exposure to ionising radiation, considerable uncertainties and divergent views remain about the health effects at low doses. The DoReMi joint programme for research focuses on the areas identified by the HLEG and MELODI as being the most promising in terms of addressing and resolving the key policy questions. By addressing the scientific basis underlying the system of radiation protection DoReMi is contributing directly to strengthening the credibility of scientific evidence relevant to the development of
radiation protection policy. Ultimately DoReMi can be expected to contribute more widely to radiation protection through engagement with International Commission on Radiological Protection and other national and international bodies. The High Level and Expert Group pointed out that many EU member states have lost key competences and are no longer capable of independently retaining their current research activities in radiation sciences, with implications for effectively fulfilling operational and policy needs and obligations. Well-sustained up-to-date research and education and training activities carried out by DoReMi and MELODI are urgently needed to ensure the European competence in radiation sciences and radiation protection.

Since the beginning of the DoReMi Network of Excellence in January 2010, there has been rapid progress in the establishment of a European research platform to focus on questions of low dose risk. The experiences on integration of research gained by MELODI and DoReMi have been exploited when preparing for the Horizon 2020. By now, Strategic Research Agendas have been prepared not only for low dose risk research but also for radioecology, emergency preparedness and dosimetry. It is now possible to bring together all these platforms under one umbrella structure, addressing research on radiation protection.

Acknowledgements: DoReMi Network of Excellence is supported by the grant agreement no. 249689 of the European Atomic Energy Community’s 7th Framework Programme.
RISK-IR: Risk, Stem cells and tissue Kinetics – Ionising Radiation

Simon Bouffler on behalf of the RISK-IR consortium

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The RISK-IR project aims to improve the knowledge of the response of stem cells to ionising radiation. Stem cells are considered to be the cells of origin for most cancers, and therefore understanding the responses to radiation in these target cell populations will improve knowledge of the processes that contribute to radiation carcinogenesis. Understanding such mechanistic aspects of radiation carcinogenesis is particularly important in the context of low dose (<100 mSv low LET radiation) radiation risk estimation. The magnitude of risk at these levels is uncertain and based largely on extrapolation of risk assessed in populations exposed to higher levels of radiation using a linear non-threshold model.

Basic understanding of stem cell biology has improved rapidly in recent years but the application of this knowledge and associated technical developments has not yet been fully explored. RISK-IR brings together partners with expertise in basic stem cell biology and those with interests in mechanistic and quantitative understanding of radiation cancer risk. The research undertaken addresses several areas of uncertainty in the mechanistic understanding of low dose radiation carcinogenesis:

- The identification and enumeration of stem/progenitor or cells at risk
- Understanding low dose radiosensitivity of stem cells and tissues
- Improved understanding of mechanisms of age-dependant cancer risk
- Improving understanding of tissue specific differences in cancer risk
- Identification of key events and individual susceptibility factors associated with cancer development

The project is organised into four work packages (WPs) as follows:

- WP1, Management and co-ordination
- WP2, Cells at risk and cancer pathways
- WP3, Age dependency, radiation quality dependency and species/tissue specificity of responses
- WP4 Low dose sensitivity and tissue kinetics.
Work started in November 2012 and the project will end in April 2017. At this stage the achievements of RISK-IR partners include:

- Demonstration of stem cell sensitivity in vitro and in vivo to doses in the range 10-100 mGy in a range of tissue types
- Finding indications of non-linear dose responses in hematopoietic stem cells, characterised by a dose range showing hypersensitivity in terms of cytotoxicity
- Development of unique models of iPS in which to explore in more detail the role of radiation in reprogramming
- Finding indications that even low dose exposures can contribute to stem cell exhaustion
- Early proof of principle work on cell fate tracking models to investigate radiation effects with demonstrated sensitivity at low doses

Work will continue through to the project end date of April 2017, and it is expected that by then we will have improved knowledge of the impact of low dose radiation on stem cells and developed a deeper understanding of how these effects contribute to radiation carcinogenesis.

Acknowledgements

The co-ordinator wishes to thank all partners for their contributions to the success of the RISK-IR project. Funding is provided by the European Union Euratom Seventh Framework Programme RISK-IR project under grant agreement nº323267.
Combining epidemiology and radiobiology to assess cancer risks in the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with total doses in the order of 100 mSv or below (EpiRadBio)

Peter Jacob on behalf of the EpiRadBio consortium

Helmholtz Zentrum München, Institute of Radiation Protection, 85764 Neuherberg, Germany

In order to provide a rationale scientific basis for the risk assessment underlying current radiation protection, the key objectives of EpiRadBio were to

- perform measurements of telomere lengths, array-based comparative genomic hybridisation and other ‘omics’ technologies with cancer tissue, normal tissue and blood samples from members of well characterized radio-epidemiological cohorts in order to identify key processes of carcinogenesis in humans exposed to low-dose or low-dose-rate radiation
- analyze radiation responses of stem cells and low dose perturbation of intercellular communication in 2D and 3D models using human normal breast and lung epithelial cells in order to elucidate further key processes of carcinogenesis and supplement the studies of samples from epidemiological cohorts
- integrate the new radiobiological results in models of carcinogenesis in order to include this knowledge in an evaluation of key epidemiological data
- derive cancer risks including individual risk factors after exposures to ionizing radiation with cumulative equivalent doses in the order of 100 mSv or below for supporting radiation protection.

Main achievements include

- a biobank of blood samples from 371 donors belonging to the French Hemangioma Cohort comprising cytogenetic slides with metaphase spreads of T- and B-lymphocytes and nucleated blood cells frozen in liquid nitrogen for DNA/RNA- and FACS analysis as well as for future cell culture experiments
- a multi-level characterization of the biomarker CLIP2 for radiation-induced thyroid cancer diagnosed below the age of 20 years including its dose dependence
- isolation of stem cell sub-populations from the human normal breast tissue and determination of their response to low doses of ionizing radiation
- an indication that chronic irradiation is more effective in inducing oxidative stress, epithelial-mesenchymal transition and gene amplification compared to acute irradiation
- a prediction that radiation decreases anti-carcinogenic apoptosis of transformed cells under in vivo conditions in contrast to the experimentally determined increase of this process under in vitro conditions
- an indication that at least for exposure at young ages radiation induces genomic instability and pathways to papillary thyroid cancer and to breast cancer different from spontaneous carcinogenesis
- the hypothesis based on mechanistic models of epidemiological data that a bystander effect may play a central role in the induction of lung cancer by alpha radiation
• a new method allowing a common analysis of cases that occurred before the first screening in a cohort, prevalent cases detected in the first screening, and incident cases detected in subsequent screenings

• a suggestion that lifestyle has a major influence on colon cancer with chromosomal instability but not on colon cancer with microsatellite instability

• lifetime radiation risk estimates based on models developed in the frame of the project.

• Contributions to impact expected in the call:

  • EpiRadBio contributes to low-dose risk research for an optimisation of radiation protection by improving the understanding of carcinogenic processes after exposure to low-dose radiation and delivering quantitative cancer risk estimates for exposure scenarios of relevance for radiation protection

  • Tissue sensitivity has been analysed by performing a variety of ‘omics’ technologies, to breast, lung and thyroid cancer tissues, and by quantifying cancer risks in the breast, lung, thyroid and digestive tract in a number of epidemiological studies. Thus, EpiRadBio covered risk of the most frequent and the most radiosensitive solid cancer types

  • Individual variability in radiation sensitivity has been addressed by analysing genomic instability in an exposed vs. non-exposed study nested in the French Haemangioma Cohort, and in cohort studies by taking account of familial factors (e.g. breast cancer among close relatives or influence of the number of children on breast cancer risk) and behavioural factors (e.g. smoking and alcohol consumption)

  • Cancer risks of different radiation quality types have been analysed by comparing lung cancer risks from plutonium and external radiation of Mayak workers, thyroid cancer risks from incorporated iodine and external radiation, and influences of gamma and alpha radiation in in vitro studies with 2D cell cultures including stem cells and with 3D tissue models

  • Cancer risks from internal exposure to radiation has been quantified by analysing lung cancer risks in the Mayak Worker Cohort after incorporation of plutonium, breast cancer risks in the French-Swedish-Italian Thyroid Cancer Cohort after incorporation of $^{131}$I, and thyroid cancer risks after incorporation of different radioiodines, especially of $^{131}$I, in the aftermath of the Chernobyl accident.
SOLO: epidemiological studies on Russian and UK cohorts

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SOLO was a five year, EC funded project to investigate the risks to human health of low and protracted radiation exposures, considering exposures to radionuclides within the body as well as external radiation. The project developed previous follow-up studies on two cohorts from the Southern Urals of the Russian Federation: the Mayak Worker Cohort (MWC) and the Techa River Cohort (TRC), the latter being exposed as a result of Mayak discharges of radioactivity to the Techa River. It also studied the Sellafield worker cohort (SWC) in the UK and, for the first time, has pooled cohorts to assess risks from exposures in utero (MWC and TRC) and for workers (MWC and SWC).

The reliability of risk estimates obtained from epidemiological studies is dependent on the quality of radiation dose information for cohort members. Improvements were made in external and internal dose estimates, including development of models for exposures of the foetus to plutonium-239 and strontium-90, and application of updated models to the calculation of $^{239}$Pu doses to Mayak and Sellafield workers.

Analyses of lung cancer and leukaemia in a pooled cohort of Mayak and Sellafield plutonium workers showed consistency of results with raised risks of lung cancer but not leukaemia. The dose-response and risk estimates for plutonium-239 induced lung cancer were consistent with comparable risk estimates for external gamma radiation when account is taken of the relative biological effectiveness of alpha particles emitted by plutonium-239. Analyses of ischaemic heart disease (IHD) and cerebrovascular disease (CeVD) in the MWC showed an association between external radiation exposure and morbidity but not mortality, an observation requiring further investigation.

A pooled cohort of children was defined whose mothers either worked at Mayak or lived near the Techa River before and/or during pregnancy. The pooled cohort currently lacks the statistical power to show significant results, but the absence of a positive result at this stage is important in showing that risks of in utero exposure have not been dramatically underestimated, particularly for internal radionuclides.
The ANDANTE project: progress towards a re-evaluation of the risk from scattered neutrons during proton therapy

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It is well known that proton therapy generates secondary radiation with a small but significant neutron component. The increasing number of paediatric proton treatments worldwide leads to a concern about second cancers arising in later life from the neutron exposure. However there are many difficulties involved with estimating the risk of cancer induction from exposure to neutrons. The usual approach to risk assessment is through the concept of relative biological effectiveness (RBE) of neutrons compared to photons, since the risk from photon exposure is much better known [ICRP, 2007]. The RBE for neutrons has been evaluated using cellular and animal models. But this causes considerable uncertainty when applying the method to humans. The ANDANTE project is evaluating the relative risk of cancer from neutrons compared to photons in the context of proton therapy, taking a new approach using three different disciplines in parallel:

Physics:

Charged particle spectra generated by both neutron and photon beams have been characterised using Monte Carlo simulation and measurements. A track structure model has been used to model the formation of complex lesions in DNA from the different spectra as an indicator of relative likelihood of cancer induction. An interesting result from this is the appearance of a peak in relative risk between neutrons and photons at a neutron energy of around 1 MeV. A method has been developed for reconstructing the scattered neutron doses outside the treatment volume during proton therapy, using available clinical data, in order to be able to predict second cancer risks.

Stem cell radiobiology:

Stem cells from thyroid, salivary gland, and breast tissue have been given well characterised exposures to both broad- and narrow-spectrum neutron beams, and to 200 kV X-rays. A number of endpoints have been used to estimate the relative risk of damage from neutrons compared to photons. Long term in vitro stem cell cultures (when possible), and in vivo murine transplantation studies are used to assess transformation potential. Detailed histopathological and molecular investigations are being performed of the long-term progeny of irradiated stem cells, specifically looking for pre-malignant lesions and signs of malignancy.

Epidemiology:

The results from the track structure modelling and stem cell experiments will result in an improved relative risk model for second cancer risk. This will be tested in a multi-centre prospective epidemiological study using data from paediatric proton therapy treatments. The project is developing the dose reconstruction and data analysis tools required for the study, and will put in place plans for the study to begin after the completion of the project, as a collaboration between centres in Europe and the USA. The feasibility and practicality of the proposed methodology on
real treatment machines, clinical data, and follow-up procedures is being tested in a pilot setting at 3 proton therapy centres in the USA and at the PSI proton therapy centre in Europe.

In the short term, the results from ANDANTE have so far largely endorsed the RBE-based radiation weighting factors for neutrons published by the ICRP. In the longer term, the prospective epidemiological study will provide greater certainty on predicting the second cancer risk in patients receiving proton therapy.


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Key findings of the ProCardio project

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In order to improve understanding of the risks of cardiovascular disease at low doses we undertook to:
- Design an epidemiological study of cardiac diseases among survivors of childhood cancer.
- Investigate the action of low doses and low dose rates on the heart and vessels.
- Conduct a search for biological markers of low dose effects on heart.
- Evolve mathematical procedures to better describe the dose response relationship.

Our key results can be summarized thus:

I) An epidemiological case-control study of the risk of cardiac disease following low dose exposures has been recruited, made up of 222 survivors of childhood cancer who later developed cardiac diseases and an equal number of survivors who did not develop cardiac disease. The initial analysis provides evidence for a dose-dependent risk of cardiovascular disease following radiation exposure.

II) The case-control study will be pooled with a case-control study conducted within a larger pan-European study of the health of Childhood Cancer Survivors (PanCareSurF). The pooled ProCardio / PanCareSurF study will include 900 cases and 900 controls.

III) There is evidence for a cardiovascular relative biological effectiveness (RBE\textsubscript{CVD}), with an estimated value of between 4 and 10 for HZE irradiation compared to photons.

IV) Differences in the response of surrogate markers of cardiovascular disease to low dose rate and acute exposures provide evidence that a dose and dose rate correction factor (DDREF\textsubscript{CVD}) should be considered for cardiovascular tissue.

V) There is evidence for the existence of a modest abscopal effect, where partial body exposure may increase atherosclerotic plaque formation in non-irradiated vessels of the heart.

VI) Cellular interactions are important for the development of radiation-induced atherosclerosis. Radiation accelerates the process by stimulating both monocyte adhesion to, and the infiltration of lipids through, the endothelium.

VII) MicroRNAs as well as both de-acetylated and mitochondrial respiration complex proteins are indicated as potential biomarkers of radiation-induced heart disease.

VIII) Two mathematical models of radiation action have been generated. These show a good fit with experimental data. One model predicts that plaque initiation and not plaque expansion is the key process in radiation-induced heart disease. The other model, when fitted to A-Bomb survivor data indicates a non-linearity of the dose response relationship towards higher doses.

PROCARDIO: Cardiovascular Risk from Exposure to Low-dose and Low-dose-rate Ionizing Radiation. Supported by a grant from the European Community’s Seventh Framework Programme (EURATOM) contract no. 295823 (www.procardio.eu)
Cognitive and Cerebrovascular Effects Induced by Low Dose Ionizing Radiation 'CEREBRAD' (Grant agreement: 295552)

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Epidemiological evidences about the occurrence of late cognitive and cerebrovascular diseases due to exposure to radiation early in life (in utero or during childhood) are scarce. Nevertheless, A-bomb survivor data indicate a linear dose-response curve with a threshold around 200 mGy. Thus, raising the concern regarding the uncertainty of low-dose radiation, which is in part due to the lack of sufficiently large cohorts, combined with a lack of understanding the underlying mechanisms. Moreover, the increasing use of radiation in medical diagnostics urges the need for appropriate research to define precisely the effect of low dose radiation on the brain. The FP7 CEREBRAD project for cognitive and cerebrovascular effects induced by low dose ionizing radiation (grant agreement n° 295552), aimed thus to gather sufficient scientific evidence to increase the statistical power of epidemiological data. Moreover, the project aimed to explain the related cellular and molecular events modulated early after exposure and most probably responsible for late cognitive and cerebrovascular diseases.

The main CEREBRAD findings are as follow:

I) Epidemiological evaluations of the risk of cerebrovascular disease following low dose exposures were based upon a cohort of 233 survivors of childhood cancer receiving radiation therapy before the age of 5 year, matched to an equal number of survivors not treated with radiation. The Excess of Odds Ratio (EOR) of stroke per Gy of average radiation dose to the cerebral arteries, was equal to EOR/Gy = 0.49 (95% CI: 0.22 to 1.17) in a linear model.

II) Cognitive impairments have been evaluated in a medical and in Chernobyl cohorts, in which exposure to radiation occurred either in utero, or during childhood below the age of one year, or at adult age in clean-up workers. Impairments appear to be age-dependent; in in utero exposed cohort, effects are observed below 0.1 Gy, in the medical cohort (exposure at childhood below the age of one year), impairments increased with increasing dose to the thyroid and cerebral hemispheres from thresholds of 0.12 Gy and 0.054 Gy, respectively. On the other hand, Clean-up workers demonstrated significant cognitive deficits when exposed to doses over 0.25 Gy.

III) The shape of the dose-response curve for cognitive impairments in animal models shows a linear dose-response curve with age-dependent sensitivity. In in utero exposed mice, subtle changes in behaviour can still be observed with the low dose 0.1 Gy, while early postnatal exposure showed impairments starting from 0.3 Gy on. More importantly, postnatal co-exposure with environmental toxicants (such as MeHg, nicotine and PBDE) showed defects at a dose below 0.1 Gy. In all, our data indicate there might be no threshold below which no effects are observed, warranting thus further investigations.

IV) Investigations in animal models on Blood-Brain Barrier (BBB) permeability and integrity after exposure to low and high doses of radiation indicate contribution of several components such as age at exposure, genetic background and basal inflammatory status in the response.

V) Innovative dosimetry calculations were developed on medical cohorts providing accurate retrospective estimates of doses to several brain structures and cerebral arteries. In parallel, animal
dosimetry simulations allowed to calculate energy deposition in soft tissue compared to bone in mice exposed to either external radiation or internal contamination.

VI) The cellular and molecular investigations revealed obvious effects of low-dose ionizing radiation 'LD-IR' on the brain at multiple levels. In general, we could observe a clear dose-dependent effect and could unveil different anomalies induced by the lowest X-ray dose studied (0.1 Gy) in terms of cognition, cell death and neurogenesis. Finally, mechanisms acting at low doses are different from those at high doses, while, processing of the late response could in part be mastered through epigenetic events, requiring thus additional future investigations.

VII) A dedicated computational bioinformatics platform was developed, called Brain Radiation Information Data Exchange (BRIDE). BRIDE fitted nicely the systems biology approach of CEREBRAD to explore pathways inference from omics data in the context of cognitive deficits.

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EPI-CT: challenges, achievements and perspectives

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The European collaborative epidemiological study to quantify risks for paediatric computerised tomography and to optimise doses (EPI-CT) was designed as a multinational cohort study of children and young adults who have received substantial doses of ionising radiation from CT scanning. It comprises three main parts: the epidemiological cohort study is assessing the cancer effects of radiation exposure from CT; the dosimetry package is developing sophisticated methodology for individual CT dosimetry and related uncertainty and furthering dose reduction and optimisation strategies; the biological part is exploring potential biomarkers of exposure and sensitivity to study biological mechanisms underlying hypersensitivity observed in paediatric patients exposed to CT radiation.

EPI-CT is coordinated by the International Agency for Research on Cancer (IARC) and it is based on a common protocol. To date, the study includes 1,163,571 patients from nine European countries (Belgium, Denmark, France, Germany, the Netherlands, Norway, Spain, Sweden and the UK). Data on 2,166,479 CT examinations (53.33% of those being head CT scans) have been retrieved from participating radiology departments.

Passive follow-up is being conducted by linkage to population-based cancer and mortality registries. The issues which may affect the precision of study results include missing doses from other radiological procedures, missing CTs conducted in non-participating radiology departments, confounding by CT indication and cancer predisposing conditions. Although it is impossible to evaluate impact of these potential confounders in the entire study, several participating countries are conducting sub-studies addressing these issues and their results will be taken into account.

A flexible approach for dose reconstruction was developed that can accommodate collection of data from historical sources (prior to 2000) and automatically extracted data from the Digital Imaging and Communications in Medicine (DICOM) headers of recorded images available in the Picture Archiving Communication System (PACS). Individual organ doses estimates are derived for each child from Monte-Carlo-based radiation transport calculations using hybrid phantoms of different sex and ages. To account for uncertainties due to missing input data, a simulation method is used. It maintains correlations of doses for persons within subgroups with similar exposure attributes and simulates uncertain dose-model parameters values. Each missing parameter is represented by a probability density function (PDF) representative of the state of knowledge for
the appropriate time period. For each calculation of the cohort dose distribution, values of parameters are selected from the appropriate PDFs while maintaining proper correlations between parameters.

An in vitro feasibility study to investigate age-dependent radiosensitivity by monitoring chromosomal aberrations and \( \gamma \)-H2AX foci in blood samples from newborns, young children (2–5 years) and adolescents exposed in CT scanner was conducted. Chromosomal aberrations as well as the induction of DNA double strand breaks were increased in blood samples from newborns and young children when compared to adults. Differences were also visible in the \( \gamma \)-H2AX foci assay. In vitro assessment of the \( \gamma \)-H2AX-foci assay as cellular biomarker for exposure demonstrated that it is feasible to apply in a multicentre prospective study in paediatric CT imaging. EPI-CT, the first large-scale international collaborative study, will contribute not only to estimating effects of low-level radiation in children and providing bases for the optimisation of paediatric CT protocols and patient protection but also has a potential to consolidate a European paediatric cohort for long-term follow-up.

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Public Lecture
High-throughput sequencing: getting ready for next generation radiation biology?

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The last few years have been characterized by a true revolution in molecular biology via the development of high-throughput sequencing technologies. Where previously DNA sequencing was depending on the relatively slow Sanger sequencing technology, new sequencing platforms are massively parallel, as such providing a dramatic increase in sequencing speed. Illustrative for this revolution is the sequencing efforts needed to obtain a first draft of the human genome, which took more than 10 years using Sanger sequencing, while nowadays one human genome can be sequenced in less than one week. Moreover, the applications of high-throughput sequencing platforms are not only limited anymore to the sequencing of DNA but are used for a much broader range of research questions.

Within this talk, first an overview of the state-of-the-art sequencing technologies will be given, including the advantages and disadvantages of each platform, and a very broad insight on the method of operation. Despite the fact that this information is rather technical, it is important to understand the technical limitations of each technology in order to understand the potential bias that might occur in sequencing data.

Next, based on some real-life examples in radiation and cancer biology, some general guidelines are given on which technology is preferentially used for which application. Thereby, a critical evaluation will be made for which type of research questions the new sequencing technologies might deliver advanced insights. An illustrative example within this context is the ongoing debate whether one would switch to RNA-sequencing, or stick to the well-established microarray platform.

In the last part of the talk, common pitfalls and challenges when working with high-throughput sequencing platforms are discussed. Related to this, the advisability of investing in a sequencing platform versus outsourcing this task is commented, as well as the requirements concerning technical expertise and infrastructure.

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Session 3
Low-Dose Risk
Molecular landscape of papillary thyroid carcinomas from the UkrAm cohort and modelling of carcinogenesis

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A significant increase of the incidence of papillary thyroid carcinomas (PTCs) was observed among children exposed to the radioiodine fallout of the Chernobyl accident. A first study on genomic copy number alterations (CNAs) of radiation-associated PTCs of the Ukrainian American cohort (UkrAm) revealed different molecular PTC subtypes. The analysis also included a transcriptomic analysis of UkrAm cases that split the cases into four groups confirming BRAF-like and RAS-like subgroups that were already reported in the TCGA data set in addition to novel molecular subgroups. Since the previously identified radiation-associated gain of chromosomal bands 7q11.22-11.23 (Hess et al., 2011) was also observed in 29% of UkrAm cases, we further investigated the CLIP2 marker, which is located within these chromosomal bands at the protein level by immunohistochemistry. We confirmed radiation-associated CLIP2 protein overexpression and, moreover, established a standardized procedure for CLIP2 typing. This essential step allowed integrating the marker into epidemiological studies for improved risk estimation and modeling of radiation-induced carcinogenesis. To gain knowledge about the functional role of CLIP2 in radiation-associated PTC, we reconstructed the CLIP2 interactome from global transcriptome data derived from UkrAm PTCs. Analysis of the CLIP2 interactome suggests the involvement of CLIP2 in the fundamental carcinogenic processes apoptosis, MAPK signaling, and genomic instability, indicating a functional role of CLIP2 in the radiation-associated carcinogenesis. Moreover, we showed a clear dose-response relationship for the CLIP2 radiation marker in post-Chernobyl PTCs exclusively for patients with young AaO (<20 yrs.) and young Aae (<5 yrs.) suggesting different molecular mechanisms of radiation-associated PTC tumourigenesis in these two age groups. Integration of molecular data on CLIP2 typing and copy number alterations into mechanistic risk models markedly improved these and also confirmed the role of CLIP2 in radiation-associated PTC tumourigenesis.
Radiological risk from low dose and low dose rate exposures

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Various radiation protection organizations have expressed different views regarding the magnitude of the dose and dose rate effectiveness factor (DDREF), including values of 2, 1.5 and 1 based on judgments about the radiobiology and epidemiology data. In examining this an important related question is whether the estimate of a low dose effectiveness factor (LDEF) based on dose-response curvature for a high dose-rate exposure (particularly the Life Span Study (LSS) of A-bomb survivors) is the same as the dose rate effectiveness factor (DREF) when the exposure is at a low dose rate or highly fractionated. The concept of DDREF is appropriate insofar as the LDEF and DREF are similar and greater than unity. In this presentation, the shape and uncertainty of the LSS dose-response curve will be examined briefly to characterize the LDEF, and risk estimates from epidemiologic studies of dose-related excess mortality after fractionated low dose or low dose rate (LDLDR) exposures will address the DREF. Because statistical power and statistical precision (per unit dose) are greatly limited at low doses, virtually all individual LDLDR studies provide very imprecise estimates of radiation risks. The aim of the present approach is to conduct meta-analyses of the universe of LDLDR studies that have dose-response data for the health outcomes of interest, to obtain a more precise overall estimate of risk that can be compared with corresponding data from the LSS study. Primary attention will be given to total solid cancer mortality but, since biological and environmental factors impacting various tumour sites may differ, LDLDR studies of several individual cancer sites are also reviewed. Preliminary results will be shown.
Large-scale Animal Studies for Evaluation of Dose and Dose Rate Responses

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The United States government regulates allowable radiation exposures for the workforce and general public relying, in large part, on the 7th report of the committee to estimate the Biological Effects of Ionizing Radiation (BEIR) organized by the National Research Council of the National Academies. This committee used information about Japanese atomic bomb survivors and a limited number of animal studies with controlled radiation exposures to calculate that most contemporary exposures to radiation (at low dose rate, fractionated, or at doses lower than 100 mSv) carry 1.5 fold less of a risk of carcinogenesis and mortality per Gy than exposures to atomic bomb survivors. This correction factor is known as the dose and dose rate effectiveness factor for the life span study of atomic bomb survivors (DDREFLSS). It was calculated using a linear-quadratic dose response model.

We argue that linear-quadratic formalism does not provide appropriate support for the study of radiation induced carcinogenesis and mortality. We propose that the risk of low dose and protracted exposures should not be based on the linear-quadratic formalism. Moreover, the risk of protracted exposures should not be derived from the apparent shape of acute dose responses. Instead, it should be estimated based on direct comparisons of data from acute and protracted exposures. Furthermore, it should not be assumed that low dose rate and fractionated exposures are necessarily co-linear, rather a dose rate effectiveness factor and a fractionation effectiveness factor should be developed separately. Unfortunately, it will continue to be necessary to extrapolate the risk of low dose exposures from higher dose exposures due to statistical considerations. The question of whether the risk following low dose exposures is co-linear with the risk following fractionated exposures made up of many low dose exposures should be exposed to ongoing debate. Finally, we encourage development of steps to increase the amount of data being analyzed in order to improve existing estimates. Data from animals should be analyzed across the same dose range as atomic bomb survivors (0 – 3 Gy). Also, we urge the scientific community to make more resources available through international radiobiology archives so that they may be used to contribute to better estimates of the risks of protracted exposures.
Dose-responses for cerebrovascular and heart diseases in atomic bomb survivors – an analysis involving multi-model inference techniques

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We focus on two essential detrimental health outcomes from the latest publically available data on the atomic bomb survivors of the Life Span Study (Shimizu et al. Brit Med J 340: b5349, 2010): mortalities from cerebrovascular diseases (CbVD) and heart diseases. The full follow-up period from 1950-2003 was included. The data were analyzed with 13 descriptive models such as linear-no-threshold (LNT), quadratic (Q), linear-quadratic, linear-threshold models (LTH) and a number of smooth step and U-shaped models and different categorical models. Each of these models was combined with a suitable baseline model either as excess relative risk (ERR) or as excess absolute risk (EAR) models. The number of parameters in the baseline model has been reduced using a series of likelihood-ratio tests on a 95% confidence level, i.e. baseline parameters that were not significant were eliminated. The same selection procedure was applied to the radiation-related model parameters. Thereby one sub-set of two non-nested ERR-models was identified for CbVD that all fitted the data about equally well (LNT, Q). This sub-set was then used to perform multi-model inference (MMI), an innovative statistical method of mathematically superposing different models to allow risk estimates to be based on several plausible dose–response models rather than just relying on a single model of choice. For CbVD, the simulated dose-response curve from MMI is located below the LNT model at lower doses. At higher doses, however, the dose-response curve from MMI predicts a higher risk compared to the LNT model. This is due to the contribution of the Q-model. For mortality from heart diseases the approach was analogous and yielded two sets of final non-nested models, one containing ERR-models (LNT, Q and LTH), the other one containing EAR-models (LNT, Q, LTH and a step model with a finite slope). For this detrimental health outcome, the LNT model predicts a significant risk down to very low doses. The dose-response curve from MMI, however, predicts that at the 5% significance level the risk is significant only at doses above 2.57 Gy – in contrast to the result that one would obtain by applying only the LNT model to the data. For both endpoints, the dose-response curve from MMI points to a sub-linear behaviour at low doses.

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Risk of cancer mortality in an international study of nuclear workers (INWORKS)

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Context The International Nuclear Workers Study (INWORKS) combines information on nuclear workers from France, the United States of America, and the United Kingdom. These are among the largest, oldest, and most informative cohorts of nuclear workers in the world. Information has been pooled, and an epidemiological analysis of cancer mortality conducted, to contribute to our understanding of risks of cancer mortality following exposure to protracted low doses of ionizing radiation.

Methods A combined cohort of nuclear workers badge-monitored for external radiation exposure was assembled and followed up to 2005. Vital status and causes of death were obtained from national registries. Estimates were derived of absorbed dose to the colon, expressed in gray (Gy). Poisson regression methods were applied to quantify the association between cumulative dose and mortality due to all cancer, all cancer excluding leukemia, solid cancer, and solid cancer excluding lung cancer.

Results A total of 308,297 radiation-monitored workers were included in the cohort. They were followed over a period that spans over more than 60 years. A total of 19,748 deaths due to cancer were identified, among them 17,957 were due to solid cancer, and 12,155 due to solid cancer other than lung.

Conclusion INWORKS provides a substantial basis of epidemiological information upon which to derive estimates of associations between radiation dose and cancer mortality in a population for who average cumulative doses are on the order of 20 mGy. This cohort study encompasses a long period of follow-up of workers and findings from it represent a substantial addition to the scientific basis for understanding the risks of cancer from protracted exposures to ionizing radiation.
Session 4
Factors affecting individual risk
Risk of leukaemia, lymphoma, and multiple myeloma in an international study of nuclear workers (INWORKS).

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Context There is considerable interest in, and uncertainty regarding, risks of malignant neoplasms of lymphoid and haematopoietic tissues following exposure to protracted low doses of ionizing radiation accrued at low dose rates. In the frame of an international project, INWORKS, we examined mortality due to leukaemia, lymphoma, and multiple myeloma in a large cohort of nuclear workers from France, the United States of America, and the United Kingdom.

Methods A combined cohort of nuclear workers badge-monitored for external radiation exposure was assembled and followed up to 2005. Vital status and causes of death were obtained from national registries. Recorded ionizing radiation doses were converted into estimates of dose to the active red bone marrow. Poisson regression methods were applied to quantify the association between cumulative dose and mortality due to leukaemia, lymphoma, and multiple myeloma.

Results A total of 308,297 radiation-monitored workers were included in the cohort. They were followed for a total of 8.2 million person-years over a period that spans over more than 60 years. Doses were accrued at very low rates (mean 1.1 mGy per year, standard deviation 2.6). The mean cumulative red bone marrow dose was 16 mGy. A total of 66,632 deaths were observed, among them 531 were due to leukaemia excluding chronic lymphocytic leukaemia, 814 to lymphoma, and 193 to multiple myeloma. The excess relative risk (ERR) of leukaemia mortality (excluding chronic lymphocytic leukaemia) was 2.96 per Gy (90% CI 1.17–5.21; lagged 2 years). This ERR was not attenuated when restricted to doses of less than 300 mGy (ERR = 2.96 per Gy, 90% CI 1.17–5.21) or less than 100 mGy (ERR = 2.96 per Gy, 90% CI 1.17–5.21) however, 90% CIs were much wider when based on data for the restricted dose range (figure). The observed association is most notably
due to an association between radiation dose and mortality from chronic myeloid leukaemia (ERR = 10.45 per Gy, 90% CI 4.48–19.65).

Conclusion INWORKS provides relatively precise estimates for radiation-related risks of leukaemia mortality, thanks to a long follow-up of workers exposed to low doses of ionizing radiation that were typically accrued at low dose-rates. Results show evidence of positive associations between protracted low-dose radiation exposure and leukaemia risk. This international study provides pertinent information for radiological protection.

References
Individual risk factors for breast cancer and risk transfer among populations

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Ionizing radiation is known to increase the risk of female breast cancer, one of the most frequent cancers worldwide. The risk depends on a number of factors, such as e.g. familial risk, number of children, age at first childbirth and time trends. The interaction of radiation with these factors is poorly understood. Furthermore, risk estimates are usually based on the atomic bomb survivors, which have a different background breast cancer rate than current Western populations.

To address these questions, results from studies for breast cancer incidence of 17,200 female Swedish hemangioma patients are presented, who had been exposed to ionizing radiation because of skin hemangioma. A total of 877 breast cancer cases have been observed with a follow-up until 2009. A re-evaluation of the dose estimates resulted in substantial dose reduction for a part of the highly exposed women. Compared to previous analyses of the Swedish hemangioma cohort, the changes in dose estimates increase the central value of the excess relative risk by a factor of two [1]. From an analysis with mechanistic models of carcinogenesis, indications for induction of a path with genomic instability was found.

With the Swedish Multi-Generation Register and the Swedish Cancer Register it was possible to retrieve information on breast cancers among mothers, sisters and daughters of the hemangioma patients. Thus, it was possible for the first time, not only to study familial risk on general breast cancer occurrence, but also to investigate the change of radiation risk due to familial breast cancer.

From comparisons to the atomic bomb survivors [2] suggestions for risk transfer among different populations are inferred. Results for lifetime risk estimates are presented and compared to estimates from BEIR VII, ICRP and UNSCEAR.


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Acentric fragments are associated with cancer risk in subjects occupationally or accidentally exposed to ionizing radiation: Re-analysis of a large European pooled cohort

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Physical dosimetry and biomonitoring are currently applied in the estimation of health risks after overexposures to ionizing radiation (IR). Biomonitoring by levels of genome damage has shown to be a reliable method for measurements of increased cancer risk. The aim of this study was to investigate the predictivity of chromosome acentric fragments for cancer risk in subjects exposed to IR and control subjects, and compare results with dicentric chromosomes, which are currently used as the standard biomarker of exposure to IR. The study was performed on 1726 subjects exposed to IR, and 641 control subjects. The mean follow-up period was 8 years (SD± 4.3). Subjects were analysed by chromosome aberration assay and results were compared with cancer cases proved according to histology and reported by national cancer registers. In subjects with cancer diagnosis reporting exposure to IR the presence of acentric and dicentric aberrations is associated to a significant increase in cancer risk, i.e., RR 1.78 (95% CI 1.01-3.13), and RR 1.88 (95% CI 1.09-3.24), respectively. This study provides the first evidence that acentric fragments predict cancer in subjects exposed to IR. Because automated acentric scoring can be easily introduced using fast flow cytometric combined with the whole chromosome painting, this biomarker may hold promise as a potential sensitive biomarker of exposure to IR and cancer risk.
Changes induced by radiation and iodine deficiency in thyroid cells

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Introduction: Ionizing Radiation (IR) and Iodine Deficiency (ID) are known risk factors for development of thyroid cancer, however there is little knowledge on their combined effects on thyroid cells especially when considering low doses of radiation. We hypothesize that IR and ID would have a harmful effect on thyroid cells as both of them induce ROS production in the cells, and this would eventually lead to increased apoptosis/cell death, activation of precancerous pathways such as cell survival and proliferation pathways (NF-κB, PI3/AKT and JAK/STAT).

Materials & methods: A normal thyroid cell line (FRTL-5) was subjected to low (0.1 Gy), intermediate (0.5 Gy) and high doses of radiation (3 Gy) under iodine sufficient (10⁻⁸M NaI in culture medium) or deficient condition (medium without iodine). Apoptotic as well as dead cells were assessed by flow cytometry, ROS production was monitored using a fluorescent probe, DNA damage was detected using comet assay and protein expression were evaluated using western blot.

Results: When assessed separately 24 and 48 hours post treatment, IR up to 0.5 Gy failed to induce apoptosis in the thyroid cells but a higher dose of 3Gy did. When considering the co-treatment (IR and ID), apoptosis was only increased at the highest dose of 3Gy. Regarding, late-apoptotic/dead cells, the co-treatment induced an increase in these populations 48 hours post co-treatment in a dose dependent manner. However, for both apoptosis/dead cells, no synergetic/additive increase was observed. After co-treatment, activation of markers of apoptosis (active caspase 3), cell survival (NF-κB p65 and pIKKα), cell growth (STAT-3) and cell cycle regulator (Cyclin-D1) were only observed at the highest dose of 3Gy. Although the co-treatment increased ROS production in a dose dependent manner and induced DNA damage starting from 0.1 Gy, the expression of p53, the guardian of the genome, was elevated starting from 0.5 Gy.

Conclusions: These data suggest that thyroid cells are relatively resistant to apoptosis, and show little DNA damage as induced by the co-treatment even when the latter involved intermediate or high doses of radiation. It should be noted that the majority of the cells remained alive after the co-treatment and this specific population may bear chromosomal aberrations, which may promote their malignant transformation.

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Session 5
ALLIANCE-MELODI cross cutting themes
Biomarkers of exposure and effects: Are they useful tools to understand radiation sensitivity in living organisms?

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The issue of biological effects of low doses of ionising radiation is of major concern for both human and environmental radiation protection. This has been recently highlighted after the Fukushima accident, especially with respect to the quantification of the magnitude of risk to individual (human) and population (human and biota) health at low dose rates. Gaining knowledge on low dose effects on human and non-human species will therefore provide robustness in effects predictions and decision taking.

Ionising radiation can cause directly or indirectly toxic effects on the major physiological systems, including immune system, oxidative stress, general metabolism, detoxication and neural activity. The alteration of one or several biological functions is susceptible to alter homeostasis and adaptability of the organisms, leading to impairment of their growth, reproduction and survival. However, the effects at high hierarchical levels are always preceded by early modifications in biological processes, from subtle biochemical disturbances to impaired physiological functions, allowing the measurement of biomarkers of effects. Therefore, the use of biochemical and physiological biomarkers may help to understand the significance of biomarkers at the individual level. For example, the immune system is likely to be one of among the more sensitive physiological systems to ionising radiation. The alteration of its components can lead to an increased susceptibility of the organism to infectious diseases. If DNA lesions constitute one of the primary damages of ionising radiation, effects other than genotoxicity were observed in organisms exposed to ionising radiation, including effects on oxidative stress, detoxication and general metabolism. These various effects of ionising radiation on physiological processes can have consequences on survival (particularly for early life stages), development, fecundity and behavior of organisms. Moreover, understanding the role of primary mechanisms at the cellular and sub-cellular level when organisms are exposed to low dose of ionising radiation can help to explain the potential consequences on physiological functions and health across generations using extrapolation models.

Another possible outcome of the use of biomarkers is to better understand the differences of radiation sensitivity across species and phyla, which have important implications for understanding the overall effects of radiation and for radiation protection: sensitive species may actually require special attention in monitoring and managing radiation protection, and differences in sensitivity between species can also have repercussions at higher levels (community, ecosystem), since interactions between species may be altered. This inter-species comparison will allow the characterization of molecular fingerprints specific to ionising radiation and of their similarities or differences among different species/phyla (inter- and intra-species differences).

In this context, the presentation aims to give an overview on the effects and mechanisms of action of ionising radiation on physiology of non-human species in the case of exposure situations at low doses.
The classical molecular and cellular biomarkers will be analyzed in the light of their ability (i) to demonstrate exposure of organisms in the environment (biomarkers of exposure), (ii) to indicate a potential deleterious effect (biomarkers of effects), (iii) to predict effects at higher organization levels, (iv) to investigate possible transgenerational effects and (v) to assess differences of radiation sensitivity across species and phyla (biomarkers of radiosensitivity). Examples will be shown of effects of gamma irradiation, americium and tritium on different biological models.
Metabolic heat: an *in vivo* signature of low dose radiation sensitivity

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The quantitative description of the chemi- and radiotoxicity of heavy metals and radionuclides at environmentally relevant micromolar concentrations has become a major challenge in radioecology. As a member of the Alliance, the Institute of Resource Ecology investigates the effects of heavy metals and different radioisotopes on microbial growth. In contrast to classical colony counting-based approaches that assess bacterial survival probabilities, we have established microcalorimetry as a novel radioecological *in vivo* method. Monitoring metabolic heat production from life cell populations, α-particle-induced lethality as well as metabolic adaptations to heavy metal stress can be quantitatively determined. In combination with genetic engineering, the specific metabolic pathways in heavy metal resistance can be studied as exemplified for the glutathione pathway in the attenuation of uranyl chemitoxicity. Using bacterial isolates from mining waste piles, radio- and chemitoxic effects can be distinguished and quantitated on the basis of geometrical models of α-particle trajectories in spatially confined bacterial populations. The calorimetric experiments offer unprecedented potential for *in vivo* studies of low dose effects also in higher organisms and can provide an attractive extension of experimental methods in the realm of future MELODI projects.
Studying response of plants to radiation using biomarkers of different levels of biological complexity

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The biosphere is constantly exposed to ionising radiation originating from natural or anthropogenic sources. Plants, as sessile organisms, form a particular case as they cannot hide or run away from exposure and are on the other hand, essential primary producers of oxygen and carbohydrates. The response of plants to radiation is known to be dependent on a number of parameters such as species, cultivar, exposure conditions and plant age. However the mechanisms that determine these differences in sensitivity are far from understood.

Research studying the response of plants to ionising radiation has up to now mainly focussed on umbrella endpoints such as growth and reproduction. In this presentation an overview is given of biological responses of two plant species: the terrestrial Arabidopsis thaliana and the free-floating macrophyte Lemna minor exposed to gamma radiation. For both species different endpoints at different levels of biological complexity are being compared.

For Arabidopsis thaliana plants differing in age and time of exposure were used and the response of growth, photosynthesis and the transcription of genes involved in DNA repair, cell cycle and signalling are shown. A clear shift in radiosensitivity with increasing seedling age is shown. This can possibly be linked to differences in regulation of DNA repair and cell cycle control at transcriptional level.

The second part of the talk will focus on Lemna minor plants chronically exposed to gamma radiation. Again different growth related and biochemical endpoints are being compared. It is shown that although some biochemical parameters are rapidly induced after exposure there is no or little differences between the sensitivity of the different endpoints tested. Finally, the sensitivity of both test plants in comparison to other reports in literature will be discussed

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The cosmic silence experiment: investigation of the molecular mechanisms involved in the biological response of living systems to environmental radiation

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The molecular mechanisms involved in the biological response to environmental radiation are still little known and need to be investigated. Important information can be acquired by analysing possible differences between parallel biological systems, one kept in a reference radiation environment (RRE) and one in a low radiation environment (LRE). To this purpose, the underground Gran Sasso National Laboratory (LNGS-INFN) represents a unique opportunity being cosmic radiation almost absent and neutron flux reduced by a $10^3$ factor. Studies performed on cells of different origin (yeast, rodent and human) have indicated that cell cultured at the LNGS underground laboratory are less preserved from DNA damage, and show reduced Reactive Oxygen Species (ROS) scavenging power than those cultured in an external reference laboratory at the Istituto Superiore di Sanità (ISS, Rome). The Cosmic Silence Project aims to deepen the investigation of the molecular mechanisms involved in environmental radiation response by using sensitive \textit{in vitro} (A11 hybridoma cells derived from transgenic pKZ1 mouse model) and \textit{in vivo} models (Drosophila melanogaster and pKZ1 transgenic mice), showing different levels of complexity in the phylogenetic tree. To this purpose, besides the cell culture laboratory, a new facility for housing living organisms is under construction underground at LNGS.

Recent data on A11 cells corroborate the hypothesis that environmental radiation contributes to the development and maintenance of defence mechanisms and indicates that a gamma component increase of the environmental radiation does not significantly influence the biological response. Characterization of the radiation field, in particular of the neutron component, is in progress in the LRE and RRE laboratories.
Gamma radiation affects gene expression in early life stages of Atlantic salmon.

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Gene expression analysis allows the identification of differentially expressed genes (DEGs) following exposure of organisms to environmental stressors. In the present work, early life history stages (eggs, juveniles) of Atlantic salmon (Salmo salar) have been exposed to gamma radiation (⁶⁰Co). Specific toxicological and/or biological processes were identified after mapping the salmon DEGs to mammalian orthologs and subjecting to protein-protein network and pathway analysis. Gene expression analysis were performed on gills, liver and whole embryos of Atlantic salmon. For juveniles, the results clearly showed that most of the regulated toxicity pathways were dependent on the radiation exposure dose; low gamma doses affected inorganic phosphate homeostasis, while medium gamma doses modulated the mitochondrial transmembrane potential, fatty acid metabolism, nuclear receptor signalling and hypoxic responses. The highest gamma dose regulated P53 signalling, anti-oxidative response, mitochondrial dysfunction and cell death. For the embryos, results showed a dose dependent change in number of expressed genes. Gene ontology analysis showed that DEG’s were associated with functions related to the developmental program of the embryogenesis. Specifically, this involved both stem cell differentiation, neuron, mussel, brain and eye development. These findings are probably also relevant to other gamma-exposed organisms.
Session 6
New biology
Role of microvesicles in non-targeted effects (NTE) of radiation: *in vivo* study

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A relatively new field of research has recently started to explore the role of microvesicles (MV) / exosomes in a plethora of effects including cancer metastasis and Parkinson’s disease (e.g. D’Souza-Schorey and Clancy 2012). MV’s are small membrane bound vesicles that are released into the extracellular environment. Neighbouring or distant cells recognise and endocytose these MV’s which carry a variety of signals including RNA, protein and DNA (Al-Mayah et al. 2012, Jella et al. 2014). Much of the current effort in the field of MV / exosome research is focused on studies relevant to cancer, highlighting their roles in physiological and pathological processes. However, far fewer studies pertain to the effects of radiation on cellular release and uptake mechanisms of exosomes and thus their role in mediating targeted and non-targeted effects (NTE) of ionizing radiation.

Recently we have shown radiation-induced non-targeted effects (NTE) in MCF7, a human breast epithelial cancer cell line. Genomic instability (GI) and bystander effects appear to be mediated by exosomes which are implicated in long-lived signalling of NTE and RNA and protein molecules of exosomes were found to work in a synergistic manner to initiate and transmit these effects to naïve cells and perpetuate GI in the affected cells (Al-Mayah, 2015). In order to determine the role of MV / exosomes in NTE *in vivo*, we used C57BL/6 male mice exposed to a range of X-ray doses. Microvesicles were isolated and analysed for a number of traits including size and concentration using the qNano (Izon Science™) as well as microvesicle cargo including cytosolic proteins and RNA. In parallel, isolated MV / exosomes were injected into un-irradiated recipient C57BL/6 male mice to investigate direct cellular damage (chromosomal instability, γH2AX); phenotypical and functional alterations as well as stress and inflammation markers. The results and potential implications of this work for low dose radiation research will be presented.

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References:


Telomere length distributions in members of radio-epidemiological cohorts

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Epidemiological studies of atomic bomb survivors have demonstrated the correlation between the appearance of solid cancers and leukemia and high radiation doses and dose rates with a linear non-threshold (LNT) extrapolation of cancer risk for doses above 100 mSv. The effect of low doses (below 100 mSv) is still controversial and the subject of intense study. Recent epidemiological studies suggest that cancer risk after exposures with doses comparable to the dose limits for occupationally exposed workers may be larger than assumed.

There is still a need for more in vivo studies and the identification of good biomarkers for the development of efficient models to estimate the cancer risk after exposure to low doses of ionizing radiation. There are only a few studies allowing simultaneous epidemiological studies on low doses and molecular biology studies at the same time. Cohorts allowing joint epidemiological and biological analyses are essential for direct radiation risk assessment and the study of radiation-induced cancers, which require accurate information especially for dosimetry, age at exposure, and follow-up post-irradiation. In France, children (6 months-3years old) were treated for haemangioma during early childhood with radiotherapy from 1940 to 1973. Epidemiological analysis of this cohort has demonstrated a 3-fold higher risk of developing cancer (a follow up of at least 40 years). A biobank of blood samples was established (cytogenetic slides of B- and T-lymphocytes and isolated nucleated blood cells). Telomeres play an essential role in cellular senescence and cancer development. To reveal the impact of telomere status on genetic stability and individual susceptibility for the development of cancer a long-time after low dose irradiation, we launched an exposed/non-exposed study. The effect of exposure during childhood was estimated by analysing the mean telomere length, intra- and intercellular heterogeneity, and telomeric and genetic stability of lymphocytes 40-60 years after treatment to assess the potential for telomere-driven chromosomal instability. We propose that mean telomere length and its intracellular heterogeneity might serve as an indicators for radiation induced cancer susceptibility and for the choice of follow-up needed after radiotherapy or/cancer screening.
Low doses of ionizing radiation enhance angiogenesis

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We demonstrated that low doses of ionizing radiation (IR) enhance angiogenesis. Doses of 0.1 and 0.3 Gy activate the receptor 2 of Vascular Endothelial Growth Factor (VEGF) and by this mechanism promote endothelial cell migration. Moreover, doses lower than 0.5 Gy do not inhibit endothelial cell proliferation and protect endothelium from cell death induced by inhibitors of several signalling pathways such as PI3K and MAPK.

In vivo, low doses of IR accelerate embryonic angiogenic sprouting and promote adult angiogenesis during zebrafish fin regeneration. Using mice models of leukemia and orthotopic breast cancer, low doses of IR promote tumour growth and metastasis in a VEGFR-dependent manner. In a microarray study, 0.1 Gy modulate the expression of 4000 transcripts, including those encoding for proteins required for angiogenesis.

In order to validate these findings in human, we assessed the angiogenic effects of doses lower than 0.3 Gy in biopsies collected from patients with rectal cancer after radiotherapy. According to the dosimetric plan obtained for each patient, two distinct peritoneal biopsies were surgically removed: i) a specimen exposed to low doses, located in the vicinity of the tumour and ii) an unirradiated specimen used as an internal calibrator for each patient (paired control sample). After CD31 staining, endothelial cells (ECs) were isolated by Laser Capture Microdissection microscope followed by RNA extraction, cDNA synthesis and qRT-PCR analysis. Our unpublished results show that low doses of IR induce the expression of several pro-angiogenic targets in human ECs and strongly suggest that the angiogenic balance is skewed toward a pro-angiogenic phenotype after low-dose IR exposure. Beyond ECs, other cells in the microenvironment could contribute to angiogenesis. Our unpublished results show that low doses of IR modulate the molecular profile of fibroblasts, adipocytes and inflammatory cells.

Our findings are relevant in the setting of radiotherapy, since they reveal new mechanisms to understand the pro-metastatic effects of IR and, are innovative as they are focused in the contribution of low doses of IR and their effects in the peri-tumoral area. Additionally, we are exploiting their relevance in the setting of image-guided radiation therapy.
Global gene expression and DNA methylation in testicular mouse cells after chronic exposure to continuous low dose rate gamma irradiation and Selenium deficiency

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Still, large uncertainties exist on adverse biological effects from chronic exposure to low-dose rate radiation. Selenium (Se) is an essential element necessary for antioxidant enzymes, declining blood Se levels are recorded in Se-poor areas. There is a potential interaction between Se-status and exposure to ionising radiation, giving rise to oxygen radicals that may be scavenged by antioxidant enzymes such as Se-proteins. An exposure facility, Figaro, that allows prolonged exposures of gene-modified rodents to low dose rates gamma irradiation was used. Two mouse lines, one with defective repair of certain oxidative DNA lesions (8-oxoguanine; Ogg1-defective mice) were used. Mice (F0) were bred from parents (P) given low-Se forage. Male F0 offspring were given either low-Se (0.01 mg Se/kg) or normal-Se (0.23 mg Se/kg) forage and were continuously exposed to low dose rate (1.41 mGy/h) gamma irradiation for 45 days (days 1-45) followed by a 45 day recovery period (days 46-90). We have shown that Se-deficiency gave rise to adverse effects on male reproduction that was aggravated by exposure to irradiation and to genotoxic effects in blood cells. In this part of the project the aim is to identify mechanism underlying the observed adverse effects by investigating global gene expression in testis and by addressing potential dysregulation of the germ cell epigenome. For global gene expression analyses testis RNA all groups (4/group, 8 groups) was subject to global quantitative RNA sequencing (Illumina HiSeq 2000 Sequencing). For DNA methylation studies caput epidydimal spermatozoa (day 90) was used to quantify the relative proportion of methylated and unmethylated alleles at CpG sites in a collection of DNA sequences by bisulfite modification, PCR amplification and pyrosequencing. We investigated repetitive elements, imprinted genes and other genes. Preliminary data will be presented. We contribute with novel data following chronic exposure of rodents to continuous low dose rate gamma irradiation to elucidate mechanisms underpinning observed adverse biological effects with potential for transmittance to coming generations. This work was supported by the Research Council of Norway through its Centres of Excellence funding scheme, project number 223268/F50 CERAD.
Session 7
EURADOS-MELODI cross cutting themes
Dose reconstruction in the CURE project for workers exposed to uranium

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Although uranium is ubiquitous in the nuclear fuel cycle, the health effects of occupational exposure to this radioelement are not well known. Available epidemiological and biological studies suffer from various limitations, often including the lack of standardized quantification of exposure levels. Dosimetry for uranium intakes is less direct than that for external irradiation as it is based on measurement of activity interpreted with models using hypotheses on the nature of the exposure. A dosimetric protocol was designed during the CURE project to harmonize procedures and to support epidemiological and biological research with an assessment of organ doses as realistic as possible. CURE was an 18-month concerted action involving 9 partners supported by the European Network of Excellence DoReMi. Its aim was to develop a protocol for a collaborative research project on the biological and health effects of uranium, integrating epidemiology, biology/toxicology and dosimetry.

For uranium miners and millers, exposures are determined using measurements of radioactivity in the ambient air. The size distribution of inhaled particles, the breathing rate and the duration of exposure are significant parameters. Exposure of workers involved in later stages of the nuclear fuel cycle is monitored by individual bioassay (mainly urinalysis) and interpreted using exposure scenario assumption (e.g. regarding the time of intake and the solubility of the uranium compounds involved). Reference biokinetic and dosimetric models are applied to estimate annual absorbed doses to the organs and tissues of interest for the study of potential health effects from uranium intake. In order to make the dosimetry for the study as comprehensive as possible, doses from other internal radiation exposures (e.g. radon and its progeny) and external radiation would be included, the contribution to dose would also be categorized by radiation type (alpha, beta, gamma), and the toxicological dose would be assessed in terms of uranium mass. The analysis of uncertainties associated with assessed doses was also considered but this issue will still require further investigation.

CURE demonstrated the feasibility of pooling existing uranium workers cohorts employing improved dosimetry and a molecular epidemiology approach. A protocol for a large scale collaborative project to improve the characterization of the biological and health effects of uranium exposures in Europe was produced.

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Dose reconstruction in the SOLO project for Mayak workers and former residents of the Techa River area

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The global aim of the SOLO project was to derive improved estimates of the risks of long-term health effects associated with protracted external and internal radiation exposures through studies of the Mayak plutonium facility workers and the population living along the Techa River contaminated due to radioactive releases from the Mayak. The Mayak Worker cohort and the Techa River cohort are unique in terms of their large size (tens of thousands persons), long-term follow-up (over six decades) and a wide range of organ doses (up to several Grays). The dosimetry systems \textit{Mayak Doses 2008} and \textit{TRDS-2009} were created in collaboration with the U.S. scientists to support epidemiological studies using individual estimates of external and internal doses. The dosimetry systems were based on archival records of individual histories of exposure at the workplaces and in the environment. Significant associations were found between radiation exposures and increased risks of solid cancer, leukaemia and circulatory diseases in both the worker cohort and the cohort of Techa residents.

The objective of the SOLO subproject 1 (SP1) dealing with external dose reconstruction was to develop and implement a strategy for improving and validating the dosimetry systems in order to enhance the epidemiological studies. Three retrospective dosimetry methods were used for validation of external dose estimates: electron paramagnetic resonance (EPR) on human teeth; fluorescence in situ hybridization (FISH) on human lymphocytes; and luminescence on quartz extracted from old brick buildings. The key outputs from SP1 represent improved dose estimates which enhance the power or informative nature of epidemiological studies of the Southern Urals population thereby providing a more robust scientific basis for underpinning radiation protection standards. The findings of the project can be of great importance for our understanding of health effects from protracted radiation exposures.

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PARTRAC – Recent developments towards medical applications

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PARTRAC [1] is a multi-scale biophysical research MC code for track structure based initial DNA damage & damage response modelling. It integrates physics, chemistry, detailed DNA structure and molecular biology of DNA repair to assess radiation effects on cellular level. The DNA repair module describing the repair of DNA double-strand breaks (DSB) via the non-homologous end-joining pathway has recently been extended to simulate the yields and kinetics of radiation-induced chromosome aberrations by tracking the information on the chromosome origin of ligated fragments and the presence of centromeres [2].

Recent developments of PARTRAC are aimed at addressing open problems in medical applications of ionizing radiation in diagnostics and therapy. Ongoing experiments with quasi-homogeneously distributed compared to sub-µm focused bunches of protons, lithium and carbon ions allow a separation of effects due to DNA damage complexity on nanometre scale from damage clustering on (sub-) micrometre scale [3]. These data provide an unprecedented benchmark for the DNA damage response model in PARTRAC and help understand the mechanisms leading to cell killing and chromosomal aberration induction. For slow light ions the cross section data basis has been extended to investigate radiation effects in the Bragg peak region [4]. DNA damage calculations for slowing-down carbon ions below 50 MeV showed a decreasing trend for detached DSB and a constant yield of DSB clusters per dose. PARTRAC has also been upgraded to simulate dose and biological effect enhancement by gold nanoparticles (GNPs). Dose values inside the cell nucleus as well as yields of SSB and DSB were more increased due to the presence of GNPs in the cytoplasm if the same amount of gold was applied in smaller particles [5].

Ongoing and planned development of PARTRAC include coupling with a radiation transport code (FLUKA, Geant4) and track-structure based calculations of cell killing for RBE studies within the mixed field of an ion therapy beam.

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Out-of-field dosimetry in radiotherapy for input to epidemiological studies

Roger Harrison, on behalf of EURADOS WG9

The purpose of radiotherapy is to eradicate or control tumours whilst minimizing the dose to normal tissues. It has long been recognised that radiation carcinogenesis, amongst other late effects, is an inevitable risk of radiotherapy. The determination of out-of-field doses is therefore important for the estimation of radiocarcinogenic risks which are, in turn, needed for the fundamental risk-benefit assessment required for justification of the exposure. Out-of-field doses include organ doses remote from the target volume and also organ and tissue doses in the vicinity of the field margins where dose gradients are high and a significant proportion of second cancers occur.

Equally importantly, the total world population of radiotherapy patients provides a very large cohort of people treated with a range of target and organ doses from tens of Gy (target) to tens of mGy (peripheral organs) in a controlled and well-documented way. Studies of these cohorts will become increasingly important as the Japanese lifespan study eventually comes to an end. The combined dose delivered to organs at risk from both radiotherapy and the associated planning and verification imaging, is an essential pre-requisite for epidemiological studies of these cohorts.

EURADOS Working Group 9 is engaged in the measurement of out-of-field doses for contemporary radiotherapy treatment modalities, including the investigation and critical comparison of available dosimetry techniques. Our focus is currently on paediatric radiotherapy, because of the increased risk of radiocarcinogenesis in this group and the often-good prognosis for these patients, whose survival may be sufficiently long for the expression of a second cancer. The principles are, however, equally applicable to other patient cohorts.

Comprehensive organ dose measurements of a simulated paediatric brain tumour treatment using photon radiotherapy have been made using 5 and 10 year old anthropomorphic phantoms, each treated using conformal radiotherapy, IMRT and gamma knife treatment modalities at three European radiotherapy centres. Where possible, comparisons of measurements with Treatment Planning System (TPS) calculations have been made. Organ doses have also been measured for a simulated paediatric craniospinal treatment. Out-of-field doses have been measured for an identical treatment using a European proton beam facility. A comprehensive investigation of secondary neutron doses and spectra within the treatment room has also been made together with an intercomparison of several neutron dosemeters.

Dosimetry techniques have included the use of TLD, RPL and OSL dosemeters for photon measurements, and bubble and track etch detectors where secondary neutron fields are present. This has enabled a detailed comparison of dosemeter responses to be made for a range of energy spectra and for a wide range of doses. These results form the foundation for the following further investigations:
• Integration of therapy doses and imaging doses from radiotherapy planning and verification procedures to give a complete organ and tissue dose specification for radiotherapy patients for input to epidemiological studies;

• Development of general guidance for the harmonization of out-of-field dosimetry techniques;

• Use of experimental data for testing and validation of (i) analytical models for remote organ dosimetry and (ii) TPS calculations in regions remote from the target;

• Comparison of radiotherapy modalities and treatment techniques from the standpoint of critical organ doses and the risk of second cancer induction;

• Comparative evaluation of existing and new dosemeters for organ dose measurement.

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Session 8
Infrastructures, education and training
Education and training in support of radiation protection research

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In January 2009, the report of the High Level and Expert Group on European Low Dose Risk Research identified that because many of the harmful effects of ionising radiation are long-term, a research programme to gain a better understanding and quantification of these effects must extend for decades into the future. Indeed one of the main reasons for the creation of the MELODI platform was to produce a stable and continuing structure to plan and coordinate research for the long term. An inevitable challenge for an area of science that stretches back over a century is to ensure a succession of high-level scientists to carry it forward. This is more likely to succeed if there is a strong programme of education and training to attract and hold gifted young students into the field.

The DoReMi Network of Excellence recognised the importance of E & T and dedicated a work package to the development and support of various E & T initiatives. See (http://www.doremi-noe.net/training_and_education.html).

DoReMi finishes at the end of 2015 after 6 years, and during this period, a number of important conclusions were drawn:

- The key time to attract and engage new students is at the MSc phase, through project work;
- A series of sponsored short courses on various RP subjects was very effective at giving students an experience of the research area, and encouraging networking;
- E&T must be seen as an intrinsic part of any research programme that benefits both the programme and the scientific community as a whole;
- In order to be responsive to the needs and take the best advantage of available resources a continuing dialogue is needed with the existing RP research and E&T community;
- There is a need for a continuing body that survives individual projects to motivate and coordinate the E&T effort in radiation protection research.

In response to the last of the conclusions, a continuing E & T Working Group was established by the Board of Directors under Article 10 (Committees and Working Groups) of the MELODI Internal Rules at the start of 2014. The main task of the WG has been to formulate a Strategic Agenda for E&T, setting out a plan for how E&T should be supported into the future. The first draft of the SA has been completed, and this will be presented.

With the start of the new European Joint Programme CONCERT in June 2015, the opportunity has been taken to build on the experience and successes of DoReMi to ensure E & T is given due emphasis and support within the research programme. The catalogue of initiatives within the CONCERT E & T work package will be presented.
Infrastructures

Laure Sabatier

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Infrastructures include so-called large infrastructures such as exposure facilities including those for animal and plant experiments (both laboratory and field facilities), epidemiological cohorts, biobanks, databases and analytical platforms (including e-infrastructures).

The current state of research infrastructures for low dose radiation biology research will be presented. Lessons learned from how DoReMi and the Melodi WG Infrastructures have addressed the needs of the radiation biology research community along with strategies from other projects and research platforms will be presented and taken into consideration in mapping the way forward.
Infrafrontier

**Michael Raess**, (General Management INFRAFRONTIER GmbH)

**INFRAFRONTIER - Making of a European Research Infrastructure**

INFRAFRONTIER is the European research infrastructure for the development, phenotyping, archiving and distribution of mouse models of human diseases. It enhances medical research by providing access to a unique collection of mouse models, research tools and associated data, and to state of the art technologies for mouse model development and phenotype analyses. The INFRAFRONTIER resources and services are provided by leading research institutions in 12 European countries and are used by a global community of biomedical researchers. INFRAFRONTIER was selected as one of 6 biomedical research infrastructures on the ESFRI Roadmap in 2006 and was prioritised on several national roadmaps. During the EC-funded Preparatory Phase 2008-2012, coordinated at the Helmholtz Zentrum München, the business plan, legal agreements and scientific strategy for the INFRAFRONTIER Research Infrastructure were developed. In April 2013 the INFRAFRONTIER GmbH was established as the coordination unit of INFRAFRONTIER. INFRAFRONTIER is involved in a number of European and global research initiatives, among them the International Mouse Phenotyping Consortium (IMPC) and the International Rare Diseases Research Consortium (IRDiRC).
RENEB – The Future

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The purpose of RENEB is to act as sustainable European Network of Biodosimetry, capable to perform rapid biodosimetric dose estimation in large-scale radiological scenarios. The network infrastructure is based on reliable assays and techniques combined with high performance standards of the partners and critical inclusion of biomarkers and methods. To enhance the visibility and viability of the network, RENEB will be linked to global Emergency Preparedness and Response systems as well as to the European radiation research area.

Following large-scale nuclear accidents or radiological emergencies, the medical and radiological classification (triage) of patients according to the degree of their injuries and the level of their radiation exposure will be required in the shortest possible time. In this context, biological and physical dosimetry have been identified as effective, reliable, independent and complementary tools for individual dose estimation. To enhance the capacity of single national laboratories, networking has been recognized as a sensible and important emergency response strategy and the establishment of a European network was initiated. 3.5 years after starting the project by the EU, RENEB is preparing its transition from a funded project to a self-sustainable network, ready to persist also in the future. In this regard, the benefit and capabilities of RENEB for Emergency Preparedness and Response Systems is shown together with initialized co-operations with national and international emergency and preparedness organizations such as IAEA, WHO and relevant directorates of the European Commission. Aside from accident management, the network with its established strategies to guarantee equally high performance between the partner laboratories has the ability and capacity to contribute to large-scale research approaches. Furthermore, the critical identification and integration of new and upcoming technologies will have impact on the European radiation research area. In this regard, links to leading European Platforms like NERIS, MELODI, EURADOS and ALLIANCE have been initiated and RENEB was identified as network with benefits especially for large scale research approaches such as molecular-epidemiological or follow-up studies. In this context, RENEB will act as analysis infrastructure for research projects, offer E&T activities to assure the maintenance of competence and provide a QA&QM concept that will help to actively identify, verify and integrate new techniques and biomarkers of exposure.

The well-organized and harmonized cooperative action between the RENEB labs will offer a realistic chance for a rapid and trustworthy individual dose assessment needed in a radiological large scale emergency situation. Additionally, the network will have impact on the European radiation research area. In both regards, RENEB will provide a unique infrastructure, high quality standards in application and validation of biomarkers and maintenance and advancement of scientific and technical competence.

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Setups for exposing cells and animals to gamma radiation at low and high dose rate and to mixed beams of low and high LET radiation

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Judgement on the shape of the dose-response relationship for cancer risk at low doses and/or low dose rates for adverse health effects is a critical issue for radiation protection policy. Also, an open question is if exposure of cells to a mixed beam of high and low LET radiation (a frequent exposure scenario in areas of high natural background radiation, external beam radiotherapy and in aeroplane and space travel) leads to effects that can be predicted by assuming additivity of the single beam components.

At the CRPR we designed and built radiation facilities dedicated to chronic irradiation of cells at low dose rates. We can thus study the effects of both low doses and low dose rates. A low dose rate animal irradiation facility has also recently been constructed and will be used from autumn 2015.

In addition, we also constructed an irradiation facility where cells can be simultaneously exposed to a mixed beam of X-rays and alpha particles. This facility is used to study the question if exposure to mixed beams yields a higher than predicted level of DNA damage, both in terms of quantity and quality (complex/clustered DNA damage).

We also have two high dose rate gamma irradiation sources that can be used as positive controls in low dose/dose rate studies. The facilities will be presented in more detail.

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Session 9
NERIS-MELODI cross cutting themes
Thyroid cancer surveys after nuclear emergencies

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Thyroid cancer is a rare form of cancer, accounting for less than about 1% of all cancer cases. It is about three times more common in women than in men. The baseline rate of childhood thyroid cancer is a few cases per million children per year. In addition to significant exposures to ionising radiation, certain hereditary genetic syndromes and lifestyle choices such as obesity can increase the probability of developing thyroid cancer. In the absence of a biomarker, it is not possible to distinguish a radiation-induced thyroid cancer in an individual from one that occurs due to other causes. If treated correctly, the cure rate of thyroid cancers is high. Studies have shown that after 20 years of follow-up from the date of diagnosis, 99% of individuals are asymptomatic (symptom-free).

The only proven, albeit comparatively rare environmental risk factor for the development of thyroid cancer is the exposure to ionising radiation. Several studies have pointed to an increased risk of thyroid cancer in children because of radioactive fallout from nuclear weapons or nuclear emergencies (Chernobyl). The absorbed doses to the thyroid for members of the public from the Chernobyl accident ranged from zero to greater than 1,000 mGy. Thyroid doses estimated for children in Fukushima Prefecture were less than 100 mGy. The experience available has demonstrated that an absorbed dose (i.e., >100 to 250 mGy) to the thyroid could be expected to increase the probability for developing thyroid cancer in those exposed as children (from 0 to 18 years of age) in a measurable way. Studies of the Japanese Life Span Study (LSS) identified that for those individuals with an absorbed dose to the thyroid less than 100 mGy, the probability of developing thyroid cancer is uncertain due to limited statistical power and precision. What we know is that there is a clear age and gender dependence of the risk to develop thyroid cancers after exposure to ionising radiation with a maximum of the observed incidence rates for children with age at exposure below 10 years. A time delay (latency period) of > 3 years has been observed before any excess incidence became statistically significant. The excess incidence persist for more than five decades after exposure.

Based on the available evidence from situations resulting in absorbed doses well above 250 mGy it is obvious that screening of the population could only account for some of the observed increased frequency of thyroid cancer, but certainly not all. Screening that was undertaken following nuclear accidents has resulted in the detection of thyroid nodules as well as cancer cases that would have never lead to any clinically observed health effects and, therefore, would never be detected outside such screening programmes (“screening effect”).

The assessment of thyroid cancer risk after low-to-moderate thyroid dose exposure (50-500 mGy) during childhood and adolescence is subject to ongoing research including detailed investigations.
of the sources of possible statistical uncertainties of the available screening programs as well as additional uncertainties like dose or model uncertainties and confounding.

During the early phase of nuclear emergencies rapid measurements of the activity in the bodies of people possibly internally contaminated are needed to separate them from those not contaminated and to initiate medical consultation and/or follow-up activities if necessary. Direct measurements methods for internal contamination that can be conducted during the first days of a nuclear emergency are thyroid measurements with hand-held scintillation probes (preferably NaI or CsI detectors) or transportable body monitors. Measurement data, estimated doses and information relevant to retrospective studies of population exposures must be stored in a way that permits later retrieval.

State of the art of early thyroid screening programs are based on standardized procedures, that includes both technical equipment for the measurement of the radioiodine content of the thyroid and templates for reporting, data handling and dose calculation. Equipment taken out of everyday use is preferred over standby equipment to assure that operators are trained, and instruments are functional and calibrated. The hospital setting is ideal for performing the thyroid measurements as well as a system for handling individuals based on readily available communication skills.

The conversion of the measured dose rates into organ doses requires detailed knowledge about the type(s) of incorporation (inhalation and/or ingestion) as well as about the time(s) of incorporation. This information might not be readily available with sufficient accuracy even under optimal conditions. The resulting uncertainties can be large (> than a factor of 2). Despite these uncertainties, there is an urgent need to convey the measurement result to the screened individuals in standardized language non-experts can understand. Insufficient information disclosure will result in distrust with response organizations. For no-risk or acceptable-risk results where individuals will be dismissed from further medical consultations it is advisable to use categorized statements like “No immediate health effect expected – negligible risk of long term effect” or “No immediate health effect expected – little risk of long term effect” rather than numbers of dose rates or organ doses alone. Experts in communication should be involved in choosing the appropriate wording.

In addition to early thyroid screening programs a longer term follow up of the possible impact of a nuclear emergency situation is advisable for the purpose of public reassurance as well as the early identification of thyroid cancer development (as a basis for early medical follow-up) and/or for scientific purposes (improvement of current understanding of risk factors). Children who received higher thyroid doses (>20 mGy) at the ages up to 1 year and up to 10 years at the time of the radiation exposure should be provided a regular medical control with respect to possible abnormalities of the thyroid. At thyroid doses well below 10 mGy screening would not be reasonable unless there is a demand by the parents. There is an urgent need in the affected population for individual care and counselling provided by professional services (eg. psychologists, sociologists, clinicians) to better understand and cope with the radiological situation. The basic needs for documentation and communication of the results are similar to those described for the early phase.

Long-term thyroid cancer surveys are typically based on thyroid ultrasound examinations including, if indicated, confirmatory examinations of blood, urine, fine-needle aspiration, and cytology.
measurements. One aim of such screening programs is the early identification thyroid cancer development as a basis for early medical follow-up. For situations with sufficiently high exposures the aim of thyroid screening programs is the assessment of age- and gender-specific risk factors for the development of thyroid cancer.

The scientific interpretation of the results obtained by long-term screening programs will be challenged by the scientific uncertainties of our understanding of the thyroid cancer development and the role of potential precursors identified within the cancer development as well as by uncertainties in estimating individual exposures. It is, therefore, important to improve the available dosimetric information within the exposed populations and to compare the findings in groups with different exposures including the findings in “reference” groups living in “non-exposed” areas.

Comparison of the incidence data observed in exposed populations with those in non-exposed areas provides the basis for the assessment of excess cancer risk factors. Alternatively, if available, detailed baseline risk data documented by national cancer registries might be used as a reference. Radiation risk factors can be derived from such a screening program if minimum requirements of good epidemiological praxis (consideration of statistical power of the study, stratification for age and gender, suitable comparison groups, long term follow-up, confounding factors, etc.) are met and – as a minimum - reliable information is available about individual or else the group dose of the members of the cohort under investigation.

It is strongly advisable to include, as a minimum, outline planning for activities and programs of this kind in the preparedness system for nuclear emergencies.

Priority areas for further improvement are to:

- increase the precision of methodologies to measure and/or estimate individual radiation doses to the thyroid;
- define and apply minimum standards for the establishment of state-of-the-art epidemiological studies;
- improve the availability and the quality of population-based cancer registries as well as the establishment of Tissue Banks;
- establish and implement short- and long-term communications strategies and plans for outreach to and dialogue with stakeholders, particularly the affected population.

*http://www.nhs.uk/conditions/cancer/Pages/Introduction.aspx*
PRIODAC, a project to determine the modalities and side effects of multiple administrations of stable iodine to protect people chronically exposed to radioactive iodine

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Accidents occurring in a nuclear reactor may result in environmental releases of large amounts of radioactive gases and particles that can be inhaled by people exposed to the plume and may contaminate durably the affected territories. Among the isotopes present in discharges, are particularly iodine-131 and other short-lived isotopes of iodine. In the absence of appropriate protective measures, the population exposure to iodine may lead to thyroid cancer, especially in young children. The health consequences associated with exposure to iodine may nevertheless be limited thanks to the implementation of protective measures, such as sheltering people, evacuation, restrictions in food consumption and ingestion of stable iodine tablets.

Decided in France under the authority of the “Prefet”, the administration of iodine tablets to the population is a major issue. Indeed, stable iodine tablets should be given to exposed people ideally two hours before exposure to the plume or failing that, within 24 hours at the latest. However, the recent Fukushima Dai-ichi disaster has revived questions about the conditions of stable iodine prophylaxis implementation. Indeed, this accident has shown that the "iodine doctrine" which calls to date a single dose of potassium iodide may not adequately protect people exposed to repeated discharges of radioactive iodine. Thus, even though the current French doctrine envisages the possibility of a second administration if the evacuation of people cannot be achieved rapidly, it doesn’t provide so far recommendations for repeated administrations of iodine stable.

Moreover, from the regulatory perspective, the authorization of potassium iodide tablets does not envisage repeated administrations; this authorization was given for a single dose of stable iodine repeated once only. Thus, health authorities are powerless in situations of chronic discharges of radioactive iodine because of lack of knowledge about how implementing repeated administrations of stable iodine. In addition, side effects caused by these repeated administrations are poorly understood and call for further analysis of the regulating mechanisms of the thyroid function. The difficulties posed by the rapid implementation of stable iodine prophylaxis emphasize the need for a diversification of the countermeasures’ arsenal by providing innovative preventive strategies. Finally, the iodine prophylaxis may not be effective in an exposed person and must be optimised for specific populations at risk.

The PRIODAC project aims to determine modalities of repeated administrations of stable potassium iodine (KI) in a situation of chronic radioactive releases, to assess the associated side effects, to better understand the molecular mechanisms regulating the metabolism of iodine, and eventually to revise the regulatory French authorization of 65-mg KI tablets. The project will benefit to the evolution of international practices related to the administration of KI towards a better harmonization.

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Integration of iodine tablet distribution into countermeasures strategies

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In the early phase of a nuclear accident, three basic countermeasures are available namely sheltering, evacuation and distribution of iodine tablets. To limit the dose in the first year, further management options can be implemented, trying to minimise the exposure from all pathways. Iodine prophylaxis on the other hand typically is treated separately and has a specific reference level that is independent from the one used for the first year, related to the effective dose and not to the dose of one single organ.

Within the European project NERIS-TP, that brought together 19 organisations from research and operational emergency centres and ended January 2014, a particular simulation model was developed aiming to explore the combination of countermeasures to limit the dose from all exposure pathways – including ingestion – to a given reference level. Within this model that is part of the JRODOS system, it is possible to define for example areas in which the combination of sheltering and distribution of iodine is appropriate in the early stage to limit the residual dose to a pre-defined value. On the other hand, the tool has the capability to examine the need for combination of sheltering and iodine distribution in case an evacuation is delayed.

The simulation is important for the strategic decision-making; however, several questions require answers by other means. Important in this respect are for example the questions about a possible second intake, the distribution to various age groups and the feasibility and acceptability of the proposed countermeasures. This paper discusses possible solutions, in particular how to judge about the feasibility of combined measures such as sheltering and distribution of iodine tablets.
Recent developments regarding iodine thyroid blocking in Switzerland and Germany

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As many European countries, Germany and Switzerland, decided after the Fukushima accident to review the legal and organizational framework of their nuclear emergency preparedness and response approaches. In this context, the Iodine thyroid blocking (ITB) countermeasure, especially the strategy of the pre-distribution of KI tablets presents a major challenge. Within the NERIS community, this issue has been debated and the presentation proposes to account the German and Swiss positions.

The main outcome in Switzerland was the update of the ordinance on the distribution of iodine tablets, which highlights the extension of the zone of pre-distribution to all residents from 20 km up to 50 km.

In Germany, the range of accidents considered in emergency planning was redefined after the Fukushima accident. The new definition resulted in a revision of emergency planning zones around nuclear power plants. For example, the planning for ITB for children, adolescents and pregnant woman has been extended to the entire territory of Germany. Before 2014, the ITB planning for children was limited to a maximum distance of 100 km from the nuclear power plant.

The following 3 questions need further clarification:

- What about repetitive intake of iodine tablets in the case of long term releases?
- What about indication against taking ITB (age dependence, iodine pathologies)?
- What about the expiration dates of ITB tablets?
Session 10
Radiation effects on stem cells
Radiation responses in normal human breast stem cells

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All tissues are organised in a hierarchical structure with stem cells being the key cell types underlying their development. They play defining roles in development of organisms and in homeostasis of adult tissues. Stem cells have unique properties amongst cell types namely; they have the ability to renew themselves through division and the potential to differentiate into a range of different tissue specific cell types. Stem cells are known to be long-lived leading to an increased potential for the accumulation of mutations potentially leading to genetic changes. There is also maybe significant commonalities between the role of stem cells and those of cancer stem (or cancer initiating) cells. Stem cells may be important target cells for radiation effects but knowledge is limited to a few tissues at present and the likely mechanisms underpinning their role in radiation-induced carcinogenesis. Using cell sorting approaches, targeting a range of markers including EpCAM, MUC1, CD49f CD44 and CD24 and aldofluor activity, it has been possible to isolate sub-populations of epithelial cells from normal breast tissue obtained from reduction mammoplasty patients. More importantly, the low-dose radiation response for DNA damage, proliferation, differentiation and survival has been characterised in these subpopulations and in defined 2D and 3D models which replicate the key cellular interactions of the human breast. This work forms the basis for further mechanistic studies which can feed data into biophysical models of radiation-induced carcinogenesis.

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Functions and secretome activity of human mesenchymal stem cells are affected by low dose radiation treatment

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A few findings have addressed the effect of radiation on the mesenchymal stem cells (MSC). These cells are of great interest since they can differentiate in bone, cartilage, and fat; support hematopoiesis; contribute to the homeostatic maintenance of many organs and tissues; and modulate inflammatory response.

In this study we showed that the main outcome of MSC low radiation exposure (40 mGray of X-rays), besides reduction of cell cycling, is the triggering of senescence, while the contribution to apoptosis is minimal. We also showed that low radiation affected the autophagic flux. Our results allow us to hypothesize that autophagy prevented radiation deteriorative processes, and its decline contributed to senescence along with a loss of stemness. Increase in ATM staining one-hour post-irradiation and subsequent decline to basal level at 48 hours, along with a persistent gamma-H2AX staining, indicated that MSC properly activated the DNA repair signaling system, while some damages remained unrepaired, mainly in non-cycling cells (Ki67-).

It is evident that senescence process may greatly affect also the composition of MSC secretome through a shift from a functional paracrine signalling to production of senescent-associated secreted factors that have potent autocrine and paracrine activities. Changes in secretome profiles of MSC may great impair their activities, which depends on the capability to secrete many factors. We performed a comparative analysis of human MSC secretome from control and X-ray irradiated cultures (40 mGray) and evaluated if factors secreted from irradiated MSC cultures may induce senescence, or arrest proliferation, or promote cytotoxic effects in young MSC. We carried out also a secretome analysis by LC-ESI-MS/MS to identify proteins secreted by MSC following low dose radiation. Preliminary data suggest that proteins involved in apoptosis and senescence pathways are enriched in irradiated secretomes. This study is of interest since alteration of MSC secretome may have wide repercussions on a body’s health given the huge number of cytokines, growth factors and modulators of the immune system that are produced by MSCs.

To further analyse the effects of low dose radiations on MSC we started a study on the effect of gamma rays and alpha particles (40 mGray) on MSC from bone marrow and from Wharton’s jelly of the umbilical cord. Preliminary data evidences that also these type radiations at low doses induced mainly senescence phenomena. Further study will be carried out to evaluate similarities and differences among these stressors.
α-particle-induced complex chromosome exchanges transmitted through extra-thymic lymphopoiesis in vitro

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Human exposure to high-linear energy transfer α-particles includes environmental (e.g. radon gas and its decay progeny), medical (e.g. radiopharmaceuticals) and occupational (nuclear industry) sources. The associated health risks of α-particle exposure for lung cancer are well documented however the risk estimates for leukaemia remain uncertain. To further our understanding of α-particle effects in target cells for leukaemogenesis and also to seek general markers of individual exposure to α-particles, this study assessed the transmission of chromosomal damage initially-induced in human haemopoietic stem and progenitor cells after exposure to high-LET α-particles. Cells surviving exposure were differentiated into mature T-cells by extra-thymic T-cell differentiation in vitro. Multiplex fluorescence in situ hybridisation (M-FISH) analysis of naïve T-cell populations showed the occurrence of stable (clonal) complex chromosome aberrations consistent with those that are characteristically induced in spherical cells by the traversal of a single α-particle track. In addition, newly arising de novo chromosome aberrations were detected in cells which possessed clonal markers of α-particle exposure and also in cells which did not show any evidence of previous exposure, suggesting ongoing genomic instability in these populations. Our findings support the usefulness and reliability of employing complex chromosome exchanges as indicators of past or ongoing exposure to high-LET radiation and demonstrate the potential applicability to evaluate health risks associated with α-particle exposure.
Radiation induced carcinogenesis of thyroid gland stem cells.

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Proton therapy in cancer treatment is a promising approach, since it is possible to more precisely localize the radiation dosage compared with other types of external beam radiotherapy. However, out-of-field neutron exposure of proton therapy might lead to enhanced formation of second cancers. This study addresses the Relative Biological Effect of neutron for the carcinogenesis of thyroid gland stem cells (TGSC). To study this a novel model was developed to study mouse TGSC in vitro. Cells were isolated from murine thyroid gland tissue and grown in non-adherent cultures in serum-free conditions to form thyrospheres/thyroid organoids. Primary spheres/organoids expressed thyroid specific genes confirming that the cells were derived from the thyroid gland. A part of the cells were able to form secondary spheres after passaging which could be continued for more than 10 passages with increasing efficiency confirming their self-renewal capacity and expansion potential of thyroid. Furthermore, these cells are capable of forming organoids expressing major thyroid gland lineages such as thyroglobulin and cytokeratin 14 expressing cells. Functionality was shown after transplantation of dissociated organoids underneath the kidney capsule in radioactive I131 treated hypothyroid mice. Eight weeks after transplantation, thyroid follicles were present under the kidney capsule, which grew in size in time. These follicles contained thyroid specific cell types, visualized by thyroglobulin and thyroid transcription factor-1, indicating that adult stem cells isolated from murine thyroid tissue can generate thyroid tissue in athyroid mice. This model was used to assess the radiation response of thyroid gland stem cells. Exposure of thyrosphere derived cells showed for survival a reversed RBE for neutrons and stem cell survival at low doses (<< 1 Gy) versus to high doses (≥ 1 Gy for Neutrons, > 2 Gy for X-rays). Currently, the γH2AX foci after 24 are analyzed to assess remaining DNA damage. Long-term passaging revealed changes in growth speed, stem cell marker expression and cancer associates genes changed in individual samples, albeit due to insufficient number data points not dose dependently.

Conclusions, thyroid stem cells can be cultured in vitro to assess radiation response. Thyroid stem cells have an interesting response to low doses of photon radiation and the model most likely can be used to assess carcinogenesis with sufficient experiments. The RBE of stem cell survival seems different at low doses than high doses.

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Session 11
Medical-MELODI cross cutting themes
Circulating microparticles associate to severe radiation proctitis consecutive to abdomino-pelvic radiotherapy

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Microparticles (MPs) are membrane fragments with biological activities shed from damaged or activated cells. MPs have been studied as biomarkers in several inflammatory diseases and as central players in intercellular communication. In this study we investigated the potential use of MPs as biomarkers in patients suffering from radiation proctitis consecutive to complications of abdomino-pelvic radiotherapy. In addition, we evaluated the relationship between circulating MPs and endothelium-dependent responses.

Flow cytometry analysis of platelet-free plasma from a cohort of 217 patients overexposed to irradiation indicated that circulating levels of Annexin V⁺ MPs displayed a 3-fold increase in grade 3 patients (SOMALENT scale) as compared to patients with grade 0, 1 and 2. Moreover, platelet-derived CD41⁺ MPs constituted the major sub-population compared to leukocyte, monocyte, endothelial and red blood cells in all groups. Using a clotting assay, we measured the procoagulant activity of MPs and we found that thrombin generation velocity tended to decrease in grade 3 patients compared to other groups. Finally, MPs from grade 3 patients did not affect endothelium activation in comparison to the other grades.

Our data demonstrate that high level of circulating MPs is correlated to the grade 3 patients with radiation proctitis. These results suggest that detection of circulating MPs may be valuable for the prognostic of radiotherapy complications. Eventually, this study could contribute to propose a new anti-MPs therapeutic approach for the treatment of radiation-induced pelvic disease.
Risk of secondary cancer induced by radiotherapy

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Ionising radiation is a two-edged sword with respect to cancer. On one hand, radiation has been used for the treatment of malignant tumours, alone or in combination with other modalities, for more than 100 years. On the other hand, it is well known that radiation is a carcinogenic factor. During the last decades, life expectancy for many cancer patients has increased due to improvements in both early detection and therapy methods. Late effects have therefore become a matter of concern for the long-term survivors of cancer therapy. Several studies have investigated the occurrence of secondary cancers in patients undergoing radiation therapy and concluded that radiation-induced cancers should be included among the late complications of radiotherapy even though they are considered the price of success for modern radiation treatment that results in improved survival rates and better quality of life for many patients. Thus, a question consequently arises about the quantification of the risks for late effects after radiotherapy.

Given the long latency of cancer induction that could extend over decades, the initiation of epidemiological studies that will study the radiation effects among the long-term survivors of radiotherapy is not a viable solution. It is therefore advised that theoretical modelling with the best models and parameters available should be used instead for the study of this secondary effect of radiotherapy and the quantification of the risk levels that may be associated with various treatment approaches. This presentation will give an overview of several models available in the literature for the assessment of risk of secondary cancer. It will also focus on the applicability of the models for risk of secondary cancer to advanced radiotherapy techniques and the optimisation of radiation treatment highlighting the particular challenges for radiation protection research in medicine with respect to the risk of secondary cancer.
Current developments in CT technology – potential benefits for radiation protection?

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Computed tomography (CT) constitutes a mainstay of present day radiological diagnostics. Through a number of technical innovations, the range of clinical applications of CT has been constantly extended, and new radiodiagnostic indications have become accessible. At the same time, this has also lead to an ever increasing contribution of CT examinations to the collective exposure of patients to ionizing radiation: In the year 2012, for example, 9 % of all diagnostic procedures requiring the use of ionizing radiation performed in Germany were CT examinations, contributing to 62 % of collective effective dose. On average, estimated effective doses to a standard patient range from ~2 mSv for a head up to ~25 mSv for an abdominal CT examination, although average effective doses below 1 mSv have been reported and are achievable, e.g. for chest CT exams, when employing new technology such as iterative image reconstruction algorithms.

Therefore, radiation exposure to the patients associated with CT imaging remains a matter of concern, and there is great interest in technical approaches for its reduction. Current research efforts focus on reducing radiation exposure by enabling CT imaging at lower or lowest noise levels. To this end, current detector designs aim at noise reduction by higher integration of readout electronics and circuitry into the detector backplane, and photon counting detector technology virtually without any electronic noise is under development. On the other hand, novel iterative image reconstruction techniques (IR) may be used for reconstruction of images with lower noise from intrinsically noisy low-dose acquisitions. IR, in turn, requires research on novel metrics for image quality determination adequately reflecting IR’s non-linear characteristics leading to a decoupling of image noise and radiation exposure.

The example of IR illustrates the key challenge that has to be met by any practical approach to and any technical research for radiation protection / exposure reduction in medical imaging: It is the preservation of diagnostic image quality for each specific imaging application in clinical routine, requiring dedicated research in view of applicability, limitations and consequences of radioprotective measures.

The medical scientific research agenda (CARPE-M) reflects the necessity of such research. Furthermore, it foresees research on in-vivo patient exposure, e.g. caused by diagnostic procedures such as CT imaging, and on the possibly inhomogeneous loco-regional distribution of dose through accurate dosimetric assessment (EURADOS). This research could provide insights into dose-response relationships useful for the modelling of radiation-induced health effects, serving as input for epidemiology (MELODI). Overall, CARPE-M aims at a better protection from ionizing radiation and its optimized use, especially in view of patients individually highly susceptible to low doses of radiation as identified by research of MELODI.
3D patient-specific and equipment-specific dosimetry in CT: from conceptus to the adolescent

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Several Monte Carlo codes and stylized mathematical anthropomorphic phantoms simulating pregnant female patients have been used for the simulation of radiological exposures performed on patients. CT simulation software packages have been recently developed to adapt simulation to a) scanner specific geometries and b) individual patient characteristics. These tools are capable of creating three-dimensional (3D) voxelized phantoms using patient CT images as input volume. After specifying parameters associated with scanner and scan protocol characteristics, the Monte Carlo code performs a simulation of the CT scan. The output is a series of dose images that depict radiation dose. The aim of this work is to present the benefits of three-dimensional patient-specific and equipment-specific Monte Carlo simulation for CT dosimetry.

CONCERT (Conceptus Radiation Doses and Risks from imaging with ionizing radiation) is a research project which aims to perform original research from which new findings, innovations and practical guidelines for optimal clinical management of pregnant patients needing radiologic procedures will result. Voxelized models have been created on the basis of CT image data from patients. Modern MDCT scanners have been modelled. Each scanner’s geometry, x-ray spectrum, composition and dimensions of the filters have been taken into account for the simulation. The simulation output provides dose information normalized to CTDI/100 mAs. The number of photons produced for each simulation is about $10^9$, resulting in a very low statistical inaccuracy (<1%). A patient-specific method of estimating conceptus radiation dose from CT examinations has been developed taking into account features associated with modern CT equipment.

Paediatric thorax CT examinations have also been simulated in our institution. Voxelized models have been created on the basis of CT image data from patients. Organ doses normalized to CTDI<sub>vol</sub> have been derived for main organs exposed primarily. A method of estimating organ radiation dose from CT examinations performed on children and adolescents will be presented which takes into consideration patient’s body size.

Acknowledgements: CONCERT is supported by the Greek Ministry of Education and Religious Affairs, General Secretariat for Research and Technology, Operational Program 'Education and Lifelong Learning', ARISTIA (Research project: CONCERT).
Common Strategic Research Agenda for Radiation Protection in Medicine by the European Medical Associations representing Ionising Radiation Applications in Medicine


Helmholtz Zentrum München, Munich and Otto-von-Guericke University, Magdeburg
Technical university Munich and Helmholtz Zentrum München, Munich (ESTRO)
University of Crete, Iraklion (EFOMP)
Medical University of Vienna (ESTRO)
Hôpital Européen Georges Pompidou, Paris (ESR)
University hospital Mannheim, University Heidelberg, Mannheim (EANM)
AZ Sint-Jan Brugge-Oostende, Brugge (EANM)
University College Dublin (EFRS)
Instituto Politécnico de Coimbra (EFRS)
University Heidelberg, Heidelberg (ESR)
Konstantopoulio General Hospital of Athens (EFOMP)

In the past decade, the funding scheme of the EC for radiation protection research has been changed. While in the past topics had been identified by the commission and projects had been chosen based on corresponding calls, today, large scale projects are funded which organize the calls for scientific projects themselves. This is based on so-called strategic research agendas (SRAs) of corresponding platforms like MELODI, EURADOS, NERIS, ALLIANCE. The European associations dealing with medical applications of ionizing radiation (EANM, EFOMP, EFRS, ESR, ESTRO) did not have a common platform and more important they did not have a common SRA so far. During the large scale EC project the definition of a SRA was initiated together with MELODI and EURADOS. The SRA has been developed during the past 6 month. The main research areas that has been identified, will be ordered in the following areas:

1. Measurement and quantification of radiation exposure in the field of medical applications of ionising radiation
2. Tissue reactions and biological radiation risk
3. Optimisation of radiation exposure and harmonisation of practices
4. Justification of the use of ionising radiation in medical practice
5. Infrastructure for quality assurance

In each of the areas, there are a number of research topics identified, that will be presented during the MELODI workshop.

This SRA proposal has already been discussed with some stakeholders and within the boards of the associations – corresponding changes that were requested will be implemented during the next weeks. This SRA is supposed to be updated regularly and there will be recurrent request for input from the community. It will also be a fundamental step for the building of a common medical platform using ionizing radiation in medical applications.
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Diagnostic Reference Levels in plain radiography for paediatric imaging: a Portuguese study

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Objective: To determine diagnostic reference levels (DRLs) in plain radiography for the most frequent paediatric plain radiography exams in Portugal (chest, pelvis and abdomen) and to characterise a standard paediatric patient for each age group used in literature.

Methods: Anthropometric data was collected from 9935 patients. Each age group (<1, 1-<5, 5-<10, 10-<16, ≥16) was categorised by the median values of weight, height and BMI, to define a standard patient. Exposure parameters, kerma-area product (KAP-mGy.cm²) and entrance surface air kerma (ESAK-µGy) were collected. DRLs for KAP and ESAK were defined as the 75th percentile (P75) of dose values and presented by age and weight.

Results: In each age group the P75 of KAP varied from 11 to 77 mGy.cm² for chest; 23 to 816 mGy.cm² for pelvis; 25 to 979 mGy.cm² for abdomen. The P75 of ESAK varied from 49 to 67 µGy for chest; 98 to 1129 µGy for pelvis and 70 to 1060 µGy for abdomen.

Conclusion: The P75 of dose values determined in this study were lower than those published in literature. When available, weight is the preferred parameter to categorise paediatric patients. The large ranges of dose values found in this study, demonstrates a clear need for the optimisation and harmonisation of practice.
POSTER PRESENTATIONS

Session A
Whack-A-Mole Model: Towards a Unified Description of Biological Effects Caused by Radiation Exposure

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We present a novel model to for estimating biological effects caused by artificial radiation exposure, i.e., the Whack-A-Mole (WAM) model. It is important to take into account the recovery effects during the time course of cellular reactions. The inclusion of dose-rate dependence is essential in the risk estimation of low-dose radiation, while nearly all the existing theoretical models rely on the total dose dependence only. By analysing experimental data of the relationship between the radiation dose and the induced mutation frequency of five organisms, namely, mouse, Drosophila, chrysanthemum, maize, Tradescantia, we found that all the data can be reproduced by the WAM model. Most remarkably, a scaling function, which is derived from the WAM model, consistently accounts for the observed mutation frequencies of the five organisms. This is the first rationale to account for the dose rate dependence as well as to provide a unified understanding of a general feature of organisms.
A Lifetime Study in irradiated mice

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At the adult age of 10 weeks male and female hybrid mice (C57BL/6 x C3H F1) were acutely whole-body irradiated with low doses of ionising radiation (0, 0.063, 0.125 and 0.5 Gy) using a Co\textsuperscript{60} source. Over the following 24 months, the mice were examined monthly for lens opacities by Scheimpflug imaging; and every four months for retinal effects by OCT (optical coherence tomography). Behavioural tests were performed at 4, 12 and 18 months post-irradiation to evaluate possible alterations in cognitive function. To investigate the underlying mechanisms of radiation-induced effects additional groups of mice were sacrificed at different time points (4 and 24 hours, 12, 18 and 24 months after irradiation) for pathological examinations and organ collection. Histological and immunohistochemical analysis of different organs will be done. In addition, blood samples will be used for expression profiling in the search for potential biomarkers.

To estimate the contribution of genetic susceptibility mice heterozygous for an \textit{Ercc2} mutation were compared to wild-type mice. The recessive mutation was located in the \textit{Ercc2} / \textit{Xpd} gene (c.2209T>C) leading to a Ser737Pro exchange in the protein. The Ercc2 protein has DNA helicase activity and is involved in general transcription and DNA repair.

Evaluation of irradiated lymphocytes isolated from wild-type and heterozygous mutant mice indicate a greater radiosensitivity of the \textit{Ercc2} heterozygous mice. First analysis of the Scheimpflug examinations in 0.5 Gy irradiated mice did not show significant differences in lens opacity compared to the unirradiated control group up to 24 months after irradiation. OCT data showed a reduction of the retinal thickness in irradiated heterozygous mutants. Differences in the behaviour of irradiated and unirradiated wild-type mice were observed. This study is still in progress.
Thyroid cancer mortality in Belgium from 1969 to 2010

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Background:
Thyroid cancer is one of the cancer types that has ionising radiation as a risk factor. In previous studies an elevation of the thyroid cancer mortality rate has been found around the nuclear site of Mol-Dessel, Belgium. In this study we investigate the thyroid cancer mortality in Belgium with a focus on the nuclear site in Mol-Dessel, with more years of mortality data and more specific statistical methods than the previous study.

Methods:
The data used is for each municipality of Belgium (i) the mortality for thyroid cancer in adults, and (ii) the population. Both (i) and (ii) are stratified by sex and age categories of five years and range from 1969 to 2010. The disease mapping is done via the Bayesian hierarchical model with conditional autoregressive priors, and is fitted through Markov Chain Monte Carlo sampling. We also compute the standardized mortality ratio (SMR) to compare with the previous study. The Bayesian hierarchical model is a much more specific method compared to the SMR as it allows borrowing strength from neighbouring areas via the random effects.

Results:
Some municipalities present with a higher relative risk, although in general there are no clear, significant clusters of elevated relative risks of thyroid cancer mortality in Belgium found through the Bayesian hierarchical model. The municipalities surrounding the nuclear site of Mol-Dessel show no significantly elevated relative risk of thyroid cancer mortality. The standardized mortality ratio shows many areas with an elevated risk, and is thus less suited for disease mapping and detecting excesses in disease risk.

Conclusions:
There seems to be no indication of a relationship between the nuclear sites and thyroid cancer mortality in Belgium. The data used spans a very long period and this study is one of the first of its kind combining a long period of mortality data with recently developed spatial statistical methods. The municipalities that present with an elevated relative risk warrant further investigation in future work.
Study of biological effects of long-term exposure to low dose-rate radiation with Whack-A-Mole model

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By adopting Whack-A-Mole (WAM) model, which we have recently proposed to estimate the biological effects of radiations, we show how the explicit dose rate dependence built in WAM plays a key role to estimate biological effects caused by radiation exposure. The result may replace the so-called DDREF, the concept of which has long been adopted to estimate the effects of low dose rate radiation. DDREF is conventionally derived by comparing the high dose rate and the low dose rate results within the Linear-Quadratic (LQ) model which has no dose rate dependence in itself. In WAM model, the dose rate itself is the key ingredient. This makes the most essential difference between the LQ model and WAM model. Basic properties of WAM model are discussed emphasizing the dose-rate dependence. By adopting the parameters that are determined to fit the mega mouse experiments, biological effects of long-term exposure to extremely low dose-rate radiation are discussed. In WAM model, the effects of the long-term exposure show the saturation property, which makes a clear distinction from the LNT hypothesis which predicts a linear increase of the effects with time. In the case of mouse, the saturation time is of the order of a month. That means when we expose mice to low dose-rate radiation for a long time, the effects will saturate after a month. The saturation property is very important when we estimate the effects of the long-term exposure to extremely low dose-rate radiation which we now encounter in Fukushima. A simple-minded use of the LNT like linear extrapolation will result in a serious overestimation of the effects. It is very important to obtain reliable parameters to be used to estimate the biological effects of the low dose rate radiation for human beings.

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Mechanistic multi-stage models are used to analyse lung-cancer mortality after Plutonium exposure in the Mayak-workers cohort. Besides the established two-stage model with clonal expansion, models with three mutation stages as well as a model with two distinct pathways to cancer are studied. The results suggest that three-stage models offer an improved description of the data. The best-fitting models point to a mechanism where radiation increases the rate of clonal expansion. This is interpreted in terms of changes in cell-cycle control mediated by bystander signalling or repopulation following cell killing.

To elucidate the implications of the different models for radiation risk, several exposure scenarios are studied. Models with a radiation effect at an early stage show a delayed response and a pronounced drop-off with older ages at exposure. Moreover, the dose-response relationship is strongly nonlinear, revealing a marked increase above a critical dose.
Cardiovascular risks after exposure to low dose ionizing radiation: research at SCK•CEN

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Although thought to be radioresistant in earlier days, the cardiovascular system is now recognized as being vulnerable to ionizing radiation. Indeed, epidemiological findings suggest that cardiovascular diseases may be a health risk associated with radiation exposure. However, below 0.5 Gy an increased risk cannot be evidenced by epidemiology alone, and a better understanding of the underlying biological and molecular mechanisms is needed.

At the Belgian Nuclear Research Centre (SCK•CEN) research is ongoing in the field of cardiovascular risks after exposure to low dose ionizing radiation. For our in vitro models, we use cells derived from human endothelium, the inner lining of the cardiovascular system playing a pivotal role in normal vascular functioning. Endothelial cells are also believed to be critical in radiation-related cardiovascular diseases. In our in vivo models tissue derived from the murine heart is used to perform tests.

We demonstrated for the first time that acute low doses of X-rays induce DNA damage and apoptosis in endothelial cells (Rombouts et al Int J Radiat Biol. 2013 89:841). Our results pointed to a non-linear dose-response relationship for double strand break formation in endothelial cells. Furthermore, the observed difference in radiation-induced apoptosis points to a higher radiosensitivity of the immortalized EA.hy926 endothelial cell line compared to primary human umbilical vein endothelial cells (HUVEC), which should be taken into account when using these cells as models for studying the endothelium radiation response.

In the framework of the FP7 DoReMi project we performed a microarray study on chronic low-dose rate gamma irradiation of HUVEC cells (1.4 and 4.1 mGy/h; 6 weeks) (Rombouts et al. Int J Radiat Biol. 2014 90:560). The data pointed to an early stress response followed by a more inflammation-related expression profile in the first weeks, which was believed to underlie the observed premature senescence within the consortium. In addition, the microarray data showed an increased upregulation of IGFBP5 over time in irradiated samples compared to control samples, and a downregulation of IGFBP5-inhibiting genes PAPPA2 and CNOTL6. Therefore, we hypothesized that IGFBP5 signalling is involved in radiation-induced premature senescence.

In the EU FP7 ProCardio project the long-term consequences of exposure to low dose and low dose rate radiation was investigated. Immortalized human coronary artery endothelial cells (TICAE) exposed to acute low dose X-ray irradiation were sampled up to 14 days after exposure...
revealed disturbed cell cycle and proliferation, inflammation and senescence as shown by functional validated microarray data. Also dissected ApoE-/- mice hearts were analysed after low dose or low dose rate irradiation and sampling up to 300 days after exposure. Microarray data revealed disturbances in cell growth, vascular development, inflammation, insulin-related processes and intercellular communication.

In conclusion, our findings motivate further research on the shape of the dose-response and the dose rate effect for radiation-induced vascular disease in order to refine radiation protection.

Acknowledgements: This study was funded by the EU FP7 DoReMi (grant agreement 249689) on ‘Low dose research towards multidisciplinary integration’, the EU FP7 Procardio project (grant agreement 295823) and by the Federal Agency of Nuclear Control (FANC-AFCN, Belgium) (grant agreement: CO-90-13-3289-00). Harms-Ringdahl M. has received support for these studies from the Swedish Radiation Safety Authority. Rombouts C. was supported by a doctoral SCK•CEN/Ghent University grant. Baselet B. is supported by a doctoral SCK•CEN/Université Catholique de Louvain grant.
SOPRANO: Systems oriented prediction of radiation risk

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A systems radiation biology approach must be adopted to understand the complexities of the biological processes that determine individual sensitivity to low doses of radiation. The ultimate goal of a systems approach will be to develop a model of the cellular radiation response that reflects individual differences in age, gender and genetic constitution. The systems model will be able to incorporate known and newly discovered differences between individuals to personalize risk prediction.

The SOPRANO project is an 18 month activity that will assemble the first interdisciplinary research team in Europe to undertake a systems radiation biology approach. SOPRANO is a pilot study nature for radiation research, as this will be the first project that explores the interfaces between the three key components of systems biology: data generation, bioinformatic analysis and systems modelling.

SOPRANO is a research project of the OPERRA consortium. Open Project for the European Radiation Research Area Supported by a grant from the European Community’s Seventh Framework Programme (EURATOM) contract no. 604984
Radiation-induced inflammatory pathway may be biased or covered by culturing procedures in *in vitro* models

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**Background:**
The inflammatory response, and its key molecule NF-κB, play a central role in managing the different external stimulus types and intensities, especially at a systemic level. In particular, NF-κB activation might be either protective for the organism, orchestrating the immune response against exogenous agents, or harmful, leading to cancer and other diseases if chronically altered or mutated. Due to the non-linear complex intra-cellular cascade on which the inflammatory pathway is based, NF-κB activation dynamics and mechanisms after ionizing radiation exposure are still not completely understood.

**Purpose:**
Aim of the work is to unravel the complex non-linear dynamics of the NF-κB activation following ionizing radiation exposure and also understand the effects of common laboratory procedures (i.e. change of culture medium).

**Materials & Methods:**
AG1522 human fibroblasts were exposed to different doses of γ rays (up to 5 Gy). Samples were either ethanol fixed or nuclear/cytoplasmic extracts collected at different time points after the exposure. Samples have been analysed through ELISA assays or Immunocytochemistry protocols to investigate the nuclear NF-κB-p65 transcription factor temporal dynamics and possible perturbations due to the different stressors.

**Results:**
Quantitative measurements were performed with different techniques and results interpreted within a systems biology approach. Temporal dynamics of nuclear NF-κB analysed after different combinations of stimuli (e.g. change of culture media, CO₂ and temperature variations, radiation exposures) highlighted both the robustness of the inflammatory pathway (up to 5 Gy of γ rays) and the biases that the basic culturing laboratory procedures might introduce to a human fibroblast *in vitro* model, strongly perturbing the negative feedback loop response of the NF-κB [1].


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Effect of ionizing radiation on human coronary artery endothelial cells

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In recent years there is a growing epidemiological indication of excess risk of late occurring cardiovascular disease (CVD) at low doses without a clear-cut threshold (< 0.5 Gy). For the benefit of public health, it is now of importance to understand the underlying biological mechanisms to complement these epidemiological data. It is proposed that damage to the vascular endothelium is critical in radiation related cardiovascular diseases. Therefore this study is aimed to identify the low LET radiation effect on human coronary artery endothelial cells in the frame of radiation induced CVD.

Immortalized human coronary artery endothelial cells were exposed to low doses of X-rays (0.05 and 0.1 Gy) and compared to isogenic cells either sham-irradiated or treated at higher doses (0.5 and 2 Gy). After irradiation, cells were kept in culture for either 1, 7 or 14 days followed by total RNA extraction.

Microarray analysis uncovered the induction of a gene expression profile resembling a pro-atherosclerotic state in endothelial cells after exposure to ionizing radiation. Pathways shown to be involved are inflammation, blood coagulation, initiation of hypertension related processes and cell cycle progression. Indeed, irradiation exposure leads to the production of IL6 and MCP1. In addition, ionizing radiation lowers the production of the anti-coagulant TFPI. Interestingly, even at low doses we found a dose dependent G\(_{1}/S\) phase cell cycle block.

The above described results indicate that ionizing radiation induced different pro-atherosclerotic pathways in coronary artery endothelial cells. In the future, these findings can be used to ameliorate the current radiation protection system and can help to devise cardiovascular risk-reducing strategies, which can limit the amount of patients suffering from cardiovascular disease worldwide.

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Is former Tinea Capitis radio-epilation treatment a risk factor for atherosclerotic disease?

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Introduction
Low dose radiation exposure has been associated with non-cancer side effects such as cardiovascular disease, namely carotid atherosclerosis, although this issue is still controversial. This issue seems to be more critical for childhood exposure, as children are more radiosensitive and have a longer life expectancy. As we had access to a Portuguese cohort of former Tinea Capitis patients irradiated in childhood as a therapeutic measure, we decided to evaluate carotid atherosclerotic disease as a possible long-term side effect of low dose radiation exposure.

Methods
We randomly selected and observed 363 individuals from the 1375 cohort members previously observed for thyroid cancer and head and neck skin cancer. For comparison we gathered an age-matched control (n=223) mainly composed of participants’ spouses (90%). A B-mode ultrasound imaging of carotid arteries, a panel of blood serum measurements, including a lipid profile, homocysteine, HbA1c, hsCRP, and hepatic enzymes, and anthropomorphic measurements were obtained.

Results
We have observed that the irradiated individuals had more frequently plaques in the left carotid comparing with the non-irradiated ones (40.4 vs 28.5, p=0.06). No statistically significant differences were observed between the two study groups regarding carotid intima-media thickness and percentage of stenosis. In the irradiated group, a statistically significant higher frequency of hypertension and of fasting glucose was observed. No other differences in the traditional risk factors were observed between the two groups. Comparing the individuals presenting left carotid plaques with the ones without plaques, in the non-irradiated group the statistically significant differences where mainly in the traditional cardiovascular risk factors, namely hypertension, diabetes, glucose, HbA1c, while in the irradiated group the differences were mainly in hepatic enzymes and white blood cells (total and monocytes).
Conclusion
The results obtained suggest that low dose head and neck past irradiation may increase the frequency of late left carotid plaque formation. Moreover, it appears that the low dose irradiation context may induce a different pattern of carotid atherosclerosis development that justifies further investigation.

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Molecular signatures for thyroid cancer aetiology identification in radiation exposed population

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Radiation exposure of the thyroid leads to the deregulation of molecular markers that can be identified and possibly used for aetiology prediction at least for doses > 0.1Gy. The impact of the mode of exposure, dose/dose rate on the molecular features, as well as the magnitude of the radiation exposure or the individual genetic background for the development of thyroid radiation-induced tumours is not well understood.

Using a microarray approach, our laboratory identified two discriminating transcriptomic signatures of thyroid cancer aetiology by comparing transcriptome profiles of thyroid tumours developed consecutively to external radiotherapy in childhood (external exposure), or developed in people living in the Chernobyl area in 1986 (131 iodide contamination), to which of sporadic thyroid tumours. Both signatures displayed a high robustness for aetiology prediction of prospective tumours.

Moreover, these analyses highly suggested 1) that post-radiotherapy and post-Chernobyl tumours display a common core of molecular specificity, which is independent of the mode of exposure and the dose. Especially, five genes were found in both signatures, and 2) with the release of Chernobyl dosimetry data, that tumours developing in patients exposed to a dose range of 20-50mGy (for which induction of cancers is not proven by conventional epidemiology) display molecular similarities with tumours induced by higher dose range (1-2 Gray) of exposure (which are known to induce thyroid cancers). If these observations are confirmed, the molecular signatures could then allow a case-by-case epidemiology of thyroid cancers in a context of radiation-exposure.

We are developing a research program to confirm these results, which will fully analyse and compare larger series of post-radiotherapy and post-Chernobyl tumours. Identification of the signatures will be realised by next-generation sequencing, by identifying specific aetiology markers at the transcriptome and miRNome level to identify mRNA, miRNA or mixed signatures to predict with a high robustness the etiology of thyroid tumours. The comparison, by the same methodology, of the transcriptome and miRNome deregulations between exposed and non-exposed non-tumoural thyroid tissue adjacent to the tumour will permit to identify if the exposed tissues display a radiation-induced imprinting and if this imprinting is shared with the tumoural tissue.
Radiation-induced DNA damage response in the eye lens.

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Cataract, or opacification of the lens, is one of the largest causes of visual impairment and blindness. Visually impairing radiation-induced cataract has, until recently, been considered a deterministic effect of radiation with a threshold greater than 2 Gy for acute exposures. Recent reviews of cataracts and lens opacities in radiation-exposed populations (e.g. A-bomb survivors, Chernobyl liquidators) indicate a risk at doses below the previous threshold value of 2 Gy (e.g. Bouffler et al., 2012; Ainsbury et al., 2009). The International Commission on Radiological Protection has recently reviewed epidemiological and mechanistic data and considers the threshold in absorbed dose to be 0.5 Gy. The Commission recommended an equivalent dose limit for the eye lens of 20 mSv in a year, averaged over five years (ICRP, 2012). This has now been incorporated into the revised EU Basic Safety Standards with which EU member states are required to comply by February 2018 (BSS 2015).

Recent studies of early and late changes (up to 10 months) in murine eye lenses undertaken by PHE in collaboration with the University of Durham (Markiewicz et al., 2015) have shown a differential location-dependent DNA damage response of lens epithelial cells to low doses of ionising radiation. Further studies are now being undertaken at PHE with the aim of replicating and expanding these early studies using histological sections, which better preserve the anatomy and morphology of the eye lens.

Groups of inbred mice (C57BL/6, CBA/Ca, BALB/c and 129Sv) will be exposed to acute doses of 0.01, 0.025, 0.05, 0.1 or 1 Gy x-rays; control mice will be sham-exposed. Sections will be stained for markers of DNA damage repair (γ-H2AX and 53BP1) using immunohistochemical methods at 1, 2, 4 and 24 hours post exposure. Flat-mount murine lens epithelium preparations will be scored as before and radiation-induced γH2AX foci in blood lymphocytes will also be evaluated at each time point. Microscopic quantification of the nuclear foci in the different groups will be undertaken and relevant statistical methods used to compare the foci levels in the x-ray and sham-exposed groups and between the different mouse strains, histological sections, whole lens epithelium preparations and blood lymphocytes.

The results of these studies will enable the low-dose dose response and time-course of DNA double strand break induction/repair in the lens to be investigated. Data from the four strains of mice included in this study will provide further information on the mechanisms, which contribute to radiation-induced cataractogenesis and enable the selection of a sensitive strain for future studies of the effect of radiation on the lens of the eye.
Occupational exposure on staff in catlab: the influence of using active dosimeters

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**Purpose:** Cardiac catheterization is one of the fluoroscopy-guided procedures that have highly increased in the last 20 years, with clear clinical benefits for the patients. However, the complexity of the procedures have also increased dramatically with longer exposure times and therefore to higher occupational dose for health professionals. The purpose of this study was to evaluate the occupational exposure to health professionals in cath labs and measure the impact of the use of active dosimeters.

**Methods and Materials:** During cardiac catheterization, active dosimeters were used to evaluate occupational exposure. The first and second operators were monitored using two dosimeters (one over and the other under the apron) and other staff members used one dosimeter over the apron. In phase one of the study, exposure values were registered without the users seeing the dose monitor display. In phase two, dose display monitor was placed next to the others cath lab monitors.

**Results:** On phase two the dose level reduced in all catlab professionals: on the first operator the dose decreases 33% over the apron and 45% under the apron; on the second operator the dose decreases 23% over the apron and 40% under the apron; and on other staff members, that circulates around the patient during the procedure, the dose decrease 57%.

**Conclusion:** The use of active dosimeters changed the behaviour of the staff, during the procedures of phase two. The awareness created by the active dosimeters allows a radiation protection reduction of about 40%. This reduction is even more evident amongst health professionals not directly involved in the procedure, such as nurses. The reduction of occupational exposure corresponds to a reduction of patient exposure.
New Approaches to Individual Radiosensitivity

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We report a method allowing the definition of a new surrogate parameter of radiosensitivity that may find use in the determination of human radiation susceptibility.

Our starting point is a fully unexpected and in many ways, unprecedented correlation observed between the yields of prompt DNA double strand breaks (prDSBs) and cell radiosensitivity to killing in a battery of fifteen cell lines using appropriate methodology. Commonly used methods detect prDSBs together with DSBs forming min up to 1h after IR by the conversion to strand breaks of thermally-labile, IR-induced, sugar-lesions to form tDSBs (total DSBs, tDSBs = prDSBs + tDSBs). Fluctuations in prDSBs-yields among cells from different individuals, and thus also their connection to radiosensitivity are thought to partly derive from differences in chromatin structure.

Therefore, we examined correlations between radiosensitivity to killing and chromatin organization. First experiments designed to correlate this response with global chromatin structure failed to show a correlation. We combine this analysis with measurements of γ-H2AX foci to allow comparison with alternative radiosensitivity assays and show that our assay is superior.

These results promise to generate a road map for future work culminating in the development of next generation approaches to radiation protection.

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Chronic exposure to low-dose gamma-radiation modulates inherent functions of human smooth muscle cells and endothelial cells

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Many human epidemiological and experimental studies have shown that high-dose of ionizing radiation, such as those used in radiotherapy, increases the risk of cardiovascular diseases due to injuries to the heart structure and vessels. However, the effects of low dose and low-dose-rate exposure on the development of cardiovascular disease are still unclear and remain a high priority for radiation protection research. Our laboratory has previously demonstrated that a chronic external (in vitro irradiation of vascular cells) or internal (in vivo contamination of ApoE−/− mice) exposure to low dose ionizing radiation induced an anti-inflammatory response, underlying an opposite effect between high and low radiation exposure. To extend these results, the aim of this study is to investigate a possible adaptive mechanism in vascular cells exposed to low dose gamma radiation. Human smooth muscle cells (HAoSMC) and endothelial cells (HAoEC) from aorta were irradiated at 6 mGy/h for fifteen days in a cell culture incubator equipped with 137Cs source. Cells were analysed at four different time points when cumulative dose reached 0.05, 0.5, 1 and 2 Gy. The same doses were administrated acutely. We evaluated cell function and gene expression profile of inflammation and matrix remodeling. Our results show an increase in both HAoSMC migration and in the tube-like formation in matrigel by HAoEC when dose reached 0.05 and 0.5 Gy compared to control (non irradiated cells). Conversely, these functions were decreased when HAoSMC or HAoEC were chronically irradiated with doses of 1 or 2 Gy. In comparison, whatever the acute low-dose irradiation, these functions were not changed. Evaluation of gene expression demonstrated that the pro-inflammatory genes, ICAM1, VCAM1, E-selectin, MCP-1 and Endothelin-1 and genes involved in matrix remodeling Col1-3, MMP1-2 were unchanged with acute or chronic low radiation exposure. Our results suggest that low dose of ionizing radiation induces a modulatory effect on the inherent properties of human smooth muscle cells and endothelial cells, such as migration and tube formation.

1 Ebrahimian et al. Radiat Res 2015
2 Le Galliac et al. PlosOne 2015
Radiation Effects on Murine Brain Pericytes

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Introduction:
Pericytes are main components of the brain microvascular region, having pleiotropic functions. Apart from regulating the integrity and normal homeostasis of the blood-brain barrier they play important role in brain inflammatory processes as well. The objective of our study was to investigate the radiosensitivity of pericytes as well as radiation effects on the inflammatory phenotype of murine brain pericytes.

Methods:
The level of radiation induced DNA damage and repair kinetics was studied on primary murine pericyte cells irradiated in vitro with doses ranging between 0.01 Gy and 2 Gy. Micronuclei were quantified by standard procedures. The level and resolution kinetics of DNA double strand breaks were evaluated by quantifying gammaH2AX foci both in the nuclei and micronuclei of irradiated pericytes at different time intervals after irradiation. Radiation-induced changes in the miRNome of pericytes are under evaluation. The immunoactive properties of pericytes were investigated in vivo in two inflammation models: an autoimmune inflammation induced in SJL mice and an acute septic shock-type inflammation induced in C57Bl/6 mice by LPS injection. Cell-surface markers indicating immune activation, augmentation of antigen recognition and phagocytosis as well as cytokine secretion were investigated by flow cytometry at different time points after irradiation.

Results:
The level of DNA repair kinetics in pericytes was fast and dose-dependent. However, a residual level of DNA damage was persisting even 1 week after irradiation, which was not dose-dependent any more. Irradiation augmented inflammation-induced pericyte activation, but reduced inflammation-induced increase in antigen recognition and phagocytosis and secretion of most of the investigated cytokines in SJL mice with autoimmune encephalomyelitis. In the C57Bl/6 mice with acute inflammation radiation upregulated, mostly markers related to antigen recognition and phagocytosis, as well as inflammatory cytokines but had little impact on influencing pericyte activation markers.

Conclusions: Residual DNA damage indicates the long-term carry-over of radiation injury after low doses already, which can be a source of genetic instability. Interaction between radiation and a pre-existing inflammation is dependent on the type of inflammatory reaction and the genetic background.

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Is the radiation risk of post-Chernobyl papillary thyroid cancer associated with a distinct molecular pathway?

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The statistical association between radiation exposure to ingested $^{131}$Iodine from fallout of the Chernobyl accident and an enhanced risk of contracting thyroid cancer has been well established by numerous epidemiological studies for children and young adults. In studies on the Ukrainian-American (UkrAm) cohort which only included subjects exposed below age 18 the excess relative risk decreased continuously from about 5 per Gy for mean age of cases 16 yrs. to just below 2 per Gy for mean age of cases 24 yrs.

For exposure in adulthood, there is little evidence for an increased radiation risk in Japanese a-bomb survivors. Histological analysis of tumour tissue from a number of sources showed that more than 90% of all thyroid carcinoma in radio-epidemiological studies after Chernobyl were of the papillary subtype (PTC). Molecular analysis of PTC samples revealed a DNA copy number gain, which was related to radiation exposure. Radiation-specific CLIP2 expression has been validated with dedicated protocol on a protein level. A functional form of the dose response for binary CLIP2 expression in young patients has been proposed with a logistic regression model. Genomic analysis showed a large number of copy number variations (CNVs) in PTCs from patients operated with a latency period ≥ 17 yrs. compared to those operated with latency < 17 yr.

Based on these molecular findings a biologically-based model is presented to estimate the radiation risk for PTC in the UkrAm cohort. The conceptual model design follows the hypothesis that different molecular mechanisms are involved in the pathogenesis of sporadic PTCs compared to radiation-induced PTCs in younger patients. The model links epidemiological incidence data to molecular measurements. Sporadic PTCs occur from multi-stage carcinogenesis which is associated with many CNVs, low thyroid doses, long latency and older age at operation (AaO) ≥ 20yr. For young patients (AaO < 20 yrs.) the probability for a radiation-induced PTC equals the probability of a positive CLIP2 marker. The radiation-induced pathway exhibits few CNVs and predominantly comes with short latency, young age at exposure and high thyroid doses. Risk estimates are compared with those from standard descriptive models and implications for radiation risk assessment are emphasised.

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Concerted Uranium Research in Europe (CURE): protocol for a collaborative research project integrating epidemiology, dosimetry and radiobiology.

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The health effects of occupational exposure to uranium are not well known. Experimental studies on the effects of uranium have reported impairments of the cerebral function, genotoxic, nephrotoxic and other effects, but the implications of these findings to human health are not clear. Available epidemiological studies suffer from various limitations. New studies integrating strengths from modern biology and from large epidemiological datasets would have better potential to improve the characterization of the biological and health effects of uranium.

CURE was an 18-month concerted action (July 2013- December 2014) supported by the European Network of Excellence DoReMi (http://www.doremi-noe.net/pdf/doremi_TRA/D5_17_Report_Uranium_exposure.pdf). Its aim was to elaborate a protocol for a collaborative research project on the biological and health effects of uranium, integrating epidemiology, biology/toxicology and dosimetry. It involved 9 European partners with competences in these fields. Contacts have also been established with teams involved in similar research projects outside the European Union (e.g.: USA, Kazakhstan, Russia).

CURE demonstrated the technical feasibility of the pooling of existing uranium workers and miners cohorts with improved dosimetry, and of a molecular epidemiology approach in these cohorts. CURE also produced the protocol for a large-scale collaborative project to improve the characterization of the biological and health effects of uranium in Europe. Funding needs to be found to implement the proposed project. In the future, it might be envisaged to extend collaborations with other countries outside Europe, and to other internal emitters.

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Minimizing mutation rate: low-dose hypersensitivity and induced radioresistance

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Low-dose hyperradiosensitivity (HRS) and induced radioresistance (IRR) have been extensively demonstrated in the past decade both by in vitro and in vivo experiments. It has been shown that apoptosis plays a role in HRS, while the activation of DNA repair pathways are responsible for IRR. Despite these advances in the biology of HRS/IRR response, there are only phenomenological descriptions of cell survival curves at low doses. The objective of the present study is to test whether a principle that the living system tries to minimize the mutation rate upon radiation exposure provides mechanistic explanation for the HRS/IRR response.

For this purpose, a mathematical model of mutagenesis is elaborated considering both mutations due to DNA damages caused by radiation and mutations due to additional cell divisions compensating excess cell loss by radiation. It is supposed that the number of pro-mutagenic lesions follows Poisson-distribution with an average increasing linearly by dose, and those cells are eliminated from the system, which contain the highest number of lesions. At a given dose, it can be computed, which surviving fraction results in the lowest mutation rate. These surviving fractions characterising the minimal mutation rate (at a given dose) are plotted as the function of dose.

Results show that surviving curves computed in the way above have local minima if the induction rate of pro-mutagenic lesions is not too high and the rate of spontaneous mutations is not too low. Supposing that high dose survival is mainly determined by physical grounds, survival curves obtained by the model are similar to the experimental data. Therefore, it can be concluded that the principle that the living system tries to minimize the mutation rate upon radiation exposure provides mechanistic explanation for the HRS/IRR response.

This explanation has significant implications for radiation therapy and radiation protection. Based on the model, it can be understood why HRS cannot be exploited in hyperfractionated radiation therapy. Supposing that cancer risk is proportional to mutation rate, results suggest that cancer risk at low doses are lower than that obtained from extrapolation of high dose cancer risk.

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Challenge to use the concept of Effective Dose Rate in place of Doubling Dose

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This report presents the comparison of spontaneous mutation frequencies with those induced by artificial radiation exposure of living objects such as human, mice, and Drosophila and plants, chrysanthemum, maize, Tradescantia. Spontaneous mutation is a fact of life so far as living objects are alive. It was Muller who first introduced the concept of “doubling dose”, which provided us with a tool to make across-species comparisons, and is currently used by radiation protection organizations to estimate biological Effects of Ionizing Radiation. This although the mutation frequency itself varies from species to species. This is because the order of doubling doses are almost of the same order among different experimental conditions, different species. However, we should note that it is varies with the dose rate even under the condition of the same total dose. This indicates the importance of dose rate rather than total dose.

We have performed a kinetic reaction model accounting explicit dose rate dependence, which we name “WAM model”. The key features is that WAM model reproduces the dose rate dependence quite well.

Our challenge is, based on the WAM model, to introduce the so called “effective dose rate”, the dose rate with which will induce the same total amount of spontaneous frequency. In WAM the mutation frequency is defined as

\[ \frac{d}{dt} F(t) = A - BF(t), \]  

with \( A = a_0 + a_d d, \) \( B = b_0 + b_d d \)

where \( d \) is the dose rate whose importance have been emphasized above. The term \( A, B \) represent the increase and decrease contribution to \( F \), respectively. The solution of this differential equation is easily obtained for the case where radiation exposure starts at \( t=0 \) with constant dose rate

\[ d ; F(t) = \frac{A}{B} (1 - e^{-Bt}) [F(t) - F(0)] + F(0), \]
with $F(0)$ being the control value coming from spontaneous mutation. With 4 parameters, $a_0, a_1, b_0, b_1$ which are to be determined from the data, we can predict the time development of $F$ for any given dose rate. The condition for stationary state is $F = B / A$. The model reproduces the experimental data of 5 species quite well; all of which fall on the universal scaling function with the scaled time.

The parameter $a_1$ is so called sensitivity, whereas the spontaneous mutation comes from $a_0$ term. It can be expressed as $a_0 = a_1 d_{\text{eff}}$ by introducing the notation of effective dose rate, $d_{\text{eff}}$. From the parameters obtained from the mouse data we find $d_{\text{eff}} = 1.10 \ [\text{mGy/h}]$, which is significantly larger than that coming from average natural background radiation, $0.2 \ \mu\text{Gy/hr}$. Note that the value $d_{\text{eff}} = 1.10 \ [\text{mGy/h}]$ is to be compared with those estimated from DSB counting for human, $d_{\text{eff}} = 1.38 \ [\text{mGy/h}]$ (Sugawara) or 8.4mGy/h owing to the double-strand DNA breaks caused by endogenous reactive oxygen species (Tubiana et al.) which are almost of the same order. We shall investigate and report the details of effective dose rate and compare those of other species and see how the “effective dose rate” works as an improvement of “doubling dose”.
Proteomic analysis reveals the progressive metabolic mal-adaptations after neonatal total body low-dose ionizing radiation

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Effects of low-dose total body ionizing radiation are becoming a major cause of concern for radiation protection. Epidemiological studies have shown that children and young adults are especially susceptible to radiation-induced cardiovascular disease (CVD). Long-term effects of low and moderate doses of ionizing radiation target the metabolic system directly or indirectly, especially when the body needs adaptation to metabolic tuning.

In this study NMRI mice received single doses of total body ^60^Co or ^137^Cs gamma-irradiation on postnatal day (PND) 10 and were sacrificed either at PND 11 or 7 months post-irradiation. The doses used were 0.02/0.05, 0.10, 0.50 and 1.0 Gy. Changes in cardiac and liver protein expression were quantified using Isotope Coded Protein Label (ICPL) and tandem mass spectrometry (LC-MS/MS).

Progressive structural and metabolic impairments were observed in both organs. We observed decrease in pyruvate metabolism in liver and shock response (dose dependent up-regulation of Ca^{++} ATPases Atp2a2) in heart as immediate responses on PND 11. Interestingly, the transcription factor peroxisome proliferator-activated receptor (PPAR) alpha was found to be activated in heart and deactivated in liver after 7 months. Inflammatory processes that are partly regulated by PPAR alpha were found to be affected in both organs at all doses and time points. These observations were proven by immuno-blotting and activity assays. PPAR alpha is an essential transcription factor involved in metabolic adaptations in neonates, especially for lipid metabolic processes. This study highlights PPAR alpha as the long-term key regulator of metabolic homeostasis after exposure to low and moderate dose ionizing radiation in neonatal stage.
Radiation-induced perturbations in an in vitro epithelial layer of CaCo-2 cells co-cultured in vitro with human PBMC

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Background:
Colorectal carcinoma is one of the most common malignancies in industrialized countries, among which colon carcinoma is usually treated with radiation therapy. This treatment involves a systemic response, with the recruitment of immune cells at the radiation-treated site, which might have synergistic effects combined with the radiation exposure.

Purpose:
To determine the effects of different doses of radiation on CaCo-2 cells and on the signalling proteins spectra shared with PBMC from healthy donors.

Materials & Methods:
Trans-Epithelial Electrical Resistance (TEER) was measured for up to 20 days in CaCo-2 cells plated in 0.4-micron porous membrane inserts and irradiated 7 days later with different doses relevant for radiotherapy treatments (up to 10 Gy). TEER was measured also following the combined effect of irradiation and co-culture with non-irradiated PBMC. In parallel, culture media samples were collected and the perturbed signalling spectra analysed and compared among the different conditions.

Results:
Exposure to different doses of X-rays showed a dose-dependent increase of TEER in CaCo-2 cells alone and also in co-culture with PBMC, although in the latter case a decrease in the TEER was observed immediately after the start of the co-culture. Measurements of the signalling spectra released by CaCo-2 irradiated cells compared to the corresponding control, showed different deregulated proteins when in co-culture with PBMC with respect to the non-co-cultured condition.

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Study of radiation-related circulatory diseases using animal models: Evaluating the feasibility of spontaneous hypertensive rat (SHR) as an animal model

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Data from atomic-bomb survivors indicate radiation-associated risks for circulatory diseases (CDs). These concerns have received keen attention from ICRP and UNSCEAR. We are conducting animal model studies to evaluate whether risks of CDs increase with increasing radiation dose, and to obtain information concerning biological mechanisms. The data from stroke-prone SHR rats (SHRSP) presented at MELODI 2013 indicated that irradiated rats had significantly shortened lifespans and more progressed perivascular damage in their organs than non-irradiated rats. However, SHRSP rats are not useful for evaluating the radiation-associated risks of hypertension because measuring their blood pressure after the onset of primary stroke symptoms leads to further symptoms. Thus, we proposed to use SHR rats, since SHR rats do not show any stroke symptoms. A preliminary study suggested the feasibility of evaluating radiation-associated hypertension using SHR rats, as presented at MELODI 2014. In this presentation, we add advanced evidence from an additional study using SHR rats.

Methods
We examined four endpoints: 1) blood pressure, 2) pathological phenotypes, 3) body weight, and 4) blood biomarkers. The rats were evenly placed into four exposure groups (1 Gy, 2 Gy, 4 Gy, and 0 Gy as a control), each consisting of 10 male rats. Blood pressure and body weight were measured once a week for 30 weeks after irradiation.

Results
1) The systolic blood pressure level in irradiated rats was significantly higher than that in non-irradiated rats. 2) Pathological analyses showed no significant difference between irradiated and non-irradiated rats in terms of damage to the brain, heart, and small intestine. However, hepatocytes containing lipid-like droplets were more frequently observed in the livers of rats in the irradiated group than in those of the non-irradiated group. 3) The body weight gain of the irradiated group was significantly more restrained with radiation dose than that of non-irradiated rats. 4) Several blood biomarkers altered with radiation dose.
Discussions
Our results demonstrated a significant association between radiation dose and blood pressure alterations in SHR rats. Further studies using this animal model system may contribute to the revelation of potential mechanisms underlying radiation-related CDs in A-bomb survivors.

Acknowledgements: A part of this study was supported by Grant-in-Aid for Scientific Research (Basic Research (C)) from the Japan Ministry of Education, Culture, Sports, Science and Technology, Grant from the Japan Ministry of the Environment Research Project for Nuclear-Power Disaster Influence and the Hiroshima University Collaboration Program.
An Overview of NASA’s Risk of Cardiovascular Disease from Radiation Exposure

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The association between high doses of radiation exposure and cardiovascular damage is well established. Patients that have undergone radiotherapy for primary cancers of the head and neck and mediastinal regions have shown increased risk of heart and vascular damage and long-term development of radiation-induced heart disease [1]. In addition, recent meta-analyses of epidemiological data from atomic bomb survivors and nuclear industry workers has also shown that acute and chronic radiation exposures is strongly correlated with an increased risk of circulatory disease at doses above 0.5 Sv [2]. However, these analyses are confounded for lower doses by lifestyle factors, such as drinking, smoking, and obesity.

The types of radiation found in the space environment are significantly more damaging than those found on Earth and include galactic cosmic radiation (GCR), solar particle events (SPEs), and trapped protons and electrons. In addition to the low-LET data, only a few studies have examined the effects of heavy ion radiation on atherosclerosis, and at lower, space-relevant doses, the association between exposure and cardiovascular pathology is more varied and unclear. Understanding the qualitative differences in biological responses produced by GCR compared to Earth-based radiation is a major focus of space radiation research and is imperative for accurate risk assessment for long duration space missions. Other knowledge gaps for the risk of radiation-induced cardiovascular disease include the existence of a dose threshold, low dose rate effects, and potential synergies with other spaceflight stressors. The Space Radiation Program Element within NASA’s Human Research Program (HRP) is managing the research and risk mitigation strategies for these knowledge gaps. In this presentation, we will review the evidence and present an overview of the HRP Risk of Cardiovascular Disease and Other Degenerative Tissue Effects from Radiation Exposure.

Dissimilar genetic response to adaptive regimen of radiation in male and female mice

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Radioadaptive response (RAR) is a biological defence mechanism whereby exposure to low dose ionizing radiation (IR) mitigates the detrimental effects of high dose irradiation. In vivo RAR has been demonstrated by whole-body X-ray exposure of male mice, using induction of apoptosis as the biological endpoint. However, there is no evidence showing sex-specificity in apoptosis of radioadapted cells. In this study, we report for the first time the effect of gender in RAR by estimating the levels of apoptosis on thymocytes obtained from male and female exposed to priming low dose X-ray irradiation. Our results indicate that the adaptive response associated with protection from cleaved caspase 3-mediated apoptosis was stronger in males than in females.

In the same way, we have use transcriptome profiling to investigate differences between males and females in the expression of genes involved in apoptosis upon in vivo radioadaptive exposure. A total of 1941 transcripts belonging to genes involved in apoptosis were assayed by microarray cDNA analysis. Analyses of gene-transcript data yielded a set of 21 with statistically significant transcript modulation in adapted thymocytes of male and female mice. Fifteen of these genes also showed significant differential expression among adapted thymocytes and either unirradiated thymocytes or non-primed thymocytes, and they were considered RAR-affected genes with sex-specific modulation of gene expression.

Trp53 gene seems to be essential in regulating induction of apoptosis after exposure to an adaptive regimen of radiation. However, our transcriptome analysis did not reveal sex-dependent transcript variations in Tp53 and Trp53-related genes. Since Trp53 induces apoptosis not only by target gene regulation but also by transcription independent signalling, we investigated the expression of two key phosphorylated forms of TRP53 protein. Lowest expressions of phosphoserine-18-TRP53 were found in thymocytes of primed mice compared to those of non-primed mice. In contrast, highest accumulation of phosphoserine-389-TRP53 was observed in adapted thymocytes, being these increases more pronounced in males. These results suggest for the first time a protective effect against apoptosis for phosphoserine-389-TRP53 in radioadaptive response of mouse thymocytes.
Dose-response relationship of medical ionizing radiation exposure from CT scans in childhood and subsequent risk of Leukaemia and CNS tumours: results from the German cohort study

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Computed tomography (CT) imaging is an important diagnostic tool in modern medicine. Five recent epidemiological studies raised concerns about possible cancer risks after exposure to medical ionizing radiation from CT examinations in childhood. These studies also drew some criticisms in particular with regard to the dose estimates as a detailed individual dosimetry was missing in most studies. Our primary objective is therefore to assess the estimated cumulative exposure in individuals exposed via CT scanning during childhood and to conduct a dose-response analysis.

Data for all children who received at least one electronically archived CT scan since the introduction of the radiology information system (RIS) in the participating radiology departments until 2010 were abstracted. Relevant exposure data of the CT scans were abstracted from RIS and the picture archiving and communication system (PACS). Conversion coefficients calculated in Monte Carlo simulations were used in order to estimate organ and effective doses from the exposure data and the available machine settings. We obtained cancer incidence by record linkage with the database of the German childhood cancer registry.

The cohort included information on 71,073 CT examinations in 44,584 children with 46 initially identified cancer cases. Overall, 66% of all examinations focused the head followed by chest scans (11%). In the youngest age group (< 5 years), the proportion of head CT was highest (73%) and is lower in the older age groups. To date, we have preliminary organ dose estimates for one hospital where we linked PACS and the RIS data for 1,919 head scans. We estimated organ doses per head scan for bone marrow (4.60 mGy/ scan, Median= 2.06) and brain dose (37.13 mGy/scan, Median = 11.36).

Further analysis will be estimating individual organ doses for each child. Results of this analysis will be presented at the meeting.
The Dose Response for Cerebrovascular Diseases in Workers at Mayak PA

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A detailed analysis of cerebrovascular diseases (CeVD) for the cohort of workers at Mayak Production Association (PA) is presented. This cohort is especially suitable for the analysis of radiation induced circulatory diseases, due to the detailed medical surveillance and information on several risk factors. The risk after external, typically protracted, gamma exposure is analysed, accounting for potential additional internal alpha exposure. Three different endpoints have been investigated: incidence and mortality from all cerebrovascular diseases and incidence of stroke. Particular emphasis was given to the form of the dose-response relationship and the time dependence of the radiation induced risk. Young attained age was observed to be an important, aggravating modifier of radiation risk for incidence of CeVD and stroke. For incidence of CeVD, our analysis supports a dose response sub-linear for low doses. Finally, the excess relative risk per dose was confirmed to be significantly higher for incidence of CeVD compared to CeVD mortality and incidence of stroke. We argue that this difference cannot result from potential problems in the mortality follow-up nor from differential frequency of examinations.
The effect of low-dose-rate irradiation on the brain, heart and liver proteome of ApoE (-/-) mice

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Populations chronically exposed to low doses of ionizing radiation, either occupationally or due to environmental contamination, show an increased risk of cardio- and cerebrovascular disorders. We have previously shown that a chronic low-dose rate exposure causes human endothelial cells to prematurely senesce in vitro.

To investigate the in vivo effects of low-dose-rate exposure female C57Bl/6J ApoE knockout mice were chronically irradiated using dose rates of 1 mGy/day or 20 mGy/day for 15 or 300 days, the cumulative doses being 0.3 Gy or 6.0 Gy. The hippocampus, liver, heart, and cardiac mitochondria were isolated from irradiated and sham-irradiated control mice and radiation-induced changes in each tissue were investigated using isotope-coded protein label and label-free proteomics technologies.

In the hippocampus, only the 0.3 Gy dose given at the low dose rate induced significant alteration in the synaptic long-term potentiation and depression as well as axonal guidance. This was reflected in the reduction of postsynaptic density protein 95 in the hippocampus, especially in the dentate gyrus, only at this lower dose. The dose of 6.0 Gy induced changes in the TCA cycle and general oxidative stress response in the hippocampus.

The alterations in the heart, cardiac mitochondria and liver proteome caused by chronic low-dose-rate exposure will be discussed.

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ProZES – a method and a tool for assessment of causal link between risk of cancer and preceding radiation exposure

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Methodology and corresponding software tool ProZES have been developed to model the probability of cancer following radiation exposure. ProZES was initiated to support German judicial authorities in their decision-making on cancer compensation claims after preceding occupational exposure. Probabilistic link between cancer and prior radiation exposure is expressed as a distribution of radiation-assigned share of individual probability to develop a specific type of cancer. Excess absolute risk and excess relative risk models, aggregated by multi-model inference techniques, are used to generate distributions of radiation risk, accounting for personal gender, age, diagnose, and exposure history. Uncertainty of model risk estimates includes also effects of various stochastically-formulated risk-modifying factors (like e.g. RBE, DREF, latency, and dosimetry). The risk models are specified for solid and liquid (including several types of leukaemia and related malignancies) cancers. Some solid cancers, like colon, stomach, lung, breast, and thyroid cancers, are modelled explicitly; while other less frequent cancers are characterized using models common for a group of malignancies (e.g. remaining cancers of digestive tract, cancers of urinary, genital organs, and other). The cancer risk models used in the tool originate from various epidemiological studies; however, most of them are based on the data from the Life Span Study of Japanese atomic bombing survivors. Majority of the risk models in ProZES are newly evaluated. Model risk estimates are then transferred to German population by stochastically modelling multiplicative and additive types of risk transfer, thus appropriately accounting for variability of baseline incidence rates due to different genetic, life-style and temporal patterns in the epidemiological cohorts and in the target population. The user-friendly software tool stochastically simulates and visualizes distributions of individual-specific probability of cancer causation.

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The work has been funded by BfS – German Federal Office for Radiation Protection under contracts 3607504570 and 3612570030.
EPI-CT: *in vitro* assessment of the applicability of the γ-H2AX-foci assay as cellular biomarker for exposure in a multicentre study of children in diagnostic radiology

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**Purpose**

A feasibility study on the application of the γ-H2AX foci assay as an exposure biomarker in a prospective multicentre paediatric radiology setting.

**Materials and methods**

A set of *in vitro* experiments was performed to evaluate technical hurdles related to biological sample collection in a paediatric radiology setting (small blood sample volume), processing and storing of blood samples (effect of storing blood at 4°C), the reliability of foci scoring for low doses (merge γ-H2AX/53BP1 scoring), as well as the impact of contrast agent administration as potential confounding factor. Given the exploratory nature of this study and the ethical constraints related to paediatric blood sampling, blood samples from adult volunteers were used for these experiments. In order to test the feasibility of pooling the γ-H2AX data when different centres are involved in an international multicentre study, two intercomparison studies in the low dose range (10-500 mGy) were performed.

**Results**

Determination of the number of x-ray induced γ-H2AX foci is feasible with one 2 ml blood sample pre- and post- computed tomography (CT) scan. Lymphocyte isolation and fixation on slides is necessary within 5h of blood sampling to guarantee reliable results. The possible enhancement effect of contrast medium on the induction of DNA DSB in a patient study can be ruled out if radiation doses and the contrast agent concentration are within diagnostic ranges. The intercomparison studies using *in vitro* irradiated blood samples showed that the participating laboratories, executing successfully the γ-H2AX foci assay in lymphocytes, were able to rank blind samples in order of lowest to highest radiation dose based on mean foci/cell counts. The dose response of all intercomparison data shows that a dose point of 10 mGy could be distinguished from the sham irradiated control (p = 0.006).
Conclusions
The results demonstrate that it is feasible to apply the γ-H2AX foci assay as a cellular biomarker of exposure in a multicentre prospective study in paediatric CT imaging after validating it in an in vivo international pilot study on paediatric patients.

Acknowledgements: The research leading to these results has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration (FP7/2007–2013) under grant agreement number 269912-EPI-CT: Epidemiological study to quantify risks for paediatric computerized tomography and to optimise doses.
Low dose rate gamma irradiation in combination with Selenium deficiency- effects on reproduction in male mice

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There is a substantial data gap regarding biological effects of chronic low dose rate/low dose ionising irradiation. Selenium (Se) is an essential element necessary for antioxidant enzymes. Investigation of effects Se-deficiency is particularly relevant due to declining blood Se levels in Se-poor areas. Wheat grown at NMBU with variable Se-content was used to make forage to feed mice through several generations to investigate these effects. There is a potential interaction between exposure to ionising radiation giving rise to oxygen radicals that may be scavenged by antioxidant enzymes such as Se-proteins.

Two mouse lines were used, one with defective repair of certain oxidative DNA lesions (Ogg1-defective). Mice (F0) were bred from parents (P) given low-Se forage, i.e. the Se-deficient situation was maintained through two generations. Male F0 mice given low-Se (0.01 mg Se/kg) or normal-Se (0.23 mg Se/kg) forage were continuously exposed to low dose rate (1.41 mGy/h) gamma irradiation for 45 days (1-45) followed by a 45 day recovery period (46-90). The Se-deficiency clearly led to adverse reproductive effects with reduced fertility for P mice and sterility for F0 males. Male F0 mice given normal Se-forage bred with naïve wildtype females (days 79-90), showed reduced reproductive capacity. Changes in testis weights, testis pathology, testicular sperm head counts, testicular DNA damage (strand breaks and oxidized DNA bases) levels (Comet assay), sperm chromatin structure analyses (SCSA) and epididymal sperm fluid protein carbonylation supported the reduced fertility observed. Se-deficient mice exposed to gamma irradiation showed higher DNA damage levels and more pronounced pathological changes in the testis.

In conclusion continuous chronic low dose rate gamma irradiation leads to negative effects on male reproduction, Se-deficiency leads to sterility, and there is an interaction between the two stressors. Accumulation of radiation-induced DNA damage in male germ cells may have severe consequences for the genomic integrity of fertilising sperm and increase hereditary risks.
Radiation-induced alternative transcription and splicing events and their applicability to practical biodosimetry

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When a radiological accident occurs, immediate and accurate assessment of the individual exposure dose based on an easily accessible sample, such as blood, is crucial. We aimed at developing a robust transcription-based signature for biodosimetry from human blood mononuclear cells irradiated with different doses of X-rays (0.1 and 1.0 Gy). Genome-wide radiation-induced changes in mRNA expression were evaluated both at the gene and exon level. Our analysis showed that several genes become alternatively spliced and transcribed in response to irradiation, which we validated using exon-specific qRT-PCR. Interestingly, a significant number of radiation-responsive genes were found to be genomic neighbours. Using three different classification models (Generalized Linear Models, Random Forests and Nearest Shrunken Centroids) we found that, overall, gene and exon signatures performed equally well for dose prediction, although exon signatures were slightly inferior to gene signatures when using less than 10 features. Implementation of a dedicated assay based on the identified biodosimetric dose-prediction signature may lead to improved point-of-care diagnostics for radiological accidents. Finally, our results highlight the importance of evaluating gene expression at the level of single exons for the purpose of radiation biodosimetry in particular and transcriptional biomarker research in general.

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Appraisal of an environmental radiological assessment system using a human phantom as a surrogate

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The expansion of the system of radiological protection for humans to protection of the environment against the effects of radiation exposure is at an early stage with respect to the development of a framework for the integration of human and ecological radiological risk assessment. Significant differences exist between human and environmental assessment methodologies in relation to transfers, exposures and dosimetric considerations. In exploring simplifications made within environmental radiological protection in terms of the efficacy and robustness of dose-rate predictions, a human surrogate was employed to facilitate a comparison of environmental and human assessment methodologies. The results of this comparison highlighted where the two systems are potentially amenable to possible integration and where obstacles to integration may be found. Initial stage transport models appear to be a component with potential for integration, although the extent to which this is achievable is unclear due to differences between endpoints. With respect to the transfer and dosimetry components of two typical methodologies, it appears that the efficacy of the environmental approach is radionuclide dependent. Integration in this context might take the form of exploring biokinetic models developed for humans with regards to selected animals and radionuclides. External dose assessment for environmental and human systems provide results for the surrogate that correspond quite closely providing an indication that integration in this regard is perhaps unnecessary.
Role of innate immune cells in the transmission of radiation-induced bystander signals

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Low-dose radiation induced bystander effects may result from soluble factors produced by irradiated cells to recruit innate immune cells to the irradiated tissue and initiate an inflammatory reaction. Monocyte/macrophages play a central role in inflammation: they sense signals produced by stressed/damaged cells, differentiate, and are activated to control the development and resolution of the inflammation to restore normal tissue homeostasis, structure and function.

We analyzed the influence of bystander effects on un-irradiated myeloid cells differentiation and functions, including the response to an infectious stimulus and the ability to modulate the fate of irradiated keratinocytes, in an in vitro model of radiation-induced skin inflammation.

The maturation and activation of macrophages are analysed by FACS and qRT-PCR. The effects of supernatants of macrophages matured in these conditions on the survival of irradiated keratinocytes are monitored by colony formation assay.

We found that low and high doses of radiation elicit different effects on keratinocytes. The soluble factors produced by irradiated keratinocytes are able to modulate the differentiation of non-irradiated monocytes into macrophages and their activation following an inflammatory stimulus. These macrophages are able to influence the fate of growing keratinocytes and favor their proliferation, but these effects are unable to counter radiation damage. Thus, the bystanders’ factors produced by irradiated keratinocytes are able to affect the maturation and activity of non-irradiated innate immune cells, and the outcome of the radiation response. In the future, we want to further investigate the effects of inflammatory cells on the growth and survival of irradiated keratinocytes.

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Studying the risk of mammography: a cell culture model

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Mammography is a medical and diagnostic procedure used in the screening of breast cancer. Some studies pointed out an increase of breast cancer cases in patients who had received multiple mammographies five years before diagnosis.

It has been shown that healthy human mammary epithelial cells become tumorigenic when they have alterations in three genetic elements (SV40-LT, H-Ras and hTERT) involving the ability to form tumours when inoculated into immunodeficient mice. In such scenario we evaluate the susceptibility of pre-transformed tissue (with two of the three factors altered) to become transformed due to radiation received in mammographic explorations.

To do so primary cultures have been established from healthy breast tissue. We have used this culture to obtain pre-transformed cells (SV40-LT+hTERT; SV40-LT+H-Ras; hTERT+H-Ras) by infection with lentiviral vectors containing the gene of interest with a reporter gene (fluorescent protein) or antibiotic resistance. We intend to irradiate these cultures with doses equivalent to those received during mammographic exploration and test the ability of irradiated cells to grow without anchorage dependence, their invasive capacity, growth in three dimensional cultures, the ability to form tumours in immunocompromised mice and the presence of metastasis in other organs.

We expect our results could contribute to a better assessment of benefits and risks of mammography as a screening method for breast cancer.
Differences in the accumulation of DNA damage in human mammary epithelial cells from young and aged individuals after exposure to ionizing radiation

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DNA-double strand breaks (DSBs) are among the most deleterious lesions induced by ionizing radiation in cells because if left misrepaired or unrepaired they become potentially carcinogenic. Due to the importance of these lesions, cells have developed pathways to halt the cell cycle and repair these lesions or induce apoptosis if repair is not possible. Several epidemiological studies suggest that age could be a modulating factor of organism radiosensitivity. In a recent experimental study to investigate the biological basis for these observations, we observed that after in vitro aging with accumulation of population doublings, human mammary epithelial cells (HMECs) accumulate more DSBs than their young counterparts and show decreased repair efficiency when exposed to X-rays delivered by a mammogram device or γ-rays.

Now, we want to go further in these studies and check whether in vivo aging also affects cell radiosensitivity. We have analyzed the induction and repair of DSBs in HMECs derived from donors of two age groups (<20 years and >65 years). Results show that cells from aged women have statistically significant more basal DSBs than cells from young women. After exposure to 1Gy of γ-rays, cells from both young and aged donors accumulate high amounts of damage. Twenty-four hours after irradiation cells from young donors have repaired all damage induced by radiation reaching the original basal level, whereas cells from aged donors have not been able to repair all the breaks and present significantly higher levels of DSBs. Thus, cells from aged donors are less efficient in repairing radiation-induced damage when compared to young ones, even long time after its induction.

Previous work from our group revealed a delay in 53BP1 recruitment to DSBs in in vitro aged cells. We have tested the efficiency of Homologous Recombination and Non-Homologous End Joining in cells from young and aged individuals by transfecting these cells with plasmids containing reporter cassettes for both repair pathways. Our results could be relevant for radiological protection issues, as they would allow a better evaluation of the risks and benefits of screening programs and the use of mammographies taking into account women’s age.
Adjustment for Smoking Reduces Radiation Risk - Fifth Analysis of Mortality of Nuclear Industry Workers in Japan, 1999–2010

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Many cohort studies among nuclear industry workers have been carried out to determine the possible health effects of low-level radiation. In those studies, confounding factors, for example, age was adjusted to exclude the effect of difference of mortality by age to estimate radiation risk. But there are few studies adjusting for smoking that is known as a strong factor which affects mortality.

Radiation Effects Association (REA) initiated a cohort study of nuclear industry worker’s mortality in 1990. To examine non-radiation factors confounding on the mortality risk among the radiation workers, REA have performed life-style questionnaire surveys among the part of workers at 1997 and 2003 and found the correlation between radiation dose and smoking rate. Mortality follow-up were made on 75,442 male respondents for an average of 8.3 years during the observation period 1999-2010. Estimates of Excess Relative Risk percent (ERR%) per 10mSv were obtained by using the Poisson regression. The ERR for all causes was statistically significant (1.05 (90%CI 0.31 : 1.80)), but no longer significant after adjusting for smoking (0.45 (-0.24 : 1.13)). The ERR for all cancers excluding leukaemia was not significant (0.92 (-0.30 : 2.16)), but after adjusting for smoking, it decreased (0.36 (-0.79 : 1.50)).

Thus, smoking has a large effect to obscure a radiation risk, so adjustment for smoking is important to estimate radiation risk.

keywords: cohort study, cancer, confounding factor
Genomic characterization and low-dose radiosensitivity of a new collection of 140 radiosensitive cell lines

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Over the last decade, many studies have been conducted to search for associations between genetic sequence alterations and the risk of normal tissue complications after exposure to ionizing radiation, including radiotherapy (RT) (1). In one of the largest SNP studies to date, which aimed at evaluating the SNP previously reported to be correlated to adverse effects to RT, no single SNP emerged as a reliable indicator of radio-sensitivity (2). Importantly, despite the large cohort size (1613 patients), this study contained relatively few highly radiosensitive patients. More recently, a genome-wide association study identified a susceptibility locus for late radiotherapy toxicity (3).

The objective of this project is to characterize at the genomic level a new collection of non-immortalized human fibroblast cell lines composed in majority of radiosensitive patients eliciting post-radiotherapy tissue reactions, and gathering 140 skin fibroblast cell lines:

- 100 fibroblast cell lines derived from patients, which have showed tissue over-reactions after RT.
- 12 radio-resistant fibroblast cell lines.
- 15 radio-sensitive fibroblasts cell lines from human syndromes (4 ATM/-, 1 LIG4/- cell lines and 10 cell lines from Gorlin patients)
- 13 normal fibroblast cell lines used as controls.

Normal skin biopsies were collected either from patients, addressed by anti-cancer centers and hospitals, or healthy donors. Fibroblasts were isolated from the samples and placed in culture according to standard procedures. Radio-sensitivity at the cellular level was characterized using classical assays, including micronuclei, γH2AX and pATM foci, after the clinically relevant radiation dose of 2 Gy. All the radiobiological resulting data were gathered in the protected and licensed database referenced as IDDN.FR.001.510017.000.D.P.2014.000.10300 (property of the Radiobiological Group, UMR1052 Inserm, Lyon, France).

The present study will have three major aims:

- characterizing low-dose radiosensitivity (50 to 250 mGy)
- searching for genomic anomalies by whole genome NGS sequencing
- investigating gene expression and epi-genomics, and particularly non-coding RNAs

In order to characterize this collection at the genomic level, we propose:

1) to investigate exome sequencing variants associated or linked to radio-sensitivity
2) to relate the genomic data with the level of tissue radio-sensitivity.
This genomic approach will be based on the expertise of the Genomic Institute of CEA (CNG, Evry) and realized on the ultra-high throughput sequencing platform operating Illumina sequencers (HiSeq 4000 and X Five system dedicated to whole genome sequencing), combined with appropriated bioinformatics analyses. A parallel approach of RNA sequencing will then highlight genes and pathways potentially involved in the specific and constitutive response to irradiation of these radiosensitive individuals. This collection will be later compared to a second collection of human fibroblasts cell lines (INDIRA cohort, normal tissue from 300 healthy people), in preparation, to be used as a control collection representative of individual radio-sensitivity of the healthy French population.

References:


Acknowledgments: National Research Agency- RSNR- INDIRA
Session B
Detection of reactive oxidizing species in gamma-irradiated CHO cell lines by biochemical techniques

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Reactive oxygen/nitrogen species (collectively called Reactive Oxidizing Species, ROS) are important end-points being associated with ionizing radiation (IR)-induced cytotoxicity involving all critical cellular components. We previously reported [MELODI Workshop 2014] the occurrence of IR-dependent oxidative damage in CHO cell line, measured by immuno-spin trapping technique (IST) [Mason, 2004]. This method is based on the covalent binding of 5,5-dimethyl-1-pyrroline N-oxide (DMPO) spin trap with radicals formed in oxidized targets revealed by specific antiserum against the spin trap and measured by Western blotting. Interestingly, we found that the activity of glutathione peroxidase, an antioxidant enzyme able to detoxify hydrogen peroxide, was decreased as a function of IR dose. Moreover, as an antioxidant response to ROS formation, we measured increased reduced glutathione concentration in irradiated cells with respect to untreated samples. In conclusion, our new results confirm the suitability of IST to detect the dose dependence of the occurrence of ROS-dependent oxidative damage in IR-treated cells.

REFERENCES
Low dose X-ray exposure disrupts embryonic brain development and induces a deviant learning behaviour

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Based on epidemiological data, and further substantiated by animal studies, it is now well established that high doses of irradiation received in utero severely compromise central nervous system development, leading to e.g. mental disability and microcephaly. Yet, due to the lack of statistical power from human studies and due to an insufficient understanding of underlying mechanisms, we face many uncertainties regarding non-cancer effects of low-dose radiation. In an attempt to answer to this concern, we used the C57Bl6 mouse model to investigate early and persistent nervous system defects occurring after prenatal low-dose X-ray exposure.

In contrast to high-dose exposed animals, which showed a cell cycle stalling of cortical progenitors at the G2/M checkpoint, we could not observe a proliferation deficit in embryos exposed to a low dose of 0.1 Gy. However, such low doses induced DNA double strand breaks throughout the cortex at 1h and 2h post-irradiation, followed by a transient increase in apoptosis at 6h post-irradiation. Furthermore, both low- and high- dose irradiated embryos displayed a disturbed neuronal differentiation, suggestive for a delayed migration or a premature differentiation of cortical stem/progenitor cells. Next to the early effects, we further disclosed a deviant spatial learning in adult mice prenatally exposed to doses as low as 0.1 Gy. Indeed, a detailed analysis of the Morris water maze test unveiled an inferior use of spatial strategies to find a hidden platform in these irradiated animals as compared to sham-irradiated controls.

In all, we show alterations in cortical development and an impaired spatial learning in mice in utero exposed to a low dose of X-rays. These novel insights are an exciting avenue to further explore, and are hinting towards future studies including doses even below 0.1 Gy.

Acknowledgements: This work is supported by the 7th EU framework programme project CEREBRAD (Cognitive and Cerebrovascular Effects Induced by Low Dose Ionizing Radiation).
RISKEDU: how can teachers support the development of scientific literacy among schoolchildren through teaching about risk and risk-assessment?

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Extensive use of new technical solutions leads to an increased exposure of humans to low doses of ionizing radiation (medicine, industry, energy) as well as electromagnetic fields (medicine, mobile phones), while tourism to sunny countries increases exposure to UV-radiation. The radiation may be beneficial, but it may also be harmful. Thus, exposure is always linked to a certain risk, and the trade-off between risks and benefits may be a quite complicated matter that people generally have difficulty assessing. The ability to make judgments about risk, both on a personal and societal level, is a crucial part of scientific literacy, defined as the ability to evaluate scientific information and arguments based on scientific evidence, and draw conclusions from these. Education about risks encountered in modern society should already start in schools so that young people are able to make sovereign decisions when entering adult life.

The purpose of RISKEDU is to generate knowledge about how science teaching can support the development of high-school students’ competency in making decisions based on informed risk assessment in societal issues involving exposure to threats associated with modern technologies, such as ionizing radiation from nuclear power plants, electromagnetic fields from wireless telecommunication or the rapidly growing field of biotechnology. This is accomplished through a close collaboration between researchers and practicing teachers, in which principles and tools for teaching are successively generated, tested, and refined.

The project will generate concrete as well as conceptual tools, which science teachers can use in order to transform the science curriculum content concerning decision making in societal contexts into meaningful teaching in the classroom.

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User-friendly disease mapping for Belgium

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Introduction: Many cancer types have unknown risk factors or have risk factors that are related to the living environment. In order to assess these risk factor disease mapping is one of the most widely used techniques. In disease mapping, we divide the area of interest in different subareas, and we display the disease rate for each of these subareas, potentially allowing us to identify anomalous subareas. Traditionally the standardized incidence or mortality ratio has been used to map the disease risk, however this suffers from instability when the disease under investigation is statistically rare or the population is low. In recent years, many novel statistical methods have been developed to address these problems. These new methods typically require the use of specialist software or programming. In this study, we compare these methods and combine them in an easy-to-use tool that allows epidemiologists to quickly go from data to disease map.

Methods: Any tool designed for depicting chloropleth maps consists of three different parts: 1) a back-end that performs the statistical modelling, 2) a graphical user interface where the user selects inputs, uploads the data and sees the resulting map, and 3) a framework that combines the first two parts. We have implemented different state-of-the-art disease mapping techniques in R: Bayesian hierarchical models, penalized spline smoothing, spatial filtering and copCAR. All these methods use the information from neighbouring subareas to provide a much more stable assessment of the subarea under study. The graphical user interface is written in JavaScript where the leaflet-library is responsible for the chloropleth mapping. Both user interface and R-backend are combined using the Shiny-application framework. The general process for disease mapping with our tool consists of: uploading the data of observed disease counts per Belgian municipality, choosing one of the described method for disease mapping, modelling the counts with the method, depicting the smoothed disease risk in a chloropleth map.

Results and conclusion: This tool has initially been developed to allow for simple, fast and repeatable research of the health effects of living in the vicinity of nuclear installations in Belgium. As a first application, we have mapped the thyroid cancer mortality in adults in Belgium from 1969 to 2010. The tool provides us with a map of the disease risk per municipality in Belgium, after which the epidemiologists can assess whether there is any anomaly in disease risk in the municipalities close to the nuclear installation of interest. This tool allows thus for public health surveillance based on chloropleth maps.
Eye Lens Opacities in Physicians Occupationally Exposed to Radiation

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We evaluated eye lens opacities among 78 occupationally exposed physicians and 21 non-exposed physicians with slit lamp examination using retroillumination. Cumulative radiation doses were obtained from the nationwide dose register (mean 72 mSv, median 26 mSv), based on comprehensive monitoring with dosimeters worn on chest outside lead apron.

The prevalence of posterior subcapsular (PSC) opacities was 8% (6 out of 78 subjects, 95% CI 4-16%, all unilateral) in the exposed group and 6% among the non-exposed (1/16, 1-28%, one affected subject bilateral PSC), yielding a prevalence ratio of 2.3 (95% CI 0.3-20).

For cortical opacities, the prevalence was 6% (4/78 subjects, 95% CI 2-13%) among the occupationally exposed physicians and 44% (7/16, 95% CI 23-67%), with a prevalence ratio of 0.3 (95% CI 0.1-1.1).

A dose-response analysis was also performed using binomial regression with log link, and it showed no linear relationship between cumulative radiation dose (prevalence ratio for PSC −0.20, 95% CI −0.52, +0.13).

Inclusion or exclusion of the non-exposed group, or adjustment for potential confounders (family history, medical history, medications) did not affect the results.

We could not confirm an excess of eye lens opacities among medical professionals working with ionising radiation reported in some earlier studies. An obvious difference between our material and previous studies is the lower dose level here, though in earlier investigations exposure assessment has been based on self-reported work histories instead of dose monitoring. The exposed subjects in our study included mainly radiologists (N=56) and cardiologists (N=19), while previous studies have used interventional cardiologists. This is the first study with a reference group composed of non-exposed physicians, maximizing comparability in terms of education and socio-economic factors. Dosimeters worn on the chest outside lead apron are a reasonable proxy for eye lens doses.
Proteome analysis of endothelial cells reveals persistent alteration in the RhoGDI and NO signalling pathways after irradiation

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Epidemiological data from radiotherapy patients show the damaging effect of ionising radiation on heart and vasculature. The endothelium is the main target of radiation damage and contributes essentially to the development of cardiac injury. There is accruing evidence indicating the role of endothelial dysfunction in both macro- and microvascular damage after irradiation.¹⁻⁴ However, the molecular mechanisms behind the radiation-induced endothelial dysfunction are not fully understood. In the present study, human coronary artery endothelial cell line (HCAEC) was exposed in culture to radiation doses of 0, and 0.5 Gy (X-ray). Cells were harvested 1 day and 7 days after exposure. The endothelial proteomes were analysed by ICPL labelling using both time points. The proteomics data were further studied by bioinformatics tools and validated by immunoblotting and ELISA. The analysis of the proteome profiles showed that the number of significantly deregulated proteins increased with time after irradiation suggesting progressing cellular damage. The analysis indicated alterations in the caveolar-mediated endocytosis signalling, protein ubiquitination and integrin signalling in a time-dependent manner. Proteomics and immunoblotting strongly suggest a radiation-dependent inactivation of endothelial Rho GTPase and eNOS signalling a week after irradiation. Taken together, the data gathered here using different methods provide evidences for immediate and persistent changes in the pathways involved in endothelial cell function and structure.


Establish and optimise dose levels for chest radiography in paediatric intensive care unit

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Purpose
To perform radiographs in Paediatric Intensive Care Unit (PICU) special attention must be given to radiological protection in children considering their high radiosensitivity. This study aim to analyse the values of scattered radiation during chest radiography in the PICU and optimise the procedures to reduce exposure.

Materials and Methods
Quality control of the radiography portable equipment was made and the scattered radiation during bedside chest radiography (with and without protection between beds) was measured using RaySafe Xi equipment and Educational Direct Dosimeter (EDD), respectively. Exposure parameters and dose levels were collected during children chest radiographs to allow establishing DRLs. In order to optimise the procedure experimental test were performed in a newborn and fives years old equivalent anthropomorphic phantoms. Image quality analyses based on CEC criteria was performed by 5 radiographers.

Results
The scattered radiation during bedside chest radiography was completely reduced when shielding between beds (2m) was used. The DRL for chest radiography was 1.17Gy.cm² for newborn and 4.2 Gy.cm² for 1 to 5 years old. Dose values were reduced 22% in phantom experimental tests without influence in image quality.

Conclusion
Shielding between beds should be used in order to eliminate the scattered radiation effects in other children. DRLs for chest radiography were established and optimised in the PICU.
Cerebrovascular and inflammatory effects of local X-ray irradiation in the brain of Apo E knock out mice.

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Background
Mice that are deficient in the production of Apo E are susceptible to atherosclerosis. Given the uncertainty regarding the ability of lower doses of radiation to promote inflammation and blood-brain barrier (BBB) damage, we report here the results of an in vivo mouse study to test the hypothesis that low- and medium doses of ionizing radiation, in the range of 0.1–10 Gy, would promote the progression of BBB lesion formation in mice that are genetically predisposed to atherosclerosis. We also hypothesized that radiation induces of M1-type macrophage in time-related manner and it may alter proinflammatory downstream signalling, thereby affecting regulation of inflammatory cytokines.

Results
In ApoE knock out (KO) mice the basal permeability was higher than in the wild-type mice and the BBB leakage increased by ageing. All doses accelerated the BBB damage 1 and 4 weeks after exposure. Late BBB disruption was observed only in the 10 Gy irradiated groups. Mobilization of EPCs from bone marrow (BM) occurred at 1 and 4 weeks after exposure. Circulating EPC counts increased in ApoE KO mice 1 week after irradiation, however later on the mobilization of these cells were not observed, the cerebrovascular damage remained unreparable. We obtained difference in macrophage polarization induced by irradiation in ApoE KO vs C57Bl animals, the radiation induced expression of iNOS and IL-23, were increased in ApoE KO animals suggesting that the pro-inflammatory status of ApoE deficient mice was enhanced with irradiation.

Summary
Local low dose X-ray radiation impairs temporally the integrity of the BBB following single irradiation of the brain with a dose exceeding 0.1 Gy only in the early postirradiation time periods. The harmful effect on the BBB is depending on the genetic background of exposed animals. Increased levels of IR-induced cytokines may then augment non-targeted effects in nearby cells not hit directly by ionizing radiation, as well as in cells and tissues distant from the initial irradiation site, hence propagate bystander responses.

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**Prophylaxis and management of acute radiation-induced skin dermatitis: The effects of a new anti-radiation substance BP-C2**

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BP-C2 is a novel oral metal-organic complex of the polyphenolic ligand and molybdenum developed for use as radiation protector. Tolerability of the BP-C2 in animals and human beings is excellent, with LD50 of around 7.0 g/kg body weight (animal dose).

The study aims here were to evaluate the radio prophylactic as well as the management efficacy of topical applied BP-C2 on irradiation induced skin dermatitis and to look at markers for fibrosis and inflammation in the skin.

**Methods**

In the skin radiation injury prophylactic group, white mice were treated with BP-C2 before and after radiation. In the post irradiation treatment group, mice were treated with BP-C2 three times per week for five weeks after the occurrence of skin injuries. The effects on skin injuries were investigated by H&E histopathology and by immunohistochemistry. Animals were irradiated at 30 GY in the one hind leg.

**Results**

All control mice developed severe skin injury in the leg. In contrast, no skin injury was observed in mice treated prophylactically with BP-C2. In the treatment group with BP-C2 all mice were cured after five weeks of treatment. The expression of several oncogenes was decreased significantly whereas the expression of tumour suppressing genes was increased. BP-C2 stimulated the innate immune system TNF-α, INF-γ, GM-CSF, IL-1β, IL-6, IL-25 and the adaptive immune system IL-10 and IL-12 and apoptosis. However, no differences in the epidermal content of E-cadherin were observed between the various groups indicating the safe use of BP-C2. Only a slight degree of fibrosis was developed judged from the presence of myelofibroblasts and fibronectin.

**Conclusions**

BP-C2 seems to be useful to protect patients from irradiation induced skin dermatitis and to manage severe skin dermatitis after its occurrence.
Experimental Investigation of Protective efficacy of BP-C2 in Mice and Rats

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BP-C2, developed for use as a radio protector, is an oral metal-organic compound, comprising the novel polyphenolic ligand and molybdenum. Tolerability of the BP-C2 is excellent, with LD50 of around 7.0 g/kg body weight (animal dose). Radioprotective efficacy of the BP-C2 was studied in small laboratory animals (CBA mice and Wistar rats) subjected to ionizing gamma radiation in the doses from 4.0 to 8.0 GY without bone marrow shielding, to model Acute Radiation Syndrome (ARS). 0.67% aqueous solution of the drug was administered intragastric once daily every second day at 3 dose levels: 6.0 to 156.2 mg/kg in mice and 3.0 to 93.7 mg/kg in rats. Irradiation was performed once after the fifth administration of the drug. Observation period constituted 30 days. Severity of the ARS was assessed using traditional methods based on evaluation of overall condition and behaviour and information from haematology and anatomical pathology examinations. Effect of the drug on haematology and hematopoietic system (endogenous colony forming units) was assessed. Bone marrow was examined for the total number of megakaryocytes and the ratio of nucleated cells.

In mice, the BP-C2 increased survival. The most pronounced radioprotective effect was observed at a dose of 81.0 mg/kg. The BP-C2 was most effective when radiation dose of 5.0 GY. At this dose, the survival of animals increased to 91.7% from 71% (Control group), changes were statistically significant. The BP-C2 increased weight of the spleen (56±4.0 vs. 27±2.0 in Control group) and content of Colony-Forming Units in the spleen (8.6±1.10 vs. 5.4±1.10 in Control group) at the median lethal dose of 5.7 GY.

In rats, the highest radioprotective effect of the BP-C2 was observed at the dose of 93.7 mg/kg. At this dose, all animals exposed to 4.0 GY of gamma radiation survived (75% survival in Control group) and Median Survival Time (MST) was at least 4 days longer. At 5.0 GY, the BP-C2 almost doubled the survival (91.7% vs. 50% in Control group) and resulted in extension of MST by 6.1 days. At 6.0 GY, the drug tripled the survival (75% vs. 25% in Control group) and extended the MST by 8.4. Smaller doses of the BP-C2 (3.0 and 48.5 mg/kg) also exhibited biological efficacy. At 5.0 GY and 6.0 GY, the BP-C2 in the dose of 48.5 mg/kg ensured better survival (66.7% vs. 50% in Control group and 41.7% vs 25% in Control group, respectively), even though differences in MST did not reach the level of statistical significance. At the highest radiation doses, the BP-C2 was not efficient.

Conclusions
Repeated administration of BP-C2 ensures dose dependent radioprotection against radiation in the median lethal dose range. The use of the drug seems to increase the likelihood and favourable outcome of ARS syndrome. MLD (Median Lethal Dose) in Control group constituted 5.7 GY in mice and 5.0 GY in rats. Dose Modifying Factor (DMF) of the drug constituted 1.2 in rats and 1.1 in mice.
In mice at 81.0 mg/kg of the BP-C2 and median lethal dose of radiation of 5.7 GY: Use of the BP-C2 helps to improve survival and MST. In the BP-C2 group, course of the ARS is more favourable and ARS syndromes such as pharyngeal, infectious complications, gastrointestinal are less severe than in the control group. The BP-C2 has favourable effect on weight of the test animals. The BP-C2 has favourable effect on reproductive activity of haematopoietic stem cells (endogenous CFU-S).

In rats at 93.7 mg/kg of the BP-C2 and median lethal dose of radiation of 5.0 GY: Use of the BP-C2 ensures survival 91.7% of animals (vs. 50% in Control group) and improves MST. BP-C2 protects from or improves the course of such clinical manifestations of the ARS as loss of weight, consumption of food and water, signs of hypodynamia, including adynamia, dyspeptic symptoms and diarrhoea, haemorrhagic syndrome, manifesting as sangvino serous discharge from the eyes and nasal. BP-C2 accelerates bone marrow hematopoesis recovery and exerts favourable effect on haematopoietic system of mice (spleen weight and number of CFU-S).
HTO and OBT exposure induces differential effects on haematopoiesis.

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Environmental exposure to tritium is a concern due to tritium rejects by nuclear reactors. These concerns may increase in future years due to potential increased releases by the ITER reactor. However, some concerns are raised about the reliability of the current ICRP recommendations. As a result, a collaborative project was developed between IRSN and CNL to study various aspects of the toxicity of tritium.

Tritiated water (HTO), tritiated amino acids (OBT) and low dose external gamma irradiation rate (1.3 and 31.2 µGy.h⁻¹, equivalent to tritium exposure) were applied to C57Bl/6 mice during either 1 or 8 months. Concentrations of tritium were 10 kBq.L⁻¹, 1 MBq.L⁻¹ and 20 MBq.L⁻¹ in drinking water, the lowest being relevant to the current regulatory level in Canada set at 7000 Bq.L⁻¹, WHO recommendations at 10 kBq.L⁻¹ and French regulatory level set at 100 Bq.L⁻¹. At 1 and 8 months of exposure, blood, spleen, liver, kidney and intestine were harvested and subjected to various analyses including blood cell count and differential, measurement of biochemical parameters in the plasma, and relevant genes expression quantification.

Results of blood cell count analyses indicated slight but significant decrease in RBC at one month of exposure with OBT but not with HTO, which was compensated by an increased mean corpuscular volume and a decreased mean cell haemoglobin content at 8 months. These results suggested the appearance of a macrocytic anaemia at one month of exposure with OBT, which was compensated by an increased half-life of red blood cells at 8 months of exposure. This macrocytic anaemia was confirmed by the measurement of blood parameters showing a decrease in serum iron concentration at 8 months of exposure with OBT with a dose dependent effect. Such a macrocytic anaemia may have several origins, including a central defect of haematopoiesis, a defect in EPO production by the kidneys, a defect in the capture of red blood cells in the spleen or a defect in iron metabolism in the liver and/or in the intestine. At the moment, results of gene expression analysis and cytokine measurement allowed to exclude the three first hypotheses. Work on iron metabolism is in course. However, these results already demonstrate a differential effect of HTO and OBT at the same level of exposure.
Radon Exposure and the Definition of Low Doses – the Problem of Spatial Dose Distribution

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Investigating the health effects of low doses of ionizing radiation is considered as one of the most important fields in radiological protection research. Although the definition of low dose given by a dose range seems to be clear, it leaves some open questions. For example, the time frame and the target volume in which absorbed dose is measured have to be defined. While dose rate is considered in the current system of radiological protection, the same cancer risk is associated with all exposures resulting in a given amount of energy absorbed by a single target cell or distributed among all the target cells of a given organ. However, the biological effects and so the health consequences of these extreme exposure scenarios are unlikely to be the same. Because of the heterogeneous aerosol deposition within the lungs, radon exposure makes a practical issue in radiological protection of the heterogeneous exposures. While the macroscopic dose is still within the low dose range based on the definition, local tissue doses in the order of Grays can be reached in the most exposed parts of the bronchial airways. Therefore it can be concluded that progress in low dose research needs not only low dose but also high dose experiments. A narrow interpretation of low dose research might exclude investigations with high relevance to radiological protection. Therefore studies motivated by the importance in radiological protection should be performed in the frame of low dose research even if the applied doses do not fit in the dose range used for the definition of low doses.

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EpiBrainRad: an epidemiologic study of the neurotoxicity induced by radiotherapy in high grade glioma patients.

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Context
Radiotherapy is one of the most important treatments of primary and metastatic brain tumors. Unfortunately, it can involve moderate to severe complications among which leukoencephalopathy is the most frequent and implies cognitive deficits such as memory, attention and executive dysfunctions. However, the incidence of this complication is not well established and the risk factors and process are poorly understood.

The main objective of the study EpiBrainRad (Epidemiology of Brain tumor treatment complications induced by Radiation) is to improve knowledge on radio-induced leukoencephalopathy based on pluridisciplinar approaches combining cognitive, biologic, imagery and dosimetric investigations.

Method/Design
The EpiBrainRad study is a prospective bicentric cohort study including newly diagnosed high grade gliomas patients treated by radiotherapy and concomitant-adjuvant temozolomide at Pitié Hospital and Paul Strauss Institute. About 300 patients will be included between their surgery and first day of radio-chemotherapy, and the follow-up lasts for three years after treatment. Cognitive functioning assessments, specific blood biomarkers measures and imagery are performed at different moment during the follow-up, and a specific dosimetric assessment is performed. The recruitment started in April 2015. Leukoencephalopathy incidence rate will be estimated in this population. Then, correlations between cognitive impairments and dosimetry, biomarkers ranges and anomalies on imagery will be analysed in order to better understand the onset and evolution of cognitive decrement associated with radiotherapy.

Discussion and conclusion
With an original multidisciplinary approach, the EpiBrainRad study aims to develop knowledge on radio-induced leukoencephalopathy in order to improve its early diagnosis and prevention. The main challenge is to preserve quality-of-life after cancer treatment, which implies to study risk factors and development of radiation-induced complications.
Radiolabeling DNA oligonucleotides with 68Ga for molecular PET imaging

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Objectives

Aptamers are small (5-15 kDa, 15–60 mer), synthetic oligonucleotides (DNA or RNA) that can interact with a specific target at high affinity and specificity. Aptamers exhibit significant advantages relative to protein vectors of radiopharmaceuticals in terms of size, synthesis, modifications, possible targets and immunogenicity. In this study, a DNA oligonucleotide (40-mer) was radiolabeled with 68Ga for molecular PET imaging.

Methods

A disulfide-functionalized DNA oligonucleotide was bioconjugated to the macrocyclic bifunctional chelator MMA-NOTA (maleimido-mono-amide (1,4,7-triazanonane-1,4,7-triyl)triacetic acid) in 1M ammoniumacetate buffer. The NOTA-oligonucleotide bioconjugate was radiolabeled with pre-concentrated and purified 68Ga (Mueller method) in 1M HEPES buffer at room temperature. The radiochemical yield, purity and stability were determined by radio-TLC and radio-HPLC.

Results

Quantitative bioconjugation of the disulfide-functionalized oligonucleotide with MMA-NOTA (75-fold molar excess) was achieved within 120 minutes at 40°C. Afterwards, this NOTA-oligonucleotide bioconjugate was radiolabeled with purified and pre-concentrated 68Ga with quantitative radiochemical yield, high radiochemical purity and high chelate stability. Under the tested conditions, the use of a 1470-fold molar excess (or 2.5 nmol of bioconjugate) resulted in a maximal expected specific activity of 51.1 MBq/nmol.

Conclusions

Post-processing of generator-produced 68Ga and radiolabeling conditions for DNA oligonucleotides were successfully optimized to obtain pure and stable 68Ga-radiolabeled oligonucleotides in a fast and simple way without the need for post-labeling purification steps or additional manipulations. At the moment, in vivo studies using xenografted mice are ongoing to evaluate the 68Ga-labeled oligonucleotide biodistribution profile and its potential as imaging radiopharmaceutical using PET.

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References

γH2AX expression kinetics in peripheral blood lymphocytes from cancer patients relate with acute radiation-induced toxicities

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Purpose
Predictive assays for acute radiation toxicities would prove of clinical relevance in radiation oncology. We prospectively examined the predictive role of γH2AX (double strand break ‘DSB’ DNA marker) expression kinetics in cancer patients undergoing radiotherapy.

Methods and materials
Using western blot analysis and band densitometry, we assessed the expression of γH2AX in Peripheral Blood Mononuclear Cells (PBMCs) DNA at 0h-30min-4h (33 patients) and at 0h-4h-24h (56 patients), following ex vivo irradiation with 2Gy. Appropriate ratios were used to characterize each patient. Early radiotherapy toxicity was correlated with the above molecular variables.

Results
The γH2AX-ratio_{30min} (band density of irradiated/non-irradiated cells at 30min) revealed a significant inverse association (p=0.0008, r=0.54). We further estimated the DSB DNA repair rate from 30min to 4h, by calculating the ‘relative γH2AX-ratio’. This RγH2AX-ratio was measured by dividing the γH2AX-ratio_{4h}/γH2AX-ratio_{30min}. A significant direct association of RγH2AX-ratio_{4h/30min} with high toxicity grade was noted (p=0.003, r=0.49).

Conclusions
Our results suggest that western blot densitometry analysis provides two important markers of normal tissue radiosensitivity. Low γH2AX-ratio_{30min} was linked with high toxicity grade, suggesting that poor γH2AX repair activity within a time frame of 30min after irradiation predict for poor radiation tolerance. On the other hand, low RγH2AX-ratio_{4h/30min}, thus rapid γH2AX content restoration at 4h after irradiation, compatible with efficient DSB repair ability, predict for increased radiation tolerance.
Medical Application of X-Irradiation during 1950s for *Tinea Capitis* (TC) treatment and its delayed radiation response impacts on non-coding RNA genome

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Until discovery of Griseofulvin in 1960, the only TC treatment available during 1950s was based on X-ray induced scalp hair removal, followed by the topical application of anti mycotic remedies. Irradiation doses applied were standardised through Kienbock-Adamson protocol, while the whole procedure was considered medical fully safe with low dose X-irradiation protocol and represented international standard of care of that time.

In 2012, a Dark.Risk international consortium was formed for joint research to the FP7 Framework. The research was foreseen to assist in putting together the Serbian TC cohort, and later on to focus on studying effects of X-irradiation to generate a predictive model of radiation risk. Major goal is the understanding of the interrelationship between radiation exposure, non-coding RNA (ncRNA) genome and health outcomes.

The response to radiation elicits large-scale changes to the expression of ncRNAs belonging to the long non-coding (lncRNA) and microRNA (miRNA) families. Their alterations are dependent on dose-, time- and tissue type. *In silico* modelling predicts that these ncRNAs act through a multitude of interconnecting regulatory pathways. We have shown that simple miRNA expression has some ability to predict outcomes, such as miR-21 or miR-221 characterisation as biomarkers for breast cancer correlation with distant metastasis. Furthermore, we have shown that suppressive epigenetic influence of specific lncRNA (*PARTICLE*) implicates an expanding role for such ncRNAs in global cellular methylation and intercellular communication in response to low dose irradiation exposure. Blood samples from 20 healthy individuals and 31 X-ray irradiated TC individuals were collected. Specific miRNAs (miR-21; miR-221) and lncRNAs (PARTICLE) as candidate biomarkers were detected in plasma samples after irradiation treatment. Consequently, we have begun to follow individual regulatory pathways responding to radiation in order to establish which of them will have a functional relevance in a systems model.

The roles of ncRNAs in the DNA-damage/repair response are only beginning to be unravelled. While their crucial existence is now un-disputed, no doubt the long non-coding genome will continue to surprise and reveal unexpected layers of cellular regulatory complexity.

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Low Doses of X-rays Induce Prolonged and ATM-independent Retention of γH2AX Foci in Human Gingival Mesenchymal Stem Cells

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Effects of low dose radiation on human mesenchymal stem cells (MSCs) are still poorly characterized. Here we examine patterns of phosphorylated histone H2AX (γH2AX) and phospho-S1981 ATM (pATM) foci formation in human gingiva-derived MSCs exposed to X-rays in time-course and dose-response experiments. Both γH2AX and pATM foci accumulated linearly with dose early after irradiation (5-60 min), with a maximum induction observed at 30-60 min (37±3 and 32±3 foci/cell/Gy for γH2AX and pATM, respectively). The number of γH2AX foci produced by intermediate doses (160 and 250 mGy) significantly decreased (40-60%) between 60 and 240 min post-irradiation, indicating re-joining of DNA double-strand breaks. In contrast, γH2AX foci produced by low doses (20-80 mGy) did not change after 60 min. The number of pATM foci between 60 and 240 min decreased down to control values in a dose-independent manner. Similar kinetics was observed for pATM foci co-localized with γH2AX foci. Collectively, our results suggest differential DNA double-strand break signalling and processing in response to low vs. intermediate doses of X-rays in human MSCs. Furthermore, mechanisms governing the prolonged retention of γH2AX foci appear to be ATM-independent.
Creation of a quantitative historical job-exposure matrix for plutonium workers and feasibility of its use with reconstructed occupational histories for epidemiological purposes

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Workers occupationally exposed to radionuclides in the workplace offer a valuable opportunity to study the effects of exposures to radiation because those potentially exposed are monitored to ensure that the radiation doses received are within regulatory limits. The Sellafield workforce is one of the world’s most important groups of plutonium workers for studying the potential health risks of low dose and low dose-rate exposures to radiation. However, for several hundred early workers, the historical monitoring data available for them cannot provide the accurate and unbiased exposure assessments needed for risk analyses. As a result, it has not been possible to include a significant proportion of the early workforce in such epidemiological studies, which has led to diminished statistical power.

This study is funded by the UK Department of Health, and aims to establish a useful exposure surrogate which can be validated by available plutonium dosimetry measurements and provide a quantitative and meaningful proxy for directly estimated dosimetry to be used in future epidemiological studies of the complete Sellafield plutonium worker cohort of over 12,500 workers.

The project is currently ongoing and this poster describes the methodology and progress of the work to date.

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BACCARAT Study: Early clinical and biological predictors of radiotherapy-induced cardiac toxicity in breast cancer

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Breast radiotherapy RT used until the 1990s was responsible for increased mortality due to long-term cardiac complications. Since the 2000s improvement have appeared in dose distributions to organ at risks such as heart, but now, little is known on their cardiac toxicity. The aim of the BACCARAT study (BreAst Cancer and Cardiotoxicity induced by RA dioTherapy) is to evaluate whether 3D Conformal Radiation Therapy induces cardiac toxicity that could be detected in the first two years after treatment based on analysis of subclinical functional and anatomical cardiac lesions in myocardial and coronary levels and evolution of circulating biomarkers.

BACCARAT study consists in a monocentric prospective cohort study that will finally include 120 women treated with adjuvant RT for breast cancer, and followed for 2 years after RT. Women aged 50 to 70 years, treated for breast cancer and for whom adjuvant RT is indicated, without chemotherapy are eligible for the study. Follow-up includes measures of a panel circulating biomarkers, anatomical coronary lesions based on Coronary computed tomography angiography and functional myocardial dysfunction based on cardiac echocardiography. Absorbed doses is evaluated for whole heart and for each different parts of heart, in particular coronary arteries. Analysis will focus on dose-response relation between subclinical cardiac lesions, biomarkers, and different absorbed doses. Furthermore, this study aims to create a bio-bank of plasma of this cohort for future investigations.

This clinical research study is a novel approach to early detect cardiotoxicity of current breast RT, combining anatomical and functional heart consequences based on cardiac imaging, a panel of circulating biomarkers and a detailed heart dosimetry. With this approach, BACCARAT aims to improve understanding of the mechanisms and circumstances that underlie and could predict the development of potential heart side effects and sequelae.
Computed paediatric tomography exposure and radiation-induced cancers: Results from a national cohort study in France

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The increasing use of computed tomography (CT) scans for the paediatric population raises the question of the possible impact of such ionising radiation (IR) exposure on the occurrence of radiation-induced cancers. Recent epidemiological studies have suggested an increased risk of cancer among children receiving CT scans. In France, a nationwide study has been launched to assess cancer risks, especially leukaemia and cerebral tumours, associated with the use of CT scans in pediatrics. This study is part of the Epi-CT collaborative European project.

The cohort includes children less than 10 years old, subjected to at least one CT scan between 2000 and 2011 in 23 French University hospitals. Cumulative organ doses were estimated according to the protocols retrieved from the radiology departments. Clinical information recorded during hospitalization was used to determine whether the children had medical predisposing factors (PFs) likely to increase their risk of cancer. Cancer incidence and mortality data were retrieved through national registries.

At all, 67,274 children were included. Examinations of the head represent 57% of the CT scans. PFs to cancer were observed in 2.3% of the children. During the follow-up from 2000 to 2011, 27 children were diagnosed with cerebral tumours, 25 with leukaemia. In children without PFs, hazard ratios (HRs) of 1.07 (95%CI 1.03–1.10) for cerebral tumours (20 cases) and 1.14 (95%CI 0.97–1.34) for leukaemia (17 cases) were estimated for each increment of 10 mGy in CT X-rays organ doses. In children with PFs, HRs decreased with increasing CT exposures, possibly due to a simultaneous increase in non-cancer mortality.

These first results indicate that patients with PFs should have a very different risk of radiation-induced cancer than patients without PFs. Then, in terms of public health, the most relevant risk estimates should be analysed separately for each group.
Predicting mean heart dose in breast cancer patients who received radiotherapy

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The German BMBF-funded PASSOS project includes a retrospective cohort study on late cardiac effects in about 12500 former breast cancer patients who received external beam radiotherapy between 1998 and 2008. Radiotherapy consisted of tangential megavoltage photon beams (6MV or 10MV) with a dose prescription in the range of 46Gy, 50.4Gy (1.8Gy or 2.0Gy fractions) in combination with an optional 10Gy or 16Gy boost to the tumour bed. For a representative sample of 771 patients, heart dose from the individual radiotherapy regimen was calculated using modern treatment planning software. The calculation was based on information about the patient’s individual anatomy from three-dimensional computed tomography images. Mean heart dose ranged from 0.3Gy to 19Gy with an average mean heart dose of 4.6Gy for patients with left-sided tumours, and 1.7Gy for patients with right-sided tumours.

A dose-response analysis of the cohort data requires that heart dose for all cohort members can be estimated based on information from the available clinical patient records. To this end, we built linear, generalized linear, and generalized additive prediction models for the exact heart dose in the sample of 771 patients. Prediction error was evaluated using cross validation and bootstrap techniques, indicating coefficients of variation of about 50%, depending on the model type and the variable selection.

Estimated dose from the models can be used to investigate the association between heart dose and cardiovascular disease. However, the effect of measurement errors should be taken into account in risk models.
A Possible Warning from Fukushima: An Update

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INTRODUCTION
After the Fukushima–Daiichi nuclear power plant disaster, thyroid screening was performed in subjects who were aged ≤18 years. This study examined the relationship between the number of participants with thyroid nodules and radiation dose, using publicly available municipality level data (N=59).

METHODS
Poisson regression was applied to that data. The numbers of participants with smaller nodule (<5mm), with larger nodule (>5.1mm), and sum of them are explained by UNSCEAR estimated thyroid dose, average age at exposure, and average age at screening.

RESULTS
Although the UNSCEAR thyroid dose was insignificant for prevalence of thyroid cancer (including suspicious cases), it was positive and significant for smaller nodules (b=18.76, t-value=3.79, p<0.01), larger nodule (b=11.45, t-value=2.16, p=0.03), and sum of them (b=18.26, t-value=5.27, p<0.01).

CONCLUSIONS
Although this was an ecological study at the municipality level, our results are consistent with previous studies that confirm significant relationships with radiation exposure and prevalence of nodule (Nagataki et al. 1994; Imaizumi et al. 2015; Schneider et al. 1993). According to follow up studies of a-bomb (Imaizumi et al 2005) and Chernobyl (Hayashida et al. 2012), nodule group has larger risk of thyroid cancer. Our results might indicate an early warning for future incidence of thyroid cancer. Health follow-up for children in Fukushima is necessary.
Individual Level Data Model is More Effective to Detect Radiation Effect: Re-Analysis of A-bomb Survivor and US Nuclear Worker Data

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INTRODUCTION
Although individual level data are recorded, most of the radiation-epidemiological studies apply the Poisson regression model to tabulated data by age, sex, dose, and other covariates. This aggregation can lead to a loss of information, inefficient estimation, and weaker statistical power when detecting the risk of a low dose.

METHODS
LSS14 data (RERF 2012) that categorized dose with 22 intervals was re-analyzed to evaluate problems of aggregation. Linear and (statistically estimated) threshold model were estimated for three dose-category aggregation levels: 22, 11, and 6 intervals.

US DOE three nuclear sites (Hanford, Oak Ridge and Rocky Flats: Gilbert et al. 1993) individual level data was also re-analyzed. Hazard model was applied to the data with explanatory variables: age, sex, race, calendar year of first employment, age at first employment, site dummy, length of employment, latency dummy, and cumulative dose. Natural experiment approach was applied to take into account exposure history.

RESULTS
For LSS14 data, we confirmed aggregation leads to smaller t-value of estimates. For thresholds model, thresholds shit upward with aggregation (-23, 3, and 37mGy for 22, 11 and 6 intervals, respectively). For each aggregation levels, thresholds were insignificant and fits of linear model were better than threshold model.

For three nuclear sites data, radiation cumulative dose was positive and significant for solid cancer (beta=0.097, z=3.11, p<0.01). Nuclear workers were classified into six exposure patterns. Adding interaction term between cumulative dose and exposure pattern improved model fit (AIC=42674 vs. 42668). Workers exposed late 1950s have more risk of solid cancer mortality.

CONCLUSIONS
For the tabulated nuclear worker data, Gilbert et al. (1993) applied the Mantel-Hansel score test and relative risk model and failed to detect effect of radiation exposure. To analyze low does effects, models that utilize individual data are more effective.
Genotoxic effects of continuous chronic low dose rate gamma irradiation and Se deficiency in mice.

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Natural and man-made sources of ionizing radiation contribute to human exposures and are a recognized hazard for human health. Inadequate dietary intake of Selenium (Se), an essential trace element involved in important functions as antioxidant, may influence radiation effects. However, little is known about genotoxic effects of continuous chronic low dose rate radiation or Se deficiency, and no study exists investigating the combined effects of both stressors. Many animal studies undertaken to explore the quantitative effects of exposure to ionizing radiation have concentrated on high acute doses and dose rates. Mice were chronically exposed at low dose rates in the Figaro facility in Norway. In the first experiment wildtype (WT) and DNA repair-deficient male mice (Ogg1−/−) were exposed to low dose rate gamma radiation (IR), and also to low Se diet. Male mice were exposed to 1.41 mGy/h gamma irradiation for 45 days (1.48 Gy total dose). Mice were either fed deficient (0.01 mg Se/kg) or proficient (0.23 mg Se/kg) whole wheat grain diet. In the second experiment WT and ApcMin/+ were chronically exposed to 1,8 mGy/h to a total dose of 1.5 or 3 Gy, or to an acute dose of 3 Gy/h using X-rays. Genotoxicity was investigated by measuring DNA lesions (alkaline single cell gel electrophoresis assay), mutations (Pig-a gene mutation assay) and chromosomal fragmentations (micronucleus assay, MN). IR induced increased levels of MN, mutations and DNA lesions (ssb/als). However, levels of DNA oxidations decreased in chronically irradiated mice and were lowest in irradiated mice on low Se diet. DNA lesions levels were also assessed 45 days after cessation of radiation (at day 90). The irradiated group expressed a lower level of ssb/als compared with the non-irradiated mice. Moreover, the genotoxicity were significantly higher in acutely compared to chronically exposed mice. The study contributes to fill the knowledge gap of genotoxic effects caused by chronic stressors alone or in combination with other stressors, and to understand the difference in genotoxic effect of acute versus chronic exposure.

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The German-Swiss Fachverband für Strahlenschutz e.V. — We stand for Radiation Safety

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The German-Swiss Fachverband für Strahlenschutz e.V. is an association of scientists and professionals in all fields of radiation safety. The majority of the members are coming from Germany and Switzerland. Our members work in research centres, universities, industry, authorities, and medical institutions.

Recognized as a non-profit organisation the Fachverband is independent from any financial, economic or political interests. The basis of our work is exclusively science, technical knowledge and practical experience. The Fachverband is member of the International Radiation Protection Association (IRPA), the global umbrella organization of national radiation protection associations. The most important instrument for a permanent exchange of knowledge and expertise are the expert groups:

✓ Education
✓ Transport
✓ External dosimetry
✓ Waste
✓ Incorporation monitoring
✓ Detection limits
✓ Natural radioactive material
✓ Non-ionizing radiation
✓ Emergency preparedness
✓ Practical radiation protection
✓ Regulatory aspects
✓ Radiation effects and –biology
✓ Environmental monitoring

Additionally,

✓ Cooperation with the German association of safety engineers (VdSI)
✓ Cooperation with the Network of Competence in Radiation Research (KVSF)
✓ Cooperation with the Romande Radiation Protection Association (ARRAD)
✓ Working group for public relations

We offer an exchange of knowledge and experience in our expert groups, participation on congresses and symposia, networking, support for young scientists and professionals, contacts to national and international associations and authorities, experts opinions and external communication and a periodical StrahlenschutzPRAXIS.
DIMITRA: Dentomaxillofacial paediatric imaging: an investigation towards low dose radiation induced risks

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Cone Beam Computed Tomography (CBCT) is an emerging X-ray technology that has found wide applications in dentomaxillofacial imaging. The ability to provide high-resolution 3D images has resulted in a significant increase in the volume of dental radiology procedures. Although CBCT is associated with higher radiation risk to the patient than conventional dental X-ray imaging (intraoral or panoramic), it is considered to be ‘low dose’ imaging as defined by the High Level Expert Group (HLEG; www.hleg.de) with doses ranging from a few μSv to mSv per examination. This proposal is set to tackle important issues raised by the HLEG and the MELODI (Multidisciplinary European Low Dose Initiative) platform. In particular, as deduced by the name DIMITRA (Dentomaxillofacial paediatric imaging: an investigation towards low dose radiation induced risks), the project focuses on the uncertainties associated with radiation-induced health risks at low doses in paediatric dentistry and is a multidisciplinary effort to approach the involved risks from different yet interrelated perspectives: radiobiological characterisation, dosimetric quantification, epidemiological surveying and image quality & dose optimization. A unique Monte Carlo (MC) framework will be used to accurately calculate organ doses in dental CBCT imaging, to quantify the radiation induced risk and to feed the radiobiology team with the appropriate data towards the identification, development and validation of biomarkers for radiation induced health effects. Furthermore, it will constitute the basis upon setting up a gender and age related epidemiology study. The balance between image quality and dose levels will be explored aiming at reducing the risk through image quality optimization. It is expected that DIMITRA’s outcomes and deliverables can be presented to a wider forum via a dissemination meeting, leading to further recommendations and potential future adaptations for the use of CBCT in paediatric dentistry.

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A priori Assessment of Environmental Impacts in the Marine Environment Using an Adjoint Marine Dispersion Model

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Due to nature of the problem, a priori assessment of potential environmental impacts due to releases to the marine environment from nuclear transport or nuclear powered vessels is a matter of some difficulty but nonetheless important given public sensitivities to radioactive contamination. Using an adjoint of a 3 dimensional hydrodynamic dispersion model, a system was developed facilitating an appraisal of potential impacts from releases at any point in the north Atlantic/Arctic area combined with a measure of uncertainty in the impact magnitudes.
Enhanced levels of NORM due to human practice and the need for proper handling of NORM waste

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Sulfate-forming rocks with elevated concentrations of uranium, such as alum shale and related black shales, are often classified as NORM. Sulfate-forming rocks are found in large volumes in Oslo, the capital of Norway, and the surrounding areas. Large-scale infrastructure construction projects are taking place in the area, such as tunneling through alum shale deposits and building new government buildings. It is estimated that these projects will cause between 250 000 – 500 000 m3 of NORM waste, in the form of alum shale and similar shales. The construction processes also causes runoff of effluents with a significant concentration of uranium, and formation of sludge with a noticeable amount of uranium.

Earlier practices of handling alum shale and similar sulfate-forming rocks were to deposit it in open landfills, which were then covered by a thin layer of soil. These deposits have later become reactive and has formed reactive forms of shale which has caused radioactive pollution, acidic leachate and noticeable environmental damage. Countermeasures for reducing downstream pollution caused the formation of radioactive sludge with a high concentration of organic matter.

Depositing NORM waste in landfills with no barriers are no longer permitted. The large volume of NORM waste excavated from the Oslo area can, if not handled properly, cause severe pollution. It is important to have facilities which can store reactive sulfate-forming rocks in such a way that the risk of pollution stays within reasonable limits for the foreseeable future.
Ecological and Human Impact Assessment in the Legacy Enhanced Radiation Areas

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Naturally occurring radioactive materials (NORM) are responsible for the major contribution to the total effective dose of ionizing radiation of the world population. Mining and tailing are considered to be hazardous steps with respect to technologically enhanced naturally occurring radioactive material (TENORM) and metal contamination of the environment, and also in terms of radiation doses to man.

The Fen Complex in Norway is an area well known for its specific magmatic bedrock, rich in thorium (Th), iron (Fe), niobium (Nb) and rare earth elements (REE). In the past, intensive mining of Fe and Nb was conducted at several sites in the area, giving rise to enhanced radiation levels. The presence of metals along with $^{232}$Th and uranium ($^{238}$U) gives the multiple stressors scenario and makes the whole exposure even more complicated. Human health studies done in this area demonstrated the annual exposure doses among the highest in Europe. In the present work, the contamination status with respect to radionuclides and trace elements, as well their impact on humans and biota were investigated at legacy NORM sites.

Significant heterogeneous radionuclide ($^{232}$Th, $^{238}$U and progenies) distribution in soil was demonstrated at investigated legacy NORM sites with $^{232}$Th activity concentration levels of soil exceeding the screening levels for radioactive waste given by the Norwegian Pollution Control Act (2010). Short and long-term surveys of the outdoor terrestrial gamma dose rates and the concentrations of radon ($^{220}$Rn, $^{222}$Rn) in the air demonstrated enhanced levels (up to 9.24 μGy/h, 5000 Bq/m$^3$ and 200 Bq/m$^3$, respectively), with seasonal variations. The radiation dose rates for biota, obtained by the ERICA Tool using site-specific data, ranged from 1.3 – 23.1 μGy/h, with maximal values estimated for earthworms, lichens and bryophytes. Regarding the humans radiation doses, the most significant contribution to doses received outdoors, is from terrestrial gamma radiation. The annual outdoor human doses at the selected locations in the Fen area would be most likely within the range 0.10 – 1.54 mSv. However, varying several factors in calculations would lead to elevated annual outdoor doses, up to 10 mSv.
Microorganisms of radionuclides-contaminated soils of Chernobyl: in depth analysis of diversity and study of uranium-bacteria interactions.

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We explored the microbial diversity of Bacteria and Archaea evolving since 25 years in a radioactive-waste repository trench located in the Chernobyl exclusion zone. To assess the effect of long-term RNs exposure on diversity, microbial assemblages of soil samples highly contaminated with radionuclides (RN) such as 137Cs and uranium were compared with nearby controls using high throughput pyrosequencing of 16S rRNA genes. The analysis of 690,023 sequences evidenced high diversity in all samples with 34 bacterial and 2 archaeal phylum represented. Chloroflexi, Acidobacteria, Proteobacteria and Verrucomicrobia were the most consistently detected phyla, representing 90% of all sequences. This result demonstrates that a long-term exposure did not lead to the decrease of microbial diversity. Furthermore, principal component analysis of pyrosequencing data showed that microbial communities of RNs contaminated samples differed significantly from that of controls, suggesting the presence of RNs adapted species in the contaminated samples.

Several heterotrophic aerobic bacteria have been cultured from the contaminated samples. Among them, the strain Microbacterium sp. A9 exhibited high uranium tolerance. The interaction between this strain and uranium was investigated by a combination of spectroscopic (FTIR and TRLFS) and microscopic (TEM/EDX) approaches. Comparison of data obtained at 4 and 25°C evidenced active and passive mechanisms of uranium uptake and release. We demonstrated that after a first step of uranium and phosphate release via an active efflux mechanism, Microbacterium sp. A9 accumulates U(VI) as intracellular needle-like structures composed of autunite. The functional groups involved in the interactions with uranium were identified.
Uranium and plutonium particles released to the environment

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Following the Chernobyl accident, radioactive particles identified close to as well as far from the damaged reactor were considered to be a “peculiarity” of that specific accident. However, uranium (U) and plutonium (Pu) containing particles, ranging from submicrons to fragments, have during the history been released from a series of sources associated with the nuclear weapon and fuel cycles; nuclear weapons tests, conventional detonation of nuclear weapons and nuclear reactor explosions or fire. In addition, radioactive particles have been released via effluents or stacks from reprocessing facilities, from waste dumped at sea, from U mining and tailing sites and from the use of depleted uranium ammunition. Thus, radioactive particles have frequently been released in the past, and radioactive particles should also be expected in the future following new nuclear events.

So what is the relevance: Radioactive particles in the environment are heterogeneously distributed and can carry a substantial amount of the bulk radioactivity. Thus, radioactive particles can act as point sources and may contribute to significant doses when deposited or retained in biological tissues. Depending on the source and release conditions, particles can be difficult to dissolve and the inventory of particle contaminated areas can be underestimated. Furthermore, the ecosystem transfer will be delayed if radioactive particles are present. Due to particle weathering, associated radionuclides are mobilized over time and particle contaminated soils and sediments can act as diffuse sources in the future. Thus, linking particle characteristics to source and release conditions and to biological effects is essential to improve the predicting power of environmental impact and risk assessment models applied for particle contaminated areas, as also focused by the IAEA’s Coordinate Research Program on particles. The present paper will focus on challenges associated with dose, impact and risk evaluations of areas contaminated with radioactive particles.
OPPERA task 2.6.3. Preliminary lessons from stem cells and animal models outside the radiation field.

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(On behalf of all the participants from the task 2.6.3. workshops)

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Long-term animal and stem cell studies have played fundamental roles for studies on radiation effects providing important complementary data to those obtained from human epidemiological studies. However, further studies are needed as clearly mentioned in the MELODI Strategic Research Agenda (www.melodi-online.eu).

The objectives of the task 2.6.3. "Drawing lessons from stem cells and animal models outside the radiation field²" within the EU OPERRA (Open Project for Open Radiation Research Area) project (http://www.melodi-online.eu/operra.html) are the following:

1) To integrate research activities outside the radiation protection field to promote continuing development of suitable whole animal models for radiation carcinogenesis and non-cancer diseases at low doses which bear clear relationships to human diseases;
2) To integrate current knowledge on stem cells models into radiobiological studies at low doses.

Therefore, a thorough evaluation of animal and stem cell models is currently performed to assess diseases of relevant tissues and organs, including the immune and hematopoietic systems, cardiovascular system, central nervous system and in embryology following exposure to low dose radiation. A list of existing studies with recommendations for key animal and stem cell models is being prepared. An identification of laboratories and institutions having experience in animal models and stem cell research that are adaptable to radiation research is foreseen. A roadmap for further collaboration between radiation and non-radiation specialists is being discussed.

All presentations given and the results of discussions and the main outcomes of the two workshops hold so far are available on the EU-OPERRA Extranet.

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STORE; an infrastructure for sharing of data and resources in radiation biology and radiation epidemiology

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There has been much discussion of the issues of datasharing and accountability in the scientific literature, amidst growing concern about the irreproducibility of reported biomedical findings, caused in part by inability to obtain primary data. Funding agencies and major journals are now implementing and enforcing stringent requirements for data sharing and transparency. The sharing of data and biomaterials from publicly-funded experimental and epidemiological radiation biology adds enormous value to the original investment, yielding substantial scientific rewards by facilitating re-analysis, avoiding duplication, reducing animal use, and stimulation of new investigations.

We report a survey of availability of primary data behind a range of 260 published papers in radiation biology over a five-year period, and find that whilst availability has improved, less than 10% of publications surveyed in 2013 gave public access to the primary data, particularly where these are high throughput ‘omics data sets. This raises questions about the culture of sharing and transparency in radiation biology and the data sharing policies of key journals in the field.

A bottleneck in compliance with data sharing requirements at the moment is the lack of availability of readily searchable public databases in which many types of data can be deposited. To that end, the STORE Consortium, funded by the European Commission and the German Federal Office for Radiation Protection (BfS), has created a platform for the storage and dissemination of data from past, present and future radiobiological research, to stimulate a wider culture of sharing research data and bioresources.

The STORE database is a database infrastructure for effective resource sharing which permits users to locate materials of interest (biological materials in biobanks such as paraffin blocks and frozen tissues) and data supporting experimental and epidemiological studies, using defined vocabularies and structured metadata. Each dataset is managed by the investigator and tagged with appropriate terms to allow easy search and retrieval. Access and security can also be controlled by the investigator – data can be made universally accessible or restricted to password holders.

STORE is available, free of charge to all international users, on http://www.rbstore.eu and fp7store.eu, with the aim of supporting and rewarding data sharing for radiobiological, epidemiological research. It establishes the basis for long-term and efficient data and materials use and, through encouragement of sharing, is expected to advance scientific progress in radiobiology.
Induction of local hyperplasia provides explanation for inverse exposure rate effect and the histopathology of radon induced lung cancers

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Radon exposure is the second most important cause of lung cancer after smoking. However, the mechanisms by which it increases lung cancer risk is quite unclear. Due to the alpha-emitters radon progeny and their heterogeneous distribution in the lungs, local cell death rates can be quite high. Equilibrium between cell death and cell division implies that cell loss is compensated by increased cell division rate and/or additional number of progenitor cells, i.e. hyperplasia. The objective of the present study was to quantify how cell division rates can be reduced by the induction of hyperplasia to decide whether it explains the inverse exposure rate effect even without supposing a threshold dose rate. For this purpose, numerical model of the bronchial epithelium was developed and applied for the determination of cell nucleus hits and cell death rates. Cell division rates were obtained by supposing equilibrium between cell death and cell division at different macroscopic exposures and different number of progenitor cells. The results show that the induction of hyperplasia provides an explanation not only for inverse exposure rate effect, but also for the increase in relative frequency of small cell lung carcinoma and squamous cell carcinoma with increasing cumulative radiation exposure in uranium miners. Results highlighted that hyperplasia can occur even without previous genetic changes underlining the role of epigenetics in radon carcinogenesis. In addition, hyperplasia decreases both the microscopic dose consequences of a given macroscopic exposure, and the biological effects of a given microscopic dose exemplifying key differences between acute and chronic exposures.

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Influence of a pre- and postnatal LDR irradiation on the age-dependent neurodegeneration in a mouse model for Parkinson-Disease ("OSTINATO")

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As known from radiotherapy patients, high-dose ionizing radiation in particular to children has the potential to increase the risk of premature dementia or other neurodegenerative diseases (ND) (Kempf et al 2012). The evidence for such a link in low-dose exposed cohorts is less clear, probably because of inadequate diagnostic tools. We reason that low radiation doses to the brain, in particular early in life, may cause an accelerated development of ND conditions such as dementia or Parkinson Disease (PD). A known risk-factor for ND is oxidative stress to the neuronal tissues, and this is known to be the preferential mode of damage at chronic low-dose rate ionising radiation. We therefore set up a study to evaluate the impact of a defective repair system for ROS induced DNA damage (Ogg1 k.o.) towards the progression of neurological deficiencies in a PD mouse model (Pitx³/EYL/EYL) after pre- and postnatal chronic irradiation using the FIGARO facility. Apart from behavioral tests that show the age-related impairment of motor activity, we also measure changes in the number of dopaminergic neurons in the mesencephalon and induction of ROS-induced DNA-damage in the affected brain regions. After the first 12 month of follow up, we observed a significant impairment of motor coordination (“time to cross a beam walk”) in Pitx³ / Ogg1 double heterozygote mutants after 0.2 Gy chronic neonatal exposure. Interestingly, all motor-symptoms show a trend for impairment only after 0.2 Gy, but not after 1 Gy. Upon completion of this joint project we will gain a better insight in the contribution of low-dose rate exposure for a chronic neurodegeneration.

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P53-dependent senescence in mesenchymal stem cells under chronic normoxia is potentiated by low-dose gamma-irradiation

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Mesenchymal stem cells (MSCs) are a source of adult multipotent cells important in tissue regeneration. Murine MSCs are known to proliferate poorly in vitro under normoxia. The aim of this study is to analyse the interaction of non-physiological high oxygen and low-dose gamma-irradiation onto growth, senescence and DNA damage in murine MSC.

Tri-potent bone marrow derived (BM) MSCs from p53 wildtype and p53−/− mice were cultured under either normoxia or hypoxia (21% versus 2% O2). Long-term observations revealed a decreasing ability of wildtype mMSCs to proliferate and form colonies under extended culture in normoxia. This was accompanied by increased senescence under normoxia but not associated with telomere shortening. After an acute exposure to low-dose γ-irradiation the normoxic wildtype cells further increased the level of senescence. The number of radiation-induced γH2AX DNA repair foci was much higher in MSCs kept under normoxia, but this sensitizing effect was completely abrogated in p53−/− cells. Murine MSCs with a p53 deficiency also showed higher clonogeneity irrespective of oxygen levels. Furthermore these cells demonstrate lower senescence levels and fewer γH2AX repair foci per cell as compared to their p53 wt counterparts, both under hypoxic and normoxic conditions. These results reveal that oxygen levels together with γ-irradiation and p53 status are interconnected factors modulating growth capacity of BM MSCs in long-term culture. These efforts help to better understand and optimize handling of MSCs prior to their therapeutic use.

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CLIP2 as radiation biomarker in papillary thyroid carcinoma

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One of the major consequences of the Chernobyl nuclear power plant accident in 1986 has been a significant increase in the incidence of papillary thyroid carcinoma (PTC) among children exposed to the radiiodine fallout, particularly to iodine-131. Thus, young age at exposure is a significant risk factor for the development of radiation-induced PTC. Previously, the investigation of PTC from young exposed patients and non-exposed controls that were matched as closely as possible for potential confounders revealed a radiation-specific DNA copy number gain on chromosomal band 7q11.23 and the radiation-associated mRNA overexpression of the gene CLIP2, located on chromosome 7q11.23 (Hess et al., PNAS, 2011).

In order to investigate the potential role of CLIP2 as a radiation marker for an individual classification of PTCs into radiation-associated and sporadic tumors, we characterized the CLIP2 protein expression by immunohistochemistry. We confirmed the radiation-associated CLIP2 overexpression at the protein level in PTCs from independent tumor cohorts and established a standardized procedure for CLIP2 typing, an essential step in integrating a molecular biomarker into epidemiological studies for improved risk estimation and modeling of radiation-induced carcinogenesis (Selmansberger et al., Oncogene, 2014). Moreover, a reconstruction of the CLIP2 gene regulatory network suggests the involvement of CLIP2 in the fundamental carcinogenic processes apoptosis, MAPK signalling, and genomic instability, indicating a functional role of CLIP2 in the carcinogenesis of radiation-associated PTC. In a further study, the association between the binary CLIP2 typing and continuous thyroid dose with logistic regression was analysed. The clear dose-response relationship for the CLIP2 radiation marker in two PTC cohorts (UkrAm and Genrisk-T) for young patients with age at operation less than 20 years and age at exposure less than 5 years demonstrates the importance of this biomarker in low-dose radiation research and suggests different molecular mechanisms depending on age (Selmansberger et al., Carcinogenesis, 2015).
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