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Abstracts of the 4th International MELODI Workshop

12–14 September 2012, Helsinki, Finland

Nina Sulonen (Ed.)

Säteilyturvakeskus
Strålsäkerhetscentralen
Radiation and Nuclear Safety Authority

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STUK • **SÄTEILYTURVAKESKUS**
STRÅLSÄKERHETSCENTRALEN
RADIATION AND NUCLEAR SAFETY AUTHORITY

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The conclusions in the STUK report series are those of the authors and do not necessarily represent the official position of STUK.

Layout and compiling:
Nina Sulonen, Radiation and Nuclear Safety Authority

ISBN 978-952-478-738-3 (print)
ISBN 978-952-478-739-0 (pdf)
ISSN 0781-1705

Electronic version published: http://www.stuk.fi

Painotalo Kyriiri Oy, Helsinki 2012

Sold by:
STUK – Radiation and Nuclear Safety Authority
P.O.Box 14, FI-00881 Helsinki, Finland
Tel. +358 9 759 881
Fax +358 9 759 88500

SULONEN Nina (Ed.). Abstracts of the 4th International MELODI Workshop. 12–14 September 2012, Helsinki, Finland. STUK-A254. Helsinki 2012, 104 pp.

Keywords: radiation protection, ionizing radiation, health effects, low dose risk, research

Foreword

The Fourth International MELODI Workshop (*www.melodi2012.org*) is organized by STUK – Radiation and Nuclear Safety Authority in Helsinki, Finland, on 12–14 September 2012.

The workshop offers an update of recent low-dose research issues, and an opportunity to participate in the MELODI Low Dose Research Platform, a major step in the long term goals that the European Low-Dose Risk research intends to achieve. The main goal of MELODI (*www.melodi-online.eu*) is to develop and maintain a Strategic Research Agenda (SRA) in the field of low-dose radiation research, and to actively promote its implementation. DoReMi Network of Excellence funded by the European Commission is supporting the setting up of the Platform and addressing some of its research needs (*www.doremi-noe.net*). In line with one of the main SRA goals, a major aim of the workshop was to set all topics in an interdisciplinary context.

The Workshop abstracts cover plenary lectures as well as poster presentations related to topical discussions in breakout sessions. The theme of the first day "Low dose risk research – state of the art" provides an introduction to the MELODI activities and the SRA and an update on recent epidemiological studies and dosimetric aspects of low dose studies. Potential implications of cardiovascular disease risk for radiation protection are also addressed. Discussion on the state-of-the art of MELODI SRA took place in three break-out groups addressing epidemiological approaches, cancer mechanisms and models and infrastructures and knowledge management. The second day "Emerging scientific challenges" features the development of science and novel technologies, covering topics such as epigenetics, systems biology, stem cells as well as biomarkers that could be potentially used in molecular epidemiological studies. The associated breakout sessions explore the roadmap for future research, covering themes on biomarkers and biobanks, non-cancer effects, as well as low dose dosimetry and dose concept. The third day "Integrating the research" provides highlights of Euratom projects dedicated for research on low dose risk.

Prof. Sisko Salomaa, Chair of Scientific Programme Committee
Helsinki, 28th August, 2012

SULONEN Nina (toim.). 4. kansainvälisen MELODI-workshopin abstraktit. STUK-A254. Helsinki 2012, 104 s.

Avainsanat: säteilysuojelu, ionisoiva säteily, terveysvaikutukset, pienten annosten riskit, tutkimus

Esipuhe

Säteilyturvakeskus järjestää 4. kansainvälisen MELODI Workshopin Helsingissä 12.–14. syyskuuta 2012.

Kokous tarjoaa viimeisintä tutkimustietoa pienten annosten riskeistä sekä tilaisuuden osallistua MELODI-tutkimusyhteisön toiminnan kehittämiseen. MELODIn tavoitteena on koota yhteen eurooppalainen tutkimusyhteisö, joka työskentelee säteilysuojelun ja pienten annosten riskien parissa (*www.melodi-online.eu*). Yhtenä MELODIn päätavoitteena on kehittää strategisen tutkimuksen agenda ja edistää aktiivisesti sen toteuttamista. Euroopan komission rahoittama huippuosaamisen verkosto DoReMi tukee MELODI-tutkimusyhteisön perustamista ja osaltaan vastaa MELODIn strategiassa esitettyihin tutkimustarpeisiin (*www.doremi-noe.net*). MELODIn tutkimusagendan mukaisesti myös Helsingissä järjestettävä kokous on monitieteinen.

Kokousesitelmien tiivistelmät kattavat sekä kutsuluennot että rinnakkaisten työryhmien aiheisiin liittyvät posteriesitykset. Ensimmäisen päivän teema ”Pienten annosten tutkimuksen nykytila” sisältää katsauksen MELODIn tutkimusagendaan sekä viimeaikaisia tutkimustuloksia väestötutkimuksista ja pieniin annoksiin liittyvistä dosimetrisista kysymyksistä. Tässä yhteydessä pohditaan myös sydän- ja verisuonisairauksien merkitystä säteilysuojelun kannalta. Keskustelu MELODIn tutkimusagendan nykytilasta jatkuu rinnakkaisissa työryhmissä, joissa käsitellään epidemiologisia lähestymistapoja, syövän syntymekanismeja ja mallinnusta sekä tutkimusinfrastruktuureja ja tiedonhallintaa. Toisen päivän teema “Uudet tieteelliset haasteet” käsittelee tieteellistä ja teknologista kehitystä, aihepiirit kattavat mm. epigenetiikan, systeemibiologian, kantasolututkimuksen sekä biomarkkerit, joita mahdollisesti voitaisiin käyttää molekyyliepidemiologisissa tutkimuksissa. Näihin liittyvät työryhmät käsittelevät biomarkkereita ja biopankkeja, muita sairauksia kuin syöpää sekä pienten annosten dosimetriaan ja annoskäsitteeseen liittyviä kysymyksiä. Kolmannen päivän teema, ”Tutkimuksen integraatio” sisältää katsauksia eurooppalaisiin tutkimushankkeisiin, jotka koskevat pienten annosten riskejä.

Prof. Sisko Salomaa, Ohjelmakomitean puheenjohtaja
Helsinki, 28. elokuuta 2012

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Oral presentations

MELODI activities and the Strategic Research Agenda (SRA)

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The MELODI Association ("Multidisciplinary European Low Dose Initiative") is a platform to coordinate and promote low dose ionizing radiation (IR) research in Europe with an interface to international partners. It includes 20 research institutions but is open to other members. The main goals of MELODI are: the optimization of radiation protection in Europe, promotion and development of sustainable R&D programs on low dose radiation health risk research, training and education at the European and national level along with the dissemination of knowledge in general, as well as to facilitate access to infrastructures, and provide guidance and expertise to the European Commission. The cornerstone of MELODI is the long-term Strategic Research Agenda (SRA), a living document which is regularly updated to address changing scientific and societal needs. It is a key element of an integrative approach to improve low dose IR health risk evaluation and reduce uncertainties. The scientific community is urged to help in establishing the framework to cope with challenges from the extended use of IR in industry, medical diagnostics and therapy, and radiation accidents. Currently, the emphasis is on research focusing on radiation-induced cancers, non-cancer effects, and responsiveness of individuals and normal tissues taking into account radiation quality, dose and dose rates, specific sensitivity of cells and tissues, and internal contamination. Relevant results are anticipated from the combination of mechanistic and suitable epidemiological studies, the use of the most recent scientific knowledge and technologies and integrative modelling. "Omics" and genetic/epigenetic profiling studies are expected to provide useful biomarkers for radiation exposure, detection of damage and repair, metabolic and pathological injuries and a better understanding of cell/tissue-specific, individual and trans-generational responses. Analysis of metabolic and radiation-induced oxidative stress (and combined stresses) and intra- and intercellular signalling will help to clarify concepts of low dose and dose-rate responses including adaptive and immune responses. Ultimately, the research should lead to improved evaluation of low dose human health risk using systems biology approaches and modelling.

The latest update on atomic-bomb survivor studies

Ozasa, Kotaro; Shimizu, Yukiko; Grant, Eric J.; Sakata, Ritsu; Sugiyama, Hiromi; Soda, Midori; Kodama, Kazunori

Radiation Effects Research Foundation, JAPAN

The Life Span Study (LSS) cohort of atomic bomb survivors has been followed since 1950. As of the end of 2003, 58% of the 86,611 members with DS02 dose estimates died. The six years of additional follow-up since the last report provide more information (17% more cancer deaths), especially among those under age 10 at exposure (58% more deaths). Poisson regression methods were used to investigate the magnitude of the radiation-associated risks, the shape of the dose response, and effect modification by gender, age at exposure, and attained age.

The risk of all causes of death was positively associated with radiation dose. The sex-averaged excess relative risk per Gy (ERR/Gy) was 0.42 for all solid cancer at age 70 years after exposure at age 30 based on a linear model. The risk was thought to increase throughout life. The ERR increased by about 29% per decade decrease in age at exposure and decreased in proportion to the −0.86 power of attained age. The estimated lowest dose range with a significant ERR for all solid cancer was 0 to 0.20 Gy and a formal dose-threshold analysis indicated no threshold. Although the risk was best fit to a linear model, there were some fluctuations at low-dose levels, which would require further investigation. The risk of cancer mortality significantly increased for most major sites including stomach, lung, liver, colon, breast, gallbladder, esophagus, bladder, and ovary whereas rectum, pancreas, uterus, prostate, and kidney parenchyma did not have statistically increased risks. Both age effects for cancers of major sites were similar to those for all solid cancer, but most were not statistically significant. An increased risk of non-neoplastic diseases including the circulatory, respiratory, and digestive system was observed, but whether these are causal relationships requires further investigation. There was no evidence of a radiation effect for infectious or external causes of death.

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Epidemiological studies on natural sources of radiation and cancer risk

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Groups of people exposed to natural sources of ionising radiation provide an opportunity for epidemiological study and the potential to provide risk estimates to complement those obtained from groups exposed to man-made sources (e.g. atomic-bomb explosions and X-ray generators). Studies of underground hard-rock miners have provided estimates of the risk of lung cancer following inhalation of radon and its radioactive decay products, and this evidence has now been supplemented by that obtained from appropriately combining data from case-control studies of lung cancer and residential exposure to radon and its progeny. The concentration, for various reasons, of other naturally occurring radionuclides has also led to exposure of certain groups – studies of radium luminisers and of patients injected with thorium-based Thorotrast have provided, respectively, clear evidence of excess risks of bone tumours and liver cancer. A further example of occupational exposure is aircrew experiencing higher levels of cosmic radiation. Apart from radon, a number of studies have been conducted of groups exposed to natural background radiation in the environment, and particular attention has been paid to areas where notably high γ-ray dose-rates have been found, such as Kerala in India and Yangjiang in China. However, these studies are difficult to conduct and interpret because, *inter alia*, the predicted risk of radiation-induced cancer is small and controlling for major risk factors (such as smoking) that may be correlated with radiation exposure poses problems. One potentially important approach is the study of childhood leukaemia in relation to natural background γ-radiation: this outcome is known to be particularly sensitive to induction by radiation and there may be a reduced opportunity for confounding when compared to the study of adults. Large studies will be required to achieve adequate statistical power, but could show whether very low dose-rates increase the risk to the extent predicted.

Review of radiation-exposed cohorts potentially available for molecular epidemiological studies

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Radiation exposures from CT scans in childhood and subsequent risk of leukaemia and brain tumours

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Background: Although computed tomography (CT) has great clinical utility, serious concerns have been raised about the potential cancer risks from the associated ionising radiation, particularly for children. Our results for leukaemia and brain tumour risks from the first cohort study of CT use in children and young adults were reported earlier this year in the Lancet.

Methods: The cohort comprised patients without previous cancer diagnoses, aged <22 years at first CT, scanned in Great Britain between 1985-2002 and followed up to 2008. The absorbed brain and red bone marrow doses were estimated per scan in milligray (mGy) and analysed in relation to cancer outcomes using Poisson relative risk models.

Results: During follow-up, 74 incident leukaemias (cohort = 178,604) and 135 brain tumours (cohort = 176,587) were diagnosed. A significant radiation dose-response was found for leukaemia (p-trend = 0·010, ERR/mGy = 0.036, 95% CI: 0.005 – 0.120) and brain tumours (p-trend < 0·001, ERR/mGy = 0.023, 95% CI: 0.010 – 0.049). Compared to <5 mGy the RR for a cumulative dose of 30 + mGy (mean = 50 mGy) was 3·18 (95% CI: 1·46 – 6·94) for leukaemia and for 50 – 74mGy (mean = 60 mGy) the RR was 2·82 (95% CI: 1·34 – 6·03) for brain tumours. For perspective, in children currently 5 – 10 head CTs ≈ 50mGy cumulative red bone marrow dose and 2 – 3 head CTs ≈ 60mGy cumulative brain dose.

Conclusions: We found increased risks of both brain tumour and leukaemia with increasing dose. However, the cumulative absolute risks are small; approximately one excess case of leukaemia and one brain tumour per 10,000 head CT scans by 10 years after exposure. Therefore, if the CT is clinically justified then the benefits should outweigh the small absolute risks. Nevertheless, doses should be kept as low as reasonably achievable and alternative procedures, which do not involve ionising radiation, should be considered if appropriate. Long-term follow-up is required to study the pattern of risks into the period when common adulthood cancers occur.

Biological and physical dosimetry based dose estimation and associated uncertainty

Ainsbury, Elizabeth[1]; Trompier, Francois[2]; Fattibene, Paola[3]; Wojcik, Andrzej[4]; Kulka, Ulrike[5]

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Retrospective dosimetry incorporates a range of biological and physical techniques. The dicentric assay, which relies on the proportional relationship between the frequency of dicentric chromosomes in peripheral blood lymphocytes and radiation dose, is recognised to be the most well developed of these. The cytokinesis-blocked micronucleus (MN) assay is also regarded as an important tool for biological dose assessment. In addition, new biological indicators of dose are being developed, such as gene expression, protein and metabolomic markers. The key physical dosimetry techniques are electron paramagnetic resonance spectroscopy (EPR) which detects stable radicals induced by the ionisation process in biological or inert materials (e.g. teeth, glass), and thermally or optically stimulated luminescence which detects luminescence emitted by crystalline materials (e.g. ceramics, quartz, glass) following exposure to ionising radiation.

The ISO/IEC Guide to the Expression of Uncertainty in Measurement (GUM) is widely used as the fundamental reference document for uncertainty estimation. Nevertheless, uncertainty estimation is a constantly evolving field, and as such there are a number of alternative techniques which can be employed, not least, the powerful techniques of Monte Carlo simulation and Bayesian parameter estimation, which are now beginning to be exploited.

Retrospective dosimetry, and its associated uncertainty, is the focus of a number of current research programs. These include the EURADOS collaboration network, the RENEB (Realising the European Network in Biodosimetry) consortium and the MULTIBIODOSE project (which aims to develop multi-disciplinary biodosimetric tools to manage high scale radiological casualties). These and other studies have demonstrated the importance of these techniques, which are used both separately and in combination, in order that accurate and reliable retrospective dosimetry can be carried out following a wide range of potential exposure scenarios.

Uncertainties on internal dose assessment

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Protection of workers and members of the public from radiation is dependent on the measurement and control of radiation doses. For radionuclides that have entered the body (internal emitters), doses are determined indirectly using appropriate mathematical models that describe the distribution and delivery of dose to body tissues over time; however, the models and their parameter values are subject to uncertainty. Consideration of uncertainties on internal doses is important for two main reasons:

1. To assess the adequacy of internal radiation protection methodology. The quantification of uncertainties on internal doses can provide numerical estimates of the reliability of the protection quantities (equivalent and effective dose coefficients) used in radiation protection to assess exposures to internal emitters. Uncertainty analysis methods have been widely applied to quantify uncertainties on internal doses. Although such studies are useful for identifying important model parameters that affect the estimation of dose, it is not always clear how the distributions of effective dose that are produced by such analyses should be interpreted with respect to the intended use of effective dose in radiation protection and the use of dose coefficients as reference values. The meaning of "reliability" is clarified and strategies for assessing reliability using methods that quantify uncertainties on internal doses are discussed.

2. To quantify risk in radiation epidemiology. Studies that estimate cancer risk from internal exposures are often based on point estimates of absorbed tissue doses that do not account for uncertainties in biokinetic and dosimetric models. It is becoming increasingly recognised that ignoring these can artificially increase the statistical power of epidemiological studies of internal exposures, and furthermore, can introduce bias into the estimates of cancer risk derived from them. Drawing on epidemiological studies that include exposures to internal emitters, notably plutonium exposures of Mayak workers, methodology for the quantification of uncertainties on doses and their effect on risk estimates is discussed.

Uncertainties related to the microdosimetry of internal emitters

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One of the key issues in internal dosimetry is how information on dosimetry and biokinetics of internal emitters can be used to improve our understanding of radiation-induced effects.

Low doses of incorporated radionuclides are characterized by spatially and temporally inhomogeneous dose distributions within a tissue or organ. The spatial inhomogeneity of target cells as well as the sensitivities of various cells in a given tissue or organ may result in different health effects. Also the temporal exposure inhomogeneity, that is acute, chronic or fractionated irradiation and the dynamic behaviour of radionuclide distribution within tissues and organs may affect the biological outcome. Thus average quantities like average absorbed organ doses may not be appropriate for the estimation of biological effects of low doses.

Important radionuclides in internal dosimetry which may require a microdosimetric approach are alpha and beta emitters, such as isotopes of plutonium and strontium in skeleton or short lived radon progenies in lungs, and Auger emitters, such as iodine isotopes, in the thyroid.

One of the most important issues in low dose research is the analysis and characterization of possible thresholds in observed health effects. Decreasing the dose, its role may become negligible compared to the role of confounding factors or compared to the repair mechanisms of cells and tissues. Another consequence of inhomogeneous cellular dose distribution is that modelling of tissue response instead of single cell responses becomes even more important because of interaction among adjacent cells.

Low dose effects of high and low LET radiations are quite different. High LET radiations reach only a small number of cells depositing a high amount of energy while low LET radiations affect many cells with a small amount of energy imparted. Thus, alternative ways of assessing high and low LET exposures should be investigated such as fluence, hit probability and microdosimetric energy distributions. Improving dosimetric quantification can decrease the uncertainty on the dose effect relationships.

Implications of cardiovascular disease risk for radiation protection?

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Background

High doses of ionizing radiation are well-known to increase the risk of cardiovascular diseases including ischemic heart diseases (IHD) and cerebrovascular disease (CVD). This presentation addresses the question, whether there is sufficient evidence for cardiovascular disease risk after whole body exposures with absorbed doses of several hundred milligray to be considered in radiation protection.

Risk factors and pathogenesis

Cardiovascular diseases have been associated with the interaction of several of a large number of risk factors including smoking, hypertension, diabetes mellitus, deficit of physical activity, or age. Principal steps of arteriosclerosis, a precursor of many cardiovascular diseases are summarized in the presentation.

Response to radiation exposure

The experimental result that low-dose exposures with a dose rate of about 1 mGy/min lowers the risk of arteriosclerotic disease have not yet been confirmed by independent measurements. Different effects of moderate-dose radiation (100–1000 mGy) in heart tissue of irradiated ApoE-/- have been reported. The relation to cardiovascular risk is, however, still not clear.

Epidemiology for acute exposure

Analyses of the LSS cohort data with an LNT model resulted for cardiovascular diseases in an ERR-per-unit-dose estimate of 0.11 (95% CI: 0.05; 0.17) Gy^{-1}. A multi-model analysis of the data indicates that risks at a few hundred milligray are lower than what is predicted by the LNT model.

Epidemiology for protracted exposure

The total of LNT analyses of about ten data sets on cardiovascular disease after protracted exposure indicates similar risk-per-unit-dose estimates as those for the LSS. There is contradictory evidence on whether or not the risk per unit dose at several hundred milligray is smaller than predicted by the LNT model.

Implications for radiation protection?

According to LNT analyses for the LSS, the ERR per unit dose for cardiovascular disease is lower than for cancer by a factor of 4–5. The number of radiation associated mortality cases is, however, only lower by a factor of 2–3. Although there are indications that the LNT analyses overestimate the risk at a few hundred milligray, cardiovascular risk should be taken into account in radiation protection in order to improve credibility.

Role of epigenetic deregulation in radiation-induced genome and epigenome instability

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Radiation poses a threat to the exposed individuals and their progeny. It is known to cause genome instability that is linked to carcinogenesis. Radiation-induced genome instability manifests as elevated delayed and non-targeted mutation, chromosome aberration and gene expression changes. Its occurrence has been well-documented in the directly exposed cells and organisms.

Yet, the mechanisms by which it arises remain obscure. We hypothesized that epigenetic alterations play leading roles in the molecular etiology of the radiation-induced genome instability. Epigenetic changes comprise cytosine DNA methylation, histone modifications and small RNA-mediated events.

We have established that radiation exposure profoundly alters transcriptome, methylome and small RNAome of exposed cells and tissues.

The model of hierarchy and cross talk between different constituents of epigenetic information (DNA methylation and microRNAome), the maintenance and regulation of radiation responses and genome stability will be presented. The new model of the radiation–induced (epi)genome instability will be introduced and discussed.

Acknowledgements: Studies in the Kovalchuk laboratory were supported by the Low Dose Radiation Program / US DOE, Canadian Institutes for Health Research, and the Alberta Cancer Foundation.

Epigenetic events and radiation exposure

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Epigenetics is broadly described as mitotic and meiotic heritable changes in gene expression or its potential, without any alteration in DNA sequences. This presentation introduces three major categories of epigenetic events (CpG methylation, histone tails modification, and expression of miRNA) and their subtypes, and provides a literature review to show the involvement of epigenetic factors in many disease phenotypes. Since epidemiologic data show that a number of such phenotypes are ionizing radiation (IR)-inducible, it is argued that IR-induced genome instability in exposed as well as in 'bystander' cells may be explained by epigenomic programming and alterations in cells. In addition, recent data are reviewed to illustrate the plausibility of the hypothesis of transgenerational IR-mediated effects as well as those in somatic cells being at least partially maintained by epigenetic mechanisms. Thus, epigenetic mechanisms may explain non-targeted IR-effects, as well as IR-induced health effects without any recognizable IR-induced sequence alterations in the genome. This review also indicates that the reversibility property of epigenetic changes, together with promotion or blocking of epigenetic events by bioactive dietary components, provides the prospect of ameliorating IR-induced health effects by chemopreventive therapies. Finally, difficulties of assessing cause-effect relationships of epigenetic changes and IR-induced effects are outlined, with possible experimental as well as modelling suggestions of such mechanisms.

Modelling the responses of populations of cancer cells to therapeutic radiation exposure

Fernandez, Eric[1]; Orrell, David[1]; Fell, David[1,2]

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Anti-cancer therapies generally involve combinations of agents, one of which can be radiation treatment. Any novel agent therefore needs to be tested in combination with existing agents, starting with experiments on cells in culture, and then mouse xenograft models before testing on humans. Apart from the difficulties inherent in extrapolating results from one system to the next, there is also the problem of how to determine the most suitable order, timing and dosage of the different agent to achieve the best therapeutic effect. This is particularly the case with agents that act selectively on cells according to their phase of the cell cycle, as do most anticancer therapeutics, since the interactions between different agents depends on the interval between treatments, and can be synenergistic or antagonistic. It is impractical to test sufficient combinations experimentally, and we have therefore developed modelling approaches to allow computational exploration of different regimens to find optimal combinations. The Virtual Tumour model simulates a xenograft as a population of growing and dividing cells, with a core of quiescent or necrotic cells. We calibrate the model with experimental data including biomarker and xenograft growth curves to capture the effects of different agents on the duration of the phases of the cell cycle, and the probability of entering apoptosis from each phase. A pharmacokinetic model is used to predict the time-varying concentrations of drugs. In recent work, we have calibrated the Virtual Tumour model to simulate the responses of a variety of cell lines in culture to varying doses and schedules of radiation, and are starting to forecast the outcome of combinations of radiation treatment with an anti-cancer drug.

Molecular markers in radiation-induced thyroid cancer

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[1]Research Unit of Radiation Cytogenetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, GERMANY; [2]Human Cancer Studies Group, Department of Surgery and Cancer, Imperial College London, London, UK

An increased thyroid cancer risk in children and adolescents has been observed after the Chernobyl accident in contaminated areas even at moderate doses of 150 mGy and below. In order to gain knowledge on radiation-associated molecular mechanisms in these tumors radiation biomarkers that point to deregulated genes and pathways need to be discovered. Several studies on post-Chernobyl thyroid cancers aimed to identify radiation-specific gene expression signatures by using gene expression arrays. A very recent study showed dose-dependent alterations in gene expression in post-Chernobyl thyroid cancers. We have used an approach in which we integrated a genomic radiation marker that we previously identified by array CGH with mRNA expression data of the genes located in this genomic region. We found an exclusive correlation of gain of the chromosomal band 7q11.22–11.23 in papillary thyroid carcinomas (PTCs) from patients that were exposed to the Chernobyl radioiodine fallout at young age. CLIP2, a candidate gene from this chromosomal band was specifically overexpressed in the exposed cases at the mRNA and protein level (IHC) in tumours from exposed patients.

The novel radiation markers provide first important insights into the mechanisms of radiation-related carcinogenesis of young onset PTC and underpin the concept of radiation-specific carcinogenesis. Further validation studies on independent tumour cohorts are necessary for all of the published molecular markers. This will allow an integration of molecular markers with epidemiology in order to improve risk estimation for radiation-induced thyroid cancer.

Review of biomarkers that could potentially be used for molecular epidemiological studies in radiation-exposed cohorts

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The magnitude of health risks at low doses of ionising radiation remains controversial due to a lack of direct human evidence. It is anticipated that significant insights will emerge from the integration of epidemiological and biological research, made possible by molecular epidemiological studies incorporating biomarkers and bioassays. Biomarkers can be used for multiple purposes in epidemiological investigation including estimation or validation of received dose, of individual susceptibility and early detection of a radiation induced health effect.

In the framework of the multidisciplinary work undertaken in DoReMi, we have reviewed ionising radiation biomarkers potentially suitable for use in large-scale epidemiological studies. In addition to logistical and ethical considerations, the relevance of their use for assessing the effects of low dose ionizing radiation exposure at the cellular and physiological level was discussed.

The integration of biology with epidemiology requires enhanced discussions between the epidemiology, biology and dosimetry communities in order to determine the most important questions to be addressed in light of pragmatic considerations including the population to be investigated, the study design, the logistics of biological sample collection, processing and storing, the choice of biomarker or bioassay, as well as the awareness of potential confounding factors. Those requirements considerably reduce the number of possible candidates and explain why, currently, there is no ideal biomarker for assessing exposure, effect or susceptibility of low dose radiation exposure in population studies, although some good candidates do exist. Because multiple end points and tissues are involved in the responses to low dose radiation, it is likely that a multi-marker approach will provide information about the interplay of different possible pathways and will be needed to evaluate an individual's risk.

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Finding genetic susceptibility to radiation effects – will GWA studies help?

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There has been a veritable explosion in information from genome-wide association (GWA) studies that hold the promise in predicting risk of complex diseases and potentially radiation-associated cancers. Applying GWA study techniques to determine genetic susceptibility to radiation exposure would be aided by methods to either determine if a cancer or health outcome was truly radiation-related or that the case group was enriched for the radiation-related outcome of interest. Two GWAS studies among cases and controls with high attributable risks for radiation-related cancers have uncovered variants in the *FOXE1* gene in thyroid cancer and the *PRDM1* gene for multiple cancers sites after Hodgkin lymphoma. The thyroid cancer study was conducted among those exposed in the Chernobyl accident and confirmed an earlier report with respect to *FOXE1* in sporadic thyroid cancer suggesting the loci's importance with or without previous radiation exposure. Methods to discriminate between radiation-related and sporadic tumors will improve chances for discovery of radiation-specific variants, but these studies using e.g. gene-expression or tumor markers are new and promising rather than established. Genes and pathways involved in radiation-related cancer risk comprise a wide array of biologic processes and suggest that radiation susceptibility to increased cancer risk is likely polygenic and it may be more difficult to find many variants with small risks that interact collectively to appreciably account for individual susceptibility. Despite the complexities, technology continues to advance with the use of next generation sequencing methods such that whole genomes might be compared between those with sporadic and radiation-related cancers and unaffected individuals. While promising, these efforts represent applications still in their relative infancy and without large populations of radiation-exposed persons with biologic specimens in which to test them.

Effects of low-dose ionizing radiation on stem cells

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In recent years, there is growing evidence for involvement of stem cells (SC) in cancer initiation. Because of their long life span connected with an increased probability to accumulate genetic damage relative to differentiated cells SC of normal tissue may be important targets also for radiation-induced carcinogenesis. Generally the knowledge about effects of radiation on SC itself and on the processes involved in carcinogenesis is very rare. The influence of high doses of ionizing radiation on SC, such as causing senescence of hematopoietic SC (Wang et al., 2010; Wang et al., 2011), reduction of proliferation and induction of stress-induced premature senescence of mesenchymal SC (Cmielova et al., 2012) as well as induction of temporary G2 cell cycle arrest in human embryonic SC (Momcilović et al., 2009) was demonstrated. The effects of moderate and low doses of IR on SC are very rare investigated. There are reports revealing that moderate radiation doses can alter miRNAome of human embryonic SC (Sokolov et al., 2012). Repeated low dose ionizing radiation (LD-IR) resulted in peripheral mobilization of bone marrow SC in diabetic rats (Guo et al., 2010). In addition LD-IR can cause alterations in protein expression profile of neural SC and influences neural differentiation (Bajinskis et al., 2011). Investigations of the effect of LD-IR on SC of normal tissue as possible targets for radiation-induced carcinogenesis are basically missing. To characterize thereby involved processes two ongoing European experimental studies are in progress. Both projects, EpiRadBio (FP7-269553, 04/2011–03/2015) and ANDANTE (FP7-295970, 01/2012–12/2015), do address radiation-induced SC responses in vitro and in vivo respectively.

The presentation will give an overview on the current knowledge of radiation-induced effects on SC, paying special attention to low and moderate doses of IR and introduce the two ongoing European experimental studies.

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'CEREBRAD' – Cognitive and cerebrovascular effects induced by low dose ionising radiation

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Human brain development is a protracted process that starts in early embryogenesis and continues until nearly two years after birth. The brain is highly sensitive to ionising radiation during the foetal and early post-natal period. Data from atomic-bomb survivors show a 40% increase in the occurrence of mental retardation per Gy, with a threshold between 0.06 and 0.31 Gy foetal-dose in the most vulnerable gestational period: between week 8 and 15.

The CEREBRAD project aims to identify the potential cognitive and cerebrovascular risks of radiation doses below 100 mGy when delivered during development or to a young child (pre or postnatally). Health risks will be assessed in epidemiological studies of patients exposed in their young age to radiotherapy who received low doses to the brain or whose mother was pregnant or new mother during the Chernobyl accident. Complementary data will be gathered using animal models to uncover key biological and molecular mechanisms underlying potential cognitive and cerebrovascular effects induced by low dose radiation.

CEREBRAD will provide a stronger evidence on whether or not cognitive and cerebrovascular diseases can be caused from exposure to low radiation doses during prenatal or early postnatal periods. Hopefully, this project will help improve European regulations.

Acknowledgements: CEREBRAD is a collaborative European project funded in 2011 under the 7th EU framework programme of EURATOM (*GA 295552*).

SOLO: Highlights of progress to date

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SOLO started in March 2010 and runs to September 2014. It aims to improve estimates of the risks of long-term health effects associated with protracted external and internal low dose radiation exposures, primarily through studies of people exposed in the Southern Urals in Russia, a group of 22,000 workers at the Mayak plant and a sub-group of the 43,000 offspring of the local population who lived near the Techa River. In addition, the feasibility is being considered of examining health effects in both Mayak and UK Sellafield plutonium workers.

The work packages:

1) **External Dosimetry for Southern Urals Populations:** aims to develop improved estimates of external doses for the two cohorts.
   Progress: Work is proceeding on the validation of doses using techniques of Electron Paramagnetic Resonance on tooth samples and Fluorescence In Situ Hybridisation cytogenetics on blood samples.
2) **Epidemiological Studies of Mayak Workers:** aims to continue analyses of circulatory disease in Mayak workers, initiate analyses of chronic respiratory disease (CRD), and other examine cancer incidence in the cohort.
   Progress: Analyses of circulatory diseases have been carried out in an enlarged cohort and with extended follow-up. A first analysis has been completed of CRD morbidity. Papers have been prepared on cancer incidence in relation to plutonium exposures.
3) **Pooled Analysis for Mayak and Sellafield plutonium workers:** aims to consider the feasibility of an epidemiological study of cancer and non-cancer diseases in these two groups.
   Progress: Following **s**ubstantial delay, this study is now proceeding.
4) **Epidemiological study of cancer following *in utero* irradiation:** aims to perform an epidemiological study of cancer in children born to women resident on the Techa river or employed at Mayak.
   Progress: The Techa river *in utero* exposed cohort has been defined and follow-up is progressing well. The internal dosimetry methodology for estimating doses is being updated.

DoReMi: Radiation induced premature senescence in endothelial cells, a system biology approach

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Epidemiological studies on cohorts exposed to high doses of ionizing radiation have provided evidence for increased risks of cardio-vascular diseases. However, it is not likely that epidemiological studies will provide risk estimates for low dose and dose rate alone, but complemented with a mechanistic understanding of the cellular process induced, the gap of uncertainties may be bridged.

In this feasibility studies, that is a part of DoReMi, WP 7 Non-Cancerous effects, we are aiming for a system biology approach to reveal the process of radiation induced premature senescence of primary endothelial cells. The endothelial cellular model system (HUVEC cells) is relevant for the organ at risk, the vascular system, and the change in phenotype to senescence is related to increased risks of cardio vascular disease. The study involves a wide range of experimental endpoints divided between seven partners where modeling as will serve as the link to the system biology approach. The design of the study is performed with the purpose to reduce inter laboratory variation and allow for comparisons between the partners for the endpoints selected.

In this presentation a short summary of the results and the lessons learned so far will be presented.

The SEDENTEXCT project on dental cone beam computed tomography: outcomes and impact

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The use of Cone Beam Computed Tomography (CBCT) in dentomaxillofacial radiology has grown significantly over the last decade, with over 40 models currently on the market. However, it has been associated with higher radiation doses than conventional dental imaging. Most dental imaging happens in primary care. Furthermore, the use of dental X-ray imaging is higher in children and young people, so CBCT brings new challenges for radiation safety.

The 7th Euratom Framework project SEDENTEXCT was a collaboration of six academic institutions and one industry partner. The aim of the project was to acquire key information necessary for sound and scientifically based clinical use of CBCT. A parallel aim was to use the information to develop evidence-based guidelines dealing with justification, optimisation and referral criteria and to provide a means of dissemination and training for users of CBCT.

The achievements of the project so far are in several complimentary areas. In dosimetry, the project performed the most extensive study of different CBCT equipment, showing wide variations in dose and the value of field size limitation in dose reduction. Dosimetry and quality assurance phantoms for CBCT have been developed and marketed. It was demonstrated that some clinical uses of CBCT may have limited impact on patient management and outcomes, casting doubt on its use. Economic evaluation tools were developed and show the variation in costs of CBCT according to the healthcare context. An online training programme for CBCT users was devised. “Basic Principles” on the use of CBCT in dentomaxillofacial radiology were developed by consensus early in the project. A significant landmark was the development of comprehensive evidence-based guidelines, including a quality assurance programme for CBCT, recently published by the European Commission as “Radiation Protection 172”.

ALLEGRO to ANDANTE: An application of radiotherapy data to low-dose radiation research

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The ALLEGRO project was a Euratom-funded project running from January 2009 to March 2011 investigating the medium- to long-term risk of normal tissue harm following radiotherapy with both current and emerging technologies. The workpackages included assessment of the normal tissue radiation doses outside the treatment volume, incidence rates and modelling methods of normal tissue complications, and studies modelling the incidence of second cancers following radiotherapy. The project led to a set of targeted recommendations to clinicians, manufacturers, and researchers for steps to reduce the overall risks to normal tissue from radiotherapy. One of the findings of the project confirmed that some treatment modalities (eg. high-energy x-rays > 10 MV and protons) expose normal tissue to a dose of neutrons that may in some cases entail significant risk of second cancer. However, the magnitude of the risk is unclear because of considerable uncertainty in the relative biological effectiveness (RBE) of neutrons in the energy range at which they are generated. This was seen as an important area of further research.

Acting on this recommendation, a follow-up collaborative project, ANDANTE, was begun in January 2012. This project takes a multi-disciplinary approach to investigation of the RBE of neutrons. It includes biophysical modelling of the initial interactions of the neutron-induced secondary charged particles with cellular macro-molecules, *in vitro*/*in vivo* investigation of stem cells exposed to neutrons over a range of energies as well as a reference beam of photons, and ultimately the formulation and epidemiological testing of a neutron exposure risk model using paediatric proton therapy data. The project intends to clarify which of the many published values of RBE for neutrons apply to cancer induction at the energies, doses, and dose-rates that are received by patients during proton therapy. Since the doses fall within the usual "low-dose" category the results will potentially have a significant impact on the more general area of radiation protection risk assessment from neutrons.

Acknowledgements: The research leading to these results has received funding from the European Atomic Energy Community Seventh Framework Programme FP7/2007–2013 under grant agreements n° 231965 (ALLEGRO) and n° 295970 (ANDANTE).

Colon cancer risk among a-bomb survivors from multi-model inference with descriptive and mechanistic models

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The risk of radiation-induced colon cancer among Japanese a-bomb survivors has been investigated by applying both descriptive and mechanistic models to incidence data 1958–1998. Several models from both families described the data almost equally well. Mechanistic models are based on biologically-based (but still phenomenological) assumptions on the stages of carcinogenesis. To obtain fits to the data, which are of the same quality as those from descriptive models, cell processes such as a sequence of mutations in the early stage and radiation-induced genomic instability had to be considered in mechanistic models. In most cases risk assessment relies on a single model, selected among descriptive models, which are considered as the quasi-standard in radiation protection. To reduce the effect of the resulting model selection bias, the method of multi-model inference (MMI), which is well known in other fields of research, has been recently introduced in radiation epidemiology. A joint is derived risk from several plausible models which are weighted according to the information criterion of Akaike (AIC). By mixing descriptive and mechanistic risk models for risk estimation markedly lower point estimates are obtained, compared to the estimates of the descriptive model with the lowest AIC value. Confidence intervals in cohort strata with low statistical power are increased by up to a factor of five. MMI is recommended in risk assessment because it provides more reliable point estimates of the risk and it improves the characterization of uncertainties.

From CARDIORISK to PROCARDIO

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The CARDIORISK project lasted from 2008 to 2011. Its aim was to investigate the mechanisms of both macro-vascular and cardiac micro-vascular radiation damage in two strains of mice after local irradiation with low, intermediate and high doses. High precision irradiation set-ups were deployed to reproducibly irradiate either the heart or selected major arteries with doses ranging from 0.2 to 16 Gy. High radiation doses were explicitly included to investigate whether different mechanism are involved at low versus high radiation doses. Animals were sacrificed at pre-determined times for up to 60 weeks after irradiation. Since the animals, the heart tissue or cells were centrally prepared and provided to all members of the research consortium, molecular and cellular responses at different times could be related directly to histo-pathological and functional changes of the irradiated hearts. The most relevant and consistent finding was a dose and time dependent reduction of the microvascular density which was significant even at 2 Gy and which was preceded by a time and dose dependent loss of alkaline phosphatase expression in endothelial cells. The angiogenic capacity of cardiac endothelial cells and the mitochondrial function of cardiomyocytes was also impaired, while inflammatory, prothrombotic and adhesive markers did not exhibit a consistent pattern at doses less than 8 Gy suggesting that inflammation is not a mechanisms causing radiation-induced heart disease at low doses of <2 Gy. Moreover, there was no evidence for a role of genomic changes and clonal expansion of damaged cells.

A second Euratom project ProCardio (2012–2015) has commenced. This will integrate and expand ongoing European epidemiological studies of survivors of childhood cancer with the search for biological markers of radiation damage to the cardiovascular system. The ProCardio team includes clinical cardiologists, epidemiologists, radiation biologists, as well as proteomic and modelling experts. ProCardio will conduct a nested case-control investigation using individuals recruited specifically for the project and as part of the PanCareSurF programme. Dosimetric analysis of heart exposures will be performed for 16 different regions of the heart. Experimentally, ProCardio will identify biomarkers by exploring novel aspects of the mechanisms of cardiovascular damage by radiation, some of which were pioneered by CARDIORISK. In particular we will study the effects of radiation quality, dose rate, and cell-cell interactions within the cardiac

and endothelial environments. The new knowledge of the pathomechanisms will be used to develop alternative mathematical models to examine the dose response relationships arising from our, as well as other, epidemiological studies of cardiovascular effects at low doses.

Radiation risk models for breast cancer

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Breast cancer is the most commonly diagnosed cancer among women in most countries. The mammary gland is very sensitive to ionizing radiation and an increase of breast cancer risk after exposure has been observed in many epidemiological studies. Summary reports on breast cancer risk after exposure to ionizing radiation include e.g. BEIR VII – Phase 2 (Health Risks from Exposure to Low Levels of Ionizing Radiation, Biological Effects on Ionizing Radiation, 2006) and UNSCEAR 2006 (Effects of ionizing radiation, United Nations Scientific Committee on Effects of Atomic Radiation).

The knowledge about radiation-related breast cancer risk in women derives mainly from epidemiological studies of patients exposed to diagnostic or therapeutic medical radiation and of the Japanese atomic bomb survivors (LSS cohort). The risk models derived from the LSS cohort will be presented in some detail. Since the background breast cancer risk varies significantly between populations (Western and Asians countries) the transfer of risk estimates to other populations is particularly challenging for breast cancer and current approaches will be discussed.

Mechanistic models of carcinogenesis describe basic steps in the progress towards cancer. Thus mechanisms of potential non-targeted effect such as genomic instability can be implemented in these models and consequences for the radiation risk estimated. Furthermore the use of different models allows a more systematic estimate of model uncertainty by the method of multi-model inference. New approaches in these directions are presented for breast cancer in the LSS and the Swedish hemangioma cohort.

Estimation of radiation risk of lung cancer

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Lung cancer is one of the most frequent malignancies in many countries. Tobacco smoking is the most prominent risk factor for lung cancer. Ionizing radiation has also been established as a significant risk factor of lung cancer based on many studies of underground miners, nuclear workers, patients therapeutically exposed to radiation, and the atomic-bomb survivors. Recently residential exposure to radon has been shown to be associated with lung cancer, especially among nonsmokers. Additive and multiplicative positive interactions have been observed between radiation and smoking.

In the atomic-bomb survivor studies, the excess relative risk (ERR) of radiation for lung cancer has been described as parametric functions of the form $\rho(d)\varepsilon(s,e,a)$, in which $\rho(d)$ describes the shape of the dose-response function and $\varepsilon(s,e,a)$ describes the effect modification by sex, age at exposure, and attained age. A linear dose model ($\rho(d)=\beta d$) has generally been found to have the best fit for solid cancer including lung cancer and has been widely accepted. Different functions have been proposed to model effect modification, RERF generally uses a function of the form $\exp(\tau e + \upsilon \ln(a))\bullet\sigma s$. The latest report indicated a significantly high ERR for lung cancer using the above model (sex-averaged ERR/Gy = 0.75, 95% CI: 0.51, 1.03 for attained age of 70 years after exposure at age of 30). The ERR/Gy was larger for females than for males while the absolute excess risks were similar for both sexes. While all solid cancer and many other cancers showed an increase in the ERR/Gy in the subjects exposed at a young age, the effect of age at exposure was weak or rather inverse for lung cancer. For attained age, the ERR/Gy decreased with increasing age for lung cancer as well as other solid cancers. Interaction with smoking was also observed. Those unique aspects of radiation risk of lung cancer in the atomic-bomb survivors needs continued investigation.

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Risk models for radiation-induced leukaemia

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Leukaemia was the first cancer associated with exposure to ionising radiation when in 1944 March tentatively interpreted an increased leukaemia mortality rate in US radiologists in terms of such exposure, and in 1948 alert clinicians noted an excess of leukaemia among the survivors of the atomic-bombings of Hiroshima and Nagasaki. Since then, other epidemiological studies (mainly, but not solely, of those exposed for medical reasons) have reported a raised risk of leukaemia following exposure to radiation, and a clear link between irradiation and leukaemia now exists. Leukaemia risk models have been developed based upon data from the follow-up of the Japanese atomic-bomb survivors, supported by information from other studies. The dose-response has been found to be significantly sub-linear, a linear-quadratic model being the usual relationship that is supported by data. Notable age-at-exposure and time-since-exposure modifications of the risk are apparent in the Japanese data: following a short minimum latent period (on the basis of other studies, generally assumed to be two years) an increased risk is apparent, which is much higher among those exposed as children when compared to adult exposure, and this excess risk then attenuates with time-since-exposure. For those exposed at a young age in particular, the radiation-induced risk is expressed temporally as a "wave". Although there is no doubt that moderate and high doses increase the risk of leukaemia, how the risk is influenced by low dose or low dose-rate exposures is more contentious. However, evidence exists for an increased risk at low levels of exposure, and the increase is around the magnitude predicted by models based upon the experience of the Japanese atomic-bomb survivors, and this evidence has been strengthened by recent findings. Recently developed radiation-induced leukaemia risk models will be presented and critically discussed, especially with respect to low dose/dose-rate predictions.

Poster presentations

Correcting for measurement errors when estimating lung cancer risk associated with radon exposure among uranium miners

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In epidemiological studies, Measurement Errors (ME) can substantially bias the estimation of the parameters. A broad variety of methods for ME correction has been developed, but they have been rarely applied. The present work investigates ME associated with radon exposure in the French cohort of uranium miners and its impact on the estimated excess relative risk of lung cancer death associated with radon exposure.

The French Cohort of Uranium Miners includes more than 5000 miners chronically exposed to radon with a follow-up duration of 30 years. ME associated with radon exposure has been characterized for each individual, taking into account the evolution of uranium extraction methods and that of radiological monitoring over time. We carried out a simulation study to investigate the effects of these ME on the estimated lung cancer excess relative risk per working level month (ERR/WLM) and to assess the behavior of different methods for correcting these effects.

ME associated with radon exposure decreased over time, from more than 90% in the early 1950's to about 10% in the late 1980's. The type of ME also changed over time from mostly Berkson to classical type. Simulation results showed that ME leads to an attenuation of the ERR towards the null, with substantial bias on ERR estimates in the order of 60%. With the different ME correction methods considered (Substitution method, Estimate Calibration method, Simulation Extrapolation method), the attenuation bias was reduced substantially but not completely. About 50–60% of the bias was corrected.

This work illustrates the importance of ME correction in order to obtain more reliable ERR estimates of lung cancer risk associated with radon exposure among French uranium miners. Such risk estimates should prove of great interest in support to the determination of protection policies against radon.

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Monitoring of thyroid cancer incidence in the vicinity of nuclear sites in Belgium

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We investigated, by means of an epidemiological study, the incidence of thyroid cancer within the 20 km proximity area around nuclear sites, and whether there is evidence for an increasing thyroid cancer risk with increasing 'surrogate' exposure (residential proximity to the site, prevailing wind directions, and estimated discharges from the plants based on mathematical modelling) within the 20 km proximity area around the nuclear sites.

Results showed no increase in the incidence of thyroid cancer around the nuclear power plants of Doel and Tihange. Results for the Belgian territory in the vicinity of the French nuclear power plant of Chooz were unstable due to the limited number of incident cases. For the nuclear sites of Mol-Dessel and Fleurus, where a combination of nuclear research and industrial activities are located, a slightly increased incidence of thyroid cancer as compared to the regional average was observed, but similar and higher increased incidences were also seen in Belgium at other locations without nuclear sites.

Based on the surrogate-exposure modelling, there was no evidence for an association between the nuclear site of Mol-Dessel and the occurrence of thyroid cancer, whatever the three surrogate exposure considered. In Fleurus, there was also no evidence for an association between the nuclear site and the occurrence of thyroid cancer in function of residential proximity. For prevailing winds, the evidence may be weakly suggestive of a potential association with the site. This evidence was, however, not confirmed by a further evolved modelling, i.e. the model of discharge estimates. This part of the analyses was hampered to a great extent by the large geographical areas at which health data are available.

It may be advisable to repeat the epidemiological monitoring within five years; to make health data available at smaller geographical level; and to participate in cross-border and international collaborative initiatives.

Lung cancer mortality (1950–1999) among Eldorado uranium workers: A comparison of models of carcinogenesis and empirical excess risk models

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Lung cancer mortality risk after radon exposure among 16,236 male Eldorado uranium workers is analyzed with models of carcinogenesis and empirical models. The workers were first employed in 1932–1980 in the Beaverlodge and Port Radium uranium mines and the radium and uranium refinery and processing facility in Port Hope, Canada, and followed up for mortality from 1950 through 1999. A total of 618 lung cancer deaths was observed. The analysis is performed by means of the biologically based two-stage clonal expansion (TSCE) model and empirical excess risk models. Under the assumptions of the TSCE model, there is a strong indication that the spontaneous clonal expansion rate of premalignant cells is reduced at ages above about 60 years. Analysis of radiation-related risks shows that the principal effect for lung cancer is an increase of the clonal expansion rate of premalignant cells with the exposure rate. However, this increase is not linear as the clonal expansion rate increases strongly for low exposure rates and then becomes linear with a smaller slope at higher exposure rates. A possible explanation for this observation could be a bystander effect. In addition, a BEIR VI model and a parametric excess relative risk model linear in radon decay product exposure with attained age, time since exposure and dose rate as effect modifiers are investigated. The predictions of the excess relative risk from the different models are compared for different exposure scenarios. The dependence of lung cancer risk on exposure and the effect modifiers is studied and the uncertainty due to the use of different models is investigated.

Domestic radon exposure and risk of childhood cancer: a prospective census-based cohort study from Switzerland

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The effect of indoor radon exposure on the risk of childhood malignancies is poorly known. Doses from radon and its decay products are much smaller to other organs than to the respiratory tract and it is unclear whether they are high enough to cause childhood leukaemia. Until now, ecological and case-control studies on radon exposure and childhood leukaemia have found inconsistent results. Using a nationwide cohort study, we aimed to investigate whether indoor radon concentration is associated with an increased risk of childhood cancer in general and of leukaemia and central nervous system (CNS) tumours in particular.

Data on the population at risk were obtained from the Swiss National Cohort (SNC). All children aged between 0 and 15 years, born before the December 4th, 2000 (date of census) and resident in Switzerland were included. The follow-up period lasted from the date of census until death, emigration, reaching the age of 16, date of diagnosis or December 31st, 2008. Information on incidence cases was obtained from the Swiss Childhood Cancer Registry (SCCR). Radon exposure was assessed for each child based on a nationwide radon prediction model (Hauri, et al., 2012). We fitted Cox regression models adjusting for environmental radiation exposure from external sources (cosmic, terrestrial and artificial ground radiation), gender, birth order within each household, socio-economic status of the parents and other potential confounders.

The mean radon concentration in the 1,300,000 children included in the analysis was 88.5 Bq/m^3, ranging from 0.7 to 490.1 Bq/m^3 (arithmetic mean). 772 children were diagnosed with childhood malignancies of which 228 had leukaemia and 205 CNS tumours.

Our preliminary results do not indicate associations between residential radon with the risk to develop malignancies, leukaemia and brain tumour during infancy.

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A record-based case-control study of natural background radiation and the incidence of childhood leukaemia and other cancers in Great Britain during 1980–2006

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Recently developed risk models for radiation-induced leukaemia predict that around 15% of childhood leukaemia incidence in Great Britain is attributable to natural background sources of ionising radiation. The uncertainties associated with this estimate are, however, substantial, not least of these being the appropriateness of generalising risks derived from groups briefly exposed to moderate and high doses (primarily the Japanese atomic-bomb survivors) to groups exposed at a very low dose-rate. Owing to the low cumulative red bone marrow (RBM) doses received in childhood from natural background radiation (~1.4 mSv per year), and the comparatively small geographical variation of these doses, large studies are required to achieve adequate statistical power to detect the predicted risk from this source of radiation. In the UK the comprehensive National Registry of Childhood Tumours, together with measurements made in a nationwide radiation survey, enables a suitably large study of the influence of external γ-ray and radon exposures upon the risk of childhood cancer, especially childhood leukaemia, and we have conducted such a case-control study of 27 447 cancer cases diagnosed in Great Britain during 1980–2006. Cumulative exposures to γ-rays and radon were calculated for cases and controls using the maternal residence at the birth of the child. The study produced an excess relative risk of childhood leukaemia of 0.12 (95% CI: 0.03, 0.22) per mSv RBM dose from background γ-radiation, which is compatible with the predictions of current risk models. No statistically significant association between childhood cancers other than leukaemia and γ-radiation, nor between childhood leukaemia or other childhood cancers and radon, was found, which is consistent with prior evidence. At present, individual γ-ray exposures are based upon the average in 459 County Districts (intermediately sized administrative units), but work is underway to improve these estimates.

Childhood leukaemia risks: Towards a better understanding of unexplained results

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Several published epidemiological studies of childhood leukaemia risk near nuclear installations recently raised questions which could not be answered by current knowledge on the effects of ionising radiation. In Germany, an increased risk of childhood leukaemia was observed in relation to the proximity of nuclear power plants (Kaatsch 2008). An increased risk was also observed near nuclear power plants in France (Sermage-Faure 2012), but this excess showed no association with the estimated distribution of radiation exposure due to gaseous discharges from the plants, and the estimated levels of exposure were far below the levels for which a detectable risk is foreseeable. As a consequence, the BfS developed an agenda for future research on childhood leukaemia in Germany (Ziegelberger 2010). In France, an experts group was also implemented, and recommendations for further actions were published (Sommelet 2011).

Based on these recommendations, BfS and IRSN decided to hold a focussed workshop, bringing together the main researchers from relevant disciplines in the field (epidemiology, biology, dosimetry and biostatistics). This workshop was organised in June 2012 under the auspices of the MELODI European association, and regrouped more than 40 participants. The goal of the workshop was twofold: a) to learn from past studies and to develop a best possible study design for answering questions on childhood leukaemia incidence close to point sources; b) to identify promising directions for future research into the causes and pathogenesis of childhood leukaemia at a European level. The meeting's concept was not focussed on radiation alone, as huge efforts have already been made in this field in the past years, and duplication of research should be avoided. The main emphasis was put on future research with interdisciplinary approaches. The results of the workshop should provide a sound basis for developing funding schemes on a national and European level.

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Mechanism of low-dose alpha particle carcinogenesis in 3D lung model – role of stroma-epithelial interactions

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Lung cancer is the most diagnosed cancer worldwide with 1.35 million new cases registered and about 1.18 million deaths each year. Smoking has been shown as the leading causative factor as the second place has been attributed to radon gas emanating to the indoor air from the ground. Epidemiological studies on radiation workers cohorts (Mayak workers, uranium miners) have shown dependence between the exposure to ionizing radiation and the lung cancer development.

We aim to study the early effects of low dose (0.1 Gy) exposure to alpha particles irradiation, similar to the natural sourses of alpha radiation, using 3D bronchial epithelial cultures EpiAirway™ (MatTek Corp., Ashland, MA) as a model system. The effect of radiation on the induction of pre-cancerous changes was investigated by analysing the alterations in the expression of cell type specific markers in the normal bronchial epithelial cells. We focused on process known as EMT (epithelial-mesenchymal transition) which is characterized by switch of epithelial polarized phenotype to mesenchymal phenotype leading to loss of cell contacts and increased cell motility. The role of the exposure of normal tissue microenvironment (stroma) has also been studied and for model we used normal primary human fibroblasts MRC-9. They were exposed to low doses of radiation and co-cultured with the EpiAirway™ tissues to allow signalling factors from the fibroblasts to influence the bronchial epithelial model. We studied the role of the TGF-β signalling in the EMT induction. The markers that we evaluated were epithelial (E-cadherin, β-catenin, ZO-1) and mesenchymal (fibronectin and vimentin). For comparison we are performing experiments on the effect from higher doses of radiation and also from blocking of the TGF-β signalling on the EMT changes in the EpiAirway™ model.

The detailed knowledge of these mechanisms could give potential biomarkers for the early stages of lung cancer. Identification of stromal signalling involved in the EMT would give targets for early prevention of alpha particles induced lung carcinogenesis.

Mechanistic insights into the hypersensitivity of Cockayne syndrome and Xeroderma pigmentosum A human fibroblasts to ionizing radiation

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In a previous study we have shown that Cockayne syndrome (CS) human primary fibroblasts are hypersensitive to ionizing radiation (IR) and oxidizing agents (D'Errico et al., 2007) and present an altered redox balance (Pascucci et al., 2012). Here we show that the hypersensitivity of CS cells to IR is detectable also at doses as low as 0.5 Gy. Moreover, we provide evidence that Xeroderma pigmentosum (XP)-A primary fibroblasts are hypersensitive to IR (including low doses) and accumulate oxidative damage in their DNA upon exposure to oxidizing agents (Parlanti et al., under revision). To gain insights into the molecular mechanisms underlying the hypersensitivity of these cells to IR, the redox status of CS and XP-A primary fibroblasts was analysed. ROS levels were measured by oxidation of the spin probe CPH to CP* by EPR. The steady-state ROS levels were significantly higher in primary fibroblasts from both CS (A and B) and XP-A donors as compared to normal. This alteration was associated with a perturbation of the oxidative metabolism in all nucleotide excision repair (NER)-defective fibroblasts as analysed by ^{1}H-NMR. Experiments are in progress to measure ROS production after IR in normal and defective cells. To check whether the radiosensitivity underlies a role of these NER proteins in the processing of IR-induced DNA lesions, the phosphorylation of H2AX, a marker of double strand breaks (DSB), was measured after exposure to 0.2 and 1 Gy. A clear increase of gamma H2AX foci per cell was detected at both doses with higher levels in CS as compared to normal cells. These data confirm the sensitivity of the phosphorylation of H2AX as a tool to measure low dose-induced DSB and suggest that CS proteins participate to the processing of DSB. The role of XPA is currently under investigation.

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A proteomic approach to identify phase-shifts in responses and processes at low doses and dose rates

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The risk projection models used to estimate risk at low doses and dose rates derived from data found at higher levels. An accurate reflection of the processes that contribute to cancer risk is therefore urgently needed. It is important to look for any 'phase-shifts' in the biological response to different radiation modes. The aim of our part (task 5.1) is to identify changes in protein regulation after low dose and dose rate irradiation in a selected cellular model.

Dose and dose rate effects were analyzed in human fibroblasts (VH10):

- Pre-irradiation only:
  dose: 40 mGy; dose rate: 4.1 mGy/h, 50 mGy/h or 24 000 mGy/h
- Pre-irradiation 2 hours prior to challenging (acute) dose:
  Pre-irradiation dose: 40 mGy , dose rates as above;
  Challenging dose: 100 mGy
  (Investigation of adaptive response effects)
- Challenging (acute) dose only:
  dose: 40 mGy, 100 mGy or 140 mGy
  All challenging doses were applied with a dose rate of 24 000 mGy/h.

Protein patterns were analyzed 3 h after exposure using the 2D-Ettan-DIGE system. Data of identified regulated proteins were evaluated by Western blot.

The results of the irradiation experiments provide evidence for a dose rate effect as demonstrated by differentially regulated proteins. A dose rate of 4.1 mGy/h had only marginal effects on protein regulation changes. However, a dose rate of 50 mGy/h showed more regulated proteins and higher regulation factors than at the dose rate of 24 000 mGy/h.

Differences in the cell response after exposure to 140 mGy in total were most pronounced comparing combined to single irradiation.

The data clearly show that low doses and low dose rates have different effects on the protein regulation in the cells. Although the protein regulation rates were only between 10–50%, the Western blots of selected proteins clearly confirmed regulation factors of > 0.2 found by 2D-DIGE.

Transcription profiling of radiaoadaptive response in mouse thymocytes

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A particular case of adaptive response in the thymus is the existence of an early radioresistance effect to thymocyte apoptosis and G1 arrest by whole-body preirradiation with low priming dose of 0.075 Gy before a single challenging dose of 1.75 Gy g-rays. However, genetic changes associated with this low dose radiation-induced adaptive protection response are not well understood.

In this work, we have investigated the adaptive response of thymocytes of C57BL/6 mice (males and females) exposed to the mentioned adaptive regimen of whole-body γ-irradiation to (a) determine how the priming dose influences subsequent transcript profiles in adapting thymocytes, and (b) identify genes that are associated with presence of low dose radiation-induced adaptive response after the challenge dose. Transcription profiles were generated from thymocyte RNA isolated from individual irradiated mice at 24 h and 1 week after whole-body γ-irradiation and unirradiated controls. RNA from these samples was probed against SurePrintG3 Mouse GE8x60K microarray (Agilent), which contains about 60,000 mouse cDNAs. Data obtained were analyzed using the GeneSpring GX11 software (Agilent). Preliminary data indicate that radiation-induced adaptive protection response of thymocytes at 24 h modulated the expression patterns of 309 genes in females, of which 77 genes showed differential expression in both sexes. No differences in the expression profiles were observed when compared the radioadaptive reponse at 1 week and control situation. Interestingly, about 70% of 309 genes with modulated expression in females showed time-dependent variation in their expression levels.

Accumulation of DNA damage in complex normal tissues after protracted low-dose radiation

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The biological consequences of low levels of radiation exposure and their effects on human health are unclear. Ionizing radiation induces a variety of lesions of which DNA double-strand breaks (DSBs) are the most biologically significant, because unrepaired or misrepaired DSBs can lead to genomic instability and cell death. Using repair-proficient mice as an *in-vivo* system we monitored the accumulation of DNA damage in normal tissues exposed to daily low-dose radiation of 100 mGy or 10 mGy. Radiation-induced foci in differentiated and tissue-specific stem cells were quantified by immunofluorescence microscopy after 2, 4, 6, 8, and 10 weeks of daily low-dose radiation and DNA lesions were characterized using transmission electron microscopy (TEM) combined with immunogold-labeling. In brain, long-living cortical neurons had a significant accumulation of foci with increasing cumulative doses. In intestine and skin, characterized by constant cell renewal of their epithelial lining, differentiated enterocytes and keratinocytes had either unchanged or only slightly increased foci levels during protracted low-dose radiation. Significantly, analysis of epidermal stem cells in skin revealed a constant increase of 53BP1 foci during the first weeks of low-dose radiation even with 10 mGy, suggesting substantial accumulations of DSBs. However, TEM analysis suggests that the 53BP1 foci are not persistently unrepaired DSBs because they do not co-localize with activated Ku70 or DNA-PKcs, core components of non-homologous end-joining. In contrast, our findings suggest that these lesions, which are predominantly located in compact heterochromatin, may reflect permanent chromatin rearrangements caused by the repair or misrepair of radiation-induced DSBs.

Gamma irradiation facility for low dose / dose rate *in vitro* biological studies

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We present a gamma irradiation facility for the exposure of cultured mammalian cells to dose rates ranging from few μGy/h to some tens of mGy/h. The study of the effects of these low dose rates have important implications both in radiation protection and in therapy (late response of healthy tissues). The irradiating system has been designed at the Istituto Superiore di Sanità (ISS), and, as of June 2012, is in the phase of construction. The realization of the facility has been made possible by a special EU funding, through a dedicated task, "LIBIS", of the EURATOM Network of Excellence "DoReMi" (7th FP).

Three especially designed holders will house, respectively, three ^{137}Cs sources with different activities, 37 MBq, 740 MBq and 18.5 GBq, forming three irradiators. Depending on the dose rate required by the experiment, one of the irradiators and the cellular sample will be placed inside a shielded incubator with controlled temperature, humidity and CO_2 atmosphere. The distance between the irradiator and the sample will be adjusted to have the chosen dose rate on the sample. The dose rate uniformity on the sample will be at least of 95%, and the percentage of 662 keV (energy of the photon released in the ^{137}Cs decay) photons impinging on the sample will be greater than 80%. Geant4 simulations will be performed in order to evaluate the dose delivered to the cells. The dose rate at sample position will also be measured before the experiments using different dosimeters (TLD, OSL, alanine and/or ionization chamber).

The main issues that will be possible to tackle with this irradiation facility are: i) comparison of the effectiveness of low dose rate chronic exposures vs acute exposures, using the ^{137}Cs gamma cell of the ISS; ii) comparison of low dose rate exposures as a function of radiation quality, made possible by the alpha particle irradiator developed at the ISS; iii) sequential irradiation studies irradiating the same sample with the two radiation types.

The PTB microbeam facility

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This microbeam facility has been in routine operation for the last 10 years and irradiations of various cell types have been carried out for a variety of collaborative projects with outside groups. The microbeam provides high-LET αparticles and low-LET protons with energies of 3 MeV to 20 MeV. This range of ions and energies allows the selection of radiation qualities with LET values between 3 keV/µm and 200 keV/µm, which covers almost entirely the range from diagnostic X-rays to naturally occurring α-radiation. The cell nucleus or cytoplasm of individual cells can be targeted with a spatial resolution of about 2 µm and irradiated with a single particle or with precisely counted multiple particles. For the study of bystander effects one can choose to target selected cells or a fraction of cells in a dish and study the radiation response in directly irradiated and bystander cells (Frankenberg et al., 2008). At present, up to 50,000 cells per hour can be automatically processed including all experimental steps (imaging, cell recognition, position analysis and irradiation). The use of reference markers allows the revisiting of each cell in a dish for later analysis of radiation responses using a variety of endpoints. Live-cell imaging of GFP- or RFP-tagged reporter genes has been established at PTB (Mosconi et al., 2011) and is readily available.

Irradiations in neutron fields with energies in the range of 0.1 MeV to 15 MeV are available at the PTB ion accelerator facility (Frankenberg et al., 2010; Schmid et al., 2003).

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STORE – a data-sharing infrastructure for radiobiology as a tool for knowledge management

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The sharing of data and biomaterials from publicly funded experimental radiation science adds enormous value to the original investment. Sharing will yield substantial scientific added value through re-analysis and new investigations. This does not only account for new technologies in data analysis. Moreover, the rapid progress in radiation biology allows analysing old material with new techniques.

The emergence of 'omics technologies has led to an explosion in the rate and volume of data generation, and data sharing through the scientific literature alone has become impossible. It is well recognised in the community that potential benefits can accrue from a wider culture of sharing to date's research data and bio resources by making data available immediately on publication and resources within a defined period. But also the use of legacy data is particularly important for radiation biology, because a large number of studies conducted between the 1950s and '90s are unrepeatable because of ethical and financial restrictions. Still, such legacy data can usefully be reanalysed in the light of new paradigms (Schofield et al., 2010).

Thus, it is not only important to rescue endangered primary data, even more crucial is to archive it at the time it is generated. This lesson has been learned by the radiobiology community, who took on that challenge 25 years ago when developing the International Radiobiological Archives, with was further developed to become the internet-based data repository ERA (Birschwilks et al., 2011).

For both purposes, rescuing endangered data from past experiments and saving data from modern studies, the STORE infrastructure has been developed. STORE allows the storage and retrieval of data from past, current and future radiobiological studies. Secure access software allows full control for the data's originator: information can be stored only, e.g. as a backup, without access for others; it can be made available to selected co-workers; it can be released to the whole scientific community. STORE can also act as a directory to physical collections of tissue samples, FFPE blocks, and slides etc. or to other relevant data bases, e.g. of the German Uranium Miners Cohort Study. For slides, STORE

offers the possibility of hosting whole histopathology slide scans as a virtual archive. It has to be emphasised that STORE is suitable for both information from radiobiological experiments and for studies amongst human populations. As an example, STORE hosts the data of the German Thorotrast study – a cohort study amongst Thorotrast patients –, the data of the respective animal experiments and a pointer to biomaterial from these experiments.

Knowledge management does not only imply making data and biomaterial available. Information will only turn into knowledge if it is linked to an action or to a thought-process, though information is the necessary first step. To allow for extracting the utmost information from a platform like STORE experiences made by others based on the same information has be made available. To that end, STORE is also capable to include annotations to the information, give references to scientific publications or incorporate grey literature like lab reports. This has been done successfully in ERA (*http://era.bfs.de*).

In our case, knowledge management also includes the development of Standard Operating Procedures (SOP) for evaluating the quality of radiobiological archive tissue and defining test systems describing the usefulness of such material. The resulting data have been quantified and the procedures performing best in terms of the call rates of data points and experimental noise, compared to those from the fresh-frozen or freshly-made formalin-fixed paraffin-embedded (FFPE) control, have been validated. The SOPs have been made available (Azimzadeh et al., 2010; 2011; 2012). Last but not least, new methods appeared during the course of the project. It has been shown that these methods can also be used for archival material (Paunesku et al., 2012).

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Acknowledgements: STORE (Sustaining access to Tissue and data frOm Radiobiological Experiments) is an FP7 project funded by the EC under GA no. 232628.

RENEB – Realizing the European Network of Biological Dosimetry

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RENEB will establish a sustainable network of biological dosimetry, including 23 organisations from 16 EU-countries. It will significantly improve the accident and emergency response capabilities in case of a large-scale radiological emergency by contributing highest efficiency in the processing and scoring of biological samples for a fast and reliable dose reconstruction. The operational basis will comprise the analysis of acknowledged biomarkers for radiation exposure. Six applied techniques will be compared, standardized and harmonized within the partner laboratories to guarantee the highest possible reliability and accuracy of the dose estimation. Beyond the use for emergency cases, the network with its capability to jointly analyse large numbers of samples will also be able to contribute to a wider field of radiation research, e.g. to studies focusing on topics like radiation effects in the low dose range, individual-, age- or sex-related radiosensitivity or internal radiation exposure. The safeguard of the high quality and education & training provisions standard will be ensured by implementing a Quality Assurance & Quality Management (QA&QM) program. This will include a long-term training program with technical exercises according to the requirements of international standards and performed on a regular basis. This program is intended to be linked to already existing European and global training platforms such as those supported by ENEN, ENETRAP, ENSTTI and IAEA. Besides the training of the laboratory staff, also the testing and validation of new bioindicators will be performed. RENEB will thus contribute in manifold way to the needs of the radiation research community. In this regard, an interaction with radiation research platforms such as MELODI is envisaged.

In vivo gene expression studies confirm differential expression profiles at low doses of ionizing radiation when compared to *in vitro* ones

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A lot is known about the effects of high doses of ionizing radiation, but till now, there is a lack of clear and coherent information on the health effects of low doses. This is a matter of great importance, especially considering the increased individual and societal radiation burden caused by medical imaging that incorporates the use of X-rays.

When studying ionizing radiation, especially in the low dose region, we are faced with the limitation of technique sensitivity, except for γ-H2AX foci. DNA microarrays are widely utilized to study cellular responses of complex nature and previous studies have shown that microarrays are effective in the determination of pathways and genes that can be induced by ionizing radiation. In our study, we examined the global gene expression profiles of whole blood samples before and after exposure to a typically low and high X-irradiation dose (0.05, and 1 Gy), at a rate of 30 mGy/min (250 kV, 1.6 mA, 1 mmCu). We investigated the gene expression profiles after 8 hours. Gene Set Enrichment Analysis (GSEA) revealed two distant dose-dependent profiles. In contrast to high doses, we found that a low dose of 0.05 Gy showed higher statistical ranking of immune-related pathways that are mainly involved in response and/or secretion of growth factors, chemokines and cytokines. This response is typically related to bystander effects and tissue response. At 1 Gy however, the response was dominated by classical radiation response genes including tumor suppressor TP53, apoptosis, DNA damage and repair.

In order to validate our *in vitro* conclusions we moved to an *in vivo* study. Blood samples were collected from prostate cancer patients undergoing arc therapy before and 24 hours after the first fraction of radiotherapy. Dosimetric calculations showed that the circulating blood of these patients received a dose in the range 0.03 – 0.1 Gy. RNA was isolated and DNA microarrays were performed on a total of 7 patients. We found 890 genes that were significantly modulated between controls and irradiated samples (FDR < 5%). Pathway enrichment analysis showed the involvement of the significant genes in immune modulation,

cytokine secretion, DNA damage, and cell cycle regulation/check points. Some of these genes (XRRC4, BMX, and TNFAIP6) were confirmed using qRT-PCR. GSEA analysis revealed similar pathways as observed in the individual gene list analysis, with more emphasis on the involvement of certain immune related pathways such as toll-like receptors and MAPK. Both *in vitro* and *in vivo* results show that low doses of ionizing radiation can induce an immune response involving intercellular communication and probably elicits increased radiation-induced damage.

Acknowledgements: This work is supported by the Federal Agency of Nuclear Control (FANC-AFCN, Belgium).

EU EPI-CT project: biomarkers of radiation sensitivity for children

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In the last 10 years the use of computed tomography (CT) has grown considerably. As a result, the numbers of examinations have increased to the extent that CT has made a substantial impact on not only patient care, but also patient and population exposure from medical X-rays. This relatively high dose modality contributes up to 60% of the collective dose from diagnostic radiology in some European countries. The increasing use of paediatric CT worldwide has raised the question of possible late effects from exposure to ionising radiation. The European collaborative EPI-CT project (***Epidemiological study to quantify risks for paediatric computerized tomography and to optimise doses***) aims at studying the cancer risks and the underlying biological effects in an international cohort study. The project is coordinated by the International Agency for Research on Cancer (IARC). The overall objective is to inform about dose reduction and optimisation in paediatric CT.

The aim of the biological part of the study (Workpackage 5) is to compare *in vitro* and *in vivo* different biomarkers for radiation exposure and to test their sensitivity in clarifying the biological mechanisms behind low dose hypersensitivity observed in CT examined paediatric patients. The work is divided into a number of distinct and complementary tasks which will allow studying the effects of CT exposure using a variety of approaches, including assessment of DNA damage (through monitoring chromosomal aberrations and gamma H2AX foci), oxidative stress, gene expression and inflammatory response.

Final results of this pilot study will be used for evaluating the feasibility of conducting a larger study allowing sufficient statistical power for estimation of age- and sex-dependent radiosensitivity.

Acknowledgements: This work is supported by the EU EPI-CT grant agreement (n° 269912).

Low dose IR induces gene expression changes via DNA breaks and other pathways

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The effects of low and protracted doses of ionizing radiation on humans are only partially understood. The shape of the dose response curve at low and protracted doses cannot be measured accurately using standard epidemiological methods. However, this information is needed for rationale policy decisions for setting exposure limits. Therefore, we set out to determine a gene expression signature that operates at low and intermediate doses using a panel of DNA double strand break repair deficient mouse models.

We developed mouse models that lacked one or both of the major DNA double strand break repair pathways (nonhomologous end-joining and homologous recombination) and compared transcriptional responses in these DNA repair deficient mice with normal mice before and after irradiation with a relatively low dose of gamma radiation (200 mGy). Importantly, the changes in gene expression patterns were nearly identical in repair-deficient mice and in irradiated mice. We identified a gene expression signature that was very similar, but considerably more pronounced (up to 5-fold) in irradiated DNA repair deficient mice compared to normal mice, showing that the transcriptional responses are indeed caused by the DNA damage inflicted by radiation and that DNA repair deficient mice can be used as the proverbial 'canary in the coalmine' in the context of low dose radiation research. The identified gene expression signature includes Gadd45a, Gad45b, MDM2, BAG3, and CDKN1A (p21) and in addition allowed the identification of additional genes by correlation analysis. A distinct set of genes was consistently upregulated after low dose IR, but this did not change with DSB repair defects, suggesting that this part of the transcriptional responses is caused by other triggers than DSBs.

We conclude that this transcriptional response can be used as a very sensitive read out for low levels of radiation exposure. This will be meaningful, as several genes from this signature have functions in known pathways that regulate cell proliferation and/or senescence and apoptosis, which are all relevant for carcinogenesis and (tissue) aging. This consistent gene expression signature defines a universal marker profile relevant for the general population, providing valuable tools for biomarkers, mechanistic understanding, prevention and therapy.

The German Uranium Miners Biobank – current status and perspectives in radiation research

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The German uranium miners of the former Wismut mining company with about 400,000 employees are the largest radiation exposed miner population worldwide. 59,000 of them are included in the Wismut Cohort Study. Yet, biobanks from occupationally radiation exposed individuals do not exist or only on a small scale. Together with the German Social Accident Insurance (DGUV) and its research institute the IPA, the Federal Institute for Occupational Safety and Health (BAuA) and the Institute of epidemiology of the HMGU, the BfS collected blood from 442 healthy workers and from 81 workers with lung cancer. Additionally, DNA and RNA from tissue of lung cancer cases from the Wismut Autopsy Archive and blood from children of Wismut miners whose fathers died early on lung cancer was collected. The biological material and the epidemiological and experimental data are stored in the German Uranium Miners Biobank at the BfS. The data will be available via STORE.

From 2009 to 2011, 442 healthy volunteers were recruited for sample collection. 292 of the probands received a cumulative radon exposure of more than 750 Working Level Month (WLM), 150 probands less than 50 WLM. Blood was collected for isolation and cryoconservation of DNA, RNA, lymphocytes and plasma proteins. The samples were processed according to established SOPs and finally stored in the biobank at the BfS. A quality check on several molecular levels (DNA methylation, SNPs, mRNA, miRNA, and protein) confirmed the suitable quality of the SOPs. Detailed data on occupational exposure to radiation, dust and arsenic as well as health and smoking data are available. DNA from 250 tissue samples of lung cancer cases and RNA from a subset of 50 samples, tumor tissue and normal tissue, was extracted. Genome wide array data (Illumina 660K chip) were generated from DNA from additional 81 lung cancer cases. DNA, lymphocytes and plasma were extracted from blood of 87 children whose fathers died of early lung cancer. The biobank will contribute to systembiological research projects to investigate genetic, biochemical and metabolic factors in dependence of occupational radiation exposure and life style. It will be a powerful tool to analyse radiation risk at low doses, individual susceptibility to radiation, long term radiation exposure markers and radiation specific fingerprints in lung cancer.

Proteomics profiling of low molecular weight plasma proteins from locally irradiated individuals

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Blood plasma from radiation exposed individuals has been shown to induce chromosome damage when transferred into lymphocyte cultures of non-irradiated persons. The nature of these clastogenic factors (CF) is still mostly unknown, although evidence exist that some of them are low molecular weight (LMW) proteins. In the current work, we have applied proteomics techniques to profile some potential CFs in plasma samples of locally exposed clinical patients.

Ca 30 patients obtaining fractionated treatment were sampled: before the first fraction and directly after last irradiation fraction was delivered. Three patient groups, receiving characteristic treatment regime, were studied: marginal resected basal cell carcinoma, painful osteoarthritis of the knee and painful tendinitis of the elbow/heel. Control group consisted of ten healthy individuals and a positive control group of three radiation accident victims was also included.

Plasma samples were pre-treated to enrich LMW proteins. Proteins were analyzed using two dimensional difference gel electrophoresis (2DE-DIGE). No differences were observed in protein expression due to local radiation exposure (before vs. after) in any of the patient groups. The expression of a few proteins was shown to diverge between groups only with respect to previous steroid treatment (treated vs. non-treated: t-test $p < 0.01$, fold ratio $> \pm 1.5$). However, plasma from the radiation accident victims showed alterations in the expression of 19 proteins (t-test with FDR correction, $p < 0.05$, fold ratio $> \pm 1.5$) in comparison to the plasma from control group. Proteins included e.g. haptoglobin and serotransferrin. However, these molecules are not among the potential proteins to function as CFs.

In summary, proteomics analysis showed that local radiation treatment did not have detectable effects on protein expression levels in any of the patient groups, whereas differences were observed in the radiation accident victims in comparison to controls.

Healthy tissue sensitivity after hadrontherapy

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Hadrontherapy is a type of cancer radiation therapy, which uses beams of charged particles such as carbon ions. Compared to conventional X-ray therapy, the main advantage of hadrontherapy is the ability to more precisely localize the radiation dosage combined with an increased biological effectiveness. This high ballistic accuracy allows depositing the maximal dose to the tumor, while damage to the surrounding healthy tissue can be limited. First results obtained from prostate cancer patients treated with carbon ion therapy, show good local tumor control and survival rates. However, despite the superior precise dose localization of hadrontherapy, the surrounding healthy tissues (e.g. large intestinal tract, bladder, urethra) are inevitably co-irradiated. So far, the impact of hadrontherapy on radiation toxicity of healthy tissues (e.g. gastro-intestinal tract) which surround the target tumor is still not clear and many aspects still need to be investigated.

The main objective of this project is to study the biological response of normal colon cells (NCM460) after *in vitro* exposure to carbon ions or X-ray beams at various low to high doses. Potential quantitative and qualitative differences between carbon ion irradiation and conventional X-rays are currently being studied. In particular, the topics of interest in this study are the investigation of *acute cellular responses* such as cell cycle progression and apoptosis at various time points following the irradiation. In a later stage, these results will be correlated with data obtained by high-throughput screening of gene expression and secreted proteins (cytokines) linked to inflammation.

Obtained results will bring more insight into cellular response of non-cancerous cells to different types of radiation and the underlying molecular pathways that are involved. This research is essential to obtain a better understanding of the treatment effect on the healthy surrounding tissue.

Acknowledgements: This work is partly supported by the Federal Public Service in the context of the feasibility study 'Application of hadrontherapy in Belgium', which is part of action 30 of the Belgian cancer plan.

Micronucleus-centromere test in lymphocytes from currently active uranium miners and radon spa personnel in the Czech Republic

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The micronucleus-centromere test is one of the most sensitive methods of biodosimetry available. Here we tested its applicability in currently active uranium miners and radon spa personnel. The frequency of micronucleus-containing cells and the percentage of centromere-free micronuclei (micronuclei containing only acentric fragments) was determined in peripheral blood lymphocytes of 72 individuals working in the Rožna mine, 42 individuals working at the Jáchymov spa and 42 control individuals. There was a significant increase in the frequency of micronucleus-containing cells as well as the percentage of centromere-free micronuclei in the lymphocytes of both uranium miners and radon spa personnel when compared with the control group. For the miners, both parameters correlated with the effective dose received during work, which was in the order of several tens of mSv. No individual dosimetry data were available for the spa personnel; a comparison with the results from uranium miners suggested that accumulated effective doses were very similar in both cases. We conclude that the micronucleus-centromere test is able to pick up relatively small effective doses which are due preferentially to the inhalation of radionuclides.

Mouse lens epithelial cells and lymphocytes exhibit similar sensitivity to γ-irradiation

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In this pilot study we compared for the first time the radiation sensitivity of mouse lens epithelial cells (LECs) and mouse lymphocytes from two genetically different mouse strains, C57BL/6J and JF1, respectively. We have chosen C57BL/6J mice as a widely used reference strain and JF1 because of its different susceptibility for radiation sensitivity (Dalke et al., 2012).

LECs and mouse lymphocytes were freshly prepared. Cells were irradiated with 0.25–2 Gy from a ^{137}Cs-source at 2 Gy/min. DNA damage and repair were evaluated by alkaline comet-assay and γH2AX foci assay.

LECs and lymphocytes showed a dose-dependent increase of DNA-damage as indicated by all parameters derived from the comet assay. In particular, analyzing parameter "percentage of DNA in the comet tail", both cell types exhibit similar radiation sensitivity.

Additionally, the number of DNA double strand breaks in LECs and lymphocytes from C57BL/6J and JF1 mice were compared by γH2AX analysis: After 1 Gy irradiation repair of DNA damage proceeded faster in LECs. A significantly higher residual damage was observed in lymphocytes after 24 h. No significant difference was detected between the two mouse strains with respect to DNA repair.

Our results demonstrate that on the DNA level, lens epithelial cells and lymphocytes exhibit a similar sensitivity to ionizing radiation. Moreover, the observed difference in DNA repair between the two cell types in both strains as indicated by the data of the γH2AX assay need further experiments to identify the underlying molecular mechanisms.

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Cytogenetic endpoints in endothelial cells (HUVEC) exposed to protracted LDR irradiation

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Endothelial cells cultured under protracted irradiation were assessed with cytogenetic endpoints including chromosomal aberration (CA) and telomere length analyses. The endothelial HUVEC cell line was irradiated at Stockholm University at low dose rates (1.4, 2.4, and 4.1 mG/h). In order to follow the cytogenetic damage induced during the protracted exposure, cell harvests at time points 1, 3, 6, and 10 weeks, were performed. Another experiment was performed where cells grown for 1, 3, 6, and 10 weeks under irradiation were placed for another 10 days in non-exposure conditions before harvest.

The HUVEC cell line turned out to be very challenging for cytogenetic assays due to the variable, mainly tetraploid, chromosome number. In all, 100 cells were analysed for CA per data point. CA of both chromosome and chromatid types were observed. In general, cells harvested directly after irradiation, demonstrated an increase in aberration frequencies with longer exposure times/higher protracted dose. No such trend was observed in cells cultured without irradiation during an extra 10 days. In situ hybridization (FISH) with telomere probe was performed to assess telomere length at the corresponding culture times and dose rates. Data from both telomere length assessment and CA scoring will be presented. The work performed is part of Task 7.3 “Feasibility study towards a systems biology approach of radiation response of the endothelium” in the DoReMi project.

Systematic review and meta-analysis of circulatory disease from exposure to low-level ionizing radiation and estimated potential population mortality risks

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Background: Although high doses of ionizing radiation have long been linked to circulatory disease, evidence for an association at lower exposures remains controversial. There is emerging evidence of risks at low doses and dose rates.

Methods: We performed a systematic review and meta-analysis to summarize information on circulatory disease risks associated with moderate- and low-level whole-body ionizing radiation exposures (Little et al., 2012). We conducted Medline/ISI Thompson searches of peer-reviewed papers published since 1990 using the terms "radiation" +"heart"+ "disease" or "radiation" +"stroke" or "radiation" +"circulatory" +"disease". Radiation exposures had to be whole-body, with cumulative mean dose < 0.5 Sv, or at low dose rate. We estimated low dose population risks of circulatory disease using excess relative risk estimates from this meta-analysis and current mortality rates for nine major developed countries.

Results: Estimated excess population risks for all circulatory diseases combined ranged from 2.5% per Sv (95% CI 0.8 to 4.2) for France to 8.5% per Sv (95% CI 4.0 to 13.2) for Russia (Little et al., 2012).

Conclusions: Our review supports an association between circulatory disease mortality and low and moderate doses of ionizing radiation. Our analysis was limited by heterogeneity among studies (particularly for non-cardiac endpoints), the possibility of uncontrolled confounding in some occupational groups by lifestyle factors, and higher dose groups (>0.5 Sv) generally driving the observed trends. Our findings suggest that overall radiation-related mortality is about twice that currently estimated for cancer (4.2%–5.6% per Sv for these populations) (Little et al., 2012).

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Low dose effects on brain

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Background: Low-dose irradiation might increase the incidence of ischemic cardiovascular diseases, however, much less attention is paid to low-dose radiation effects on the microvasculature of the brain and its microenvironment. We have studied low-dose irradiation induced mitochondrial damage in the whole brain and in the endothelial cells of the brain microvasculature.

Methods: Since developing brain might be more susceptible to low-dose induced long-term alterations, we investigated 10-day old C57BL/6 mice. Animals were irradiated on their head with single doses of 0.1 or 2 Gy X-rays. 1, 3, 7 days and 1 month after irradiation mice were sacrificed, and single cell suspensions of the various brain regions (cortex, cerebellum, hipotalamus) made. Half of the brains were processed for biochemical measurements of enzyme activities of the respiratory chain in the mitochondrial membrane. The other halves were stained with Rhodamine 123 to evaluate alterations in the mitochondrial membrane potential, with Mitosox Red to determine the level of superoxide anions in the mitochondrial membrane. By costaining the cells with the endothelial specific marker CD31, we studied mitochondrial damage within the endothelial cells as well.

Results: A reduction of cytochrome C oxidase activity was detected after irradiation with 2 Gy, while 0.1 Gy had only moderate effect compared to control. The other enzymes in the respiratory chain are under investigation. A moderate, dose-dependent increase was seen in the level of mitochondrial superoxide as well as in the level of mitochondrial membrane potential damage 3 days after irradiation, which normalized after 1 week. Mitochondrial damage in the CD31 cells seems to be consistently higher than that found in the unfractionated brain cell suspension.

Conclusions: Low-dose local irradiation induces moderate but detectable mitochondrial alterations both in the brain and in the endothelial cells of the microvascular compartment of the brain.

Towards a better knowledge of cardiovascular doses following radiotherapy using hybrid computational phantoms

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A general awareness of the cardiovascular diseases following radiotherapy has emerged for few years. The implied biological mechanisms are only partially understood and a better knowledge of the cardiovascular doses is certainly needed to better establish a dose-effect relationship. To date, retrospective studies perform dose reconstruction with a representative patient or a mathematical phantom, which introduce dose error due to the non-personalization of the considered anatomy. It is all the more problematic that the heart can be located in a high dose gradient during breast or Hodgkin lymphoma radiotherapies. Here, hybrid computational phantoms are used in order to reduce dose uncertainties due to anatomical approximations (Moignier et al., 2012).

In the case of left breast radiotherapy, thoracic models adapted to the patient anatomy and including a generic detailed heart model with coronaries have been generated. When the CT scan of the patient is available, the insertion of a detailed heart model compensate for the absence of coronaries on the imaging used for treatment planning. For retrospective studies, when only orthogonal chest radiographies are available, the thoracic model is deformed to fit the orthogonal views, ensuring a better beam positioning than with a representative patient and hence a better dose assessment.

Since most of the cardiovascular diseases are due to coronary lesions it is all the more important to specify coronary doses. For this purpose, rather than using a generic heart model it is possible to use patient's coroscans showing the detailed coronary pattern. Dose reconstructions have been performed for 20 patients treated for Hodgkin lymphoma between 1996 and 2010. Inclusion of the heart anatomy in the patient 3D model enables to establish a dose mapping at the coronary level.

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Two-dimensional difference gel electrophoresis analysis of short-term response of human endothelial cell line EA.hy926 to gamma radiation

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Epidemiological studies indicate that moderate and low radiation doses to the heart area may result in an increase in cardiovascular mortality. This has been suggested to occur for instance among breast cancer patients, who are exposed therapeutically. However, it is not known how the cardiovascular damage is created by the low doses of ionizing radiation. It has been suggested that heart endothelium could be one of the targets for the ionizing radiation-induced heart injury. The aim of this study was to examine the short-term proteome level response in endothelial cells to low and moderate doses of γ-radiation.

Human endothelial cell line EA.hy926 was used as *in vitro* model in this study. Cells were exposed to γ-radiation at doses of 200 mGy and 1 Gy using Co-60 source. Cells were harvested in three time points (10 min, 30 min, and 4 hours) after the exposure. Protein expression changes were examined in total cell lysates using two-dimensional difference gel electrophoresis (2DE-DIGE). Differences in protein expression were examined using statistical testing with correction for multiple comparisons as well as based on fold ratios between exposed and control samples. No significant changes were found in protein expression in any of the examined experimental conditions. However, western blotting showed that 53BP1 protein was phosphorylated in dose- and time-dependent manner in response to γ-radiation.

Our results suggest that the exposure of EA.hy926 cells to γ-radiation doses of 200 mGy and 1 Gy does not cause acute changes in the proteome. Detected changes in the phosphorylation of 53BP1 protein indicate that the exposure caused DNA damage in cells and thus, cells responded to irradiation. Further examination of cellular proteome, using more sensitive techniques (MS/MS), is warranted and might reveal changes, which are not detected by 2DE-DIGE.

Manuscript of this study has been submitted for publication.

Identification of novel p53 target genes in the developing mouse brain

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Epidemiological data from atomic bomb survivors have shown that *in utero* exposure to low radiation doses especially between weeks 8–5 of pregnancy led to an increased incidence of severe mental retardation among these subjects later in life. We found that this can be mimicked in mice by irradiating pregnant mice at day 12 of gestation (E12) with 1 Gy of X-rays. To identify possible molecular mechanisms which are responsible for this effect, we analysed gene expression in the fetal brains after 2 h following irradiation. Results showed a dose-dependent increase in genes involved in cell cycle arrest, DNA repair and apoptosis, which are known to be mediated by the tumour suppressor p53. However, a number of upregulated genes were so far not known to be regulated by p53.

Therefore, we analysed several of these radiation-induced genes for putative p53 binding sites using a matrix scanning algorithm. For all of the tested genes, we identified at least one predicted p53 binding site ($p < 10^{-4}$) within the investigated genomic sequences. Chromatin immunoprecipitation with an antibody against phosphorylated p53 showed that these genes are indeed *in vivo* targets of p53 in response to X-irrradiation of the E12 mouse brain. To further confirm the involvement of p53 in the transcriptional activation of these genes upon irradiation, we analyzed mRNA expression in irradiated primary cultures of cortical neurons from wild-type ($p53^{+/+}$) *versus* p53-deficient ($p53^{-/-}$) mice. Expression of most of these genes was significantly induced in cells from $p53^{+/+}$ mice, whereas no difference in expression could be observed in cells from $p53^{-/-}$ mice. Thus, we have identified new p53 target genes in the fetal mouse brain that are transcriptionally activated after exposure to ionizing radiation and we therefore extend the p53 transcriptional network.

Acknowledgements: This work is supported by the EU FP7 projects CEREBRAD and DoReMi.

The endothelium response to low dose ionizing radiation

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High radiation doses (> 5 Gy) increase the risk of cardiovascular diseases (CVD). In recent years, epidemiological data support the fact that lower radiation doses increase the risk of CVD as well and this after much longer intervals than previously expected (cfr. Atomic bomb survivors). However for radiation doses below 0.5 Gy, these epidemiological findings are unclear and a better understanding of the underlying biological and molecular mechanisms is needed. The endothelium is believed to be a critical target in the development of radiation-related CVD because of its pivotal role in normal vascular functioning.

In this work, we used the immortalized endothelial cell line EA.hy926 and primary Human Umbilical Vein Endothelial cell (HUVEC) as models, to characterize the endothelial response to low (< 0.5 Gy) and medium (0.5 – 5 Gy) acute doses of X-rays (250 keV, 15 mA, 1 mm Cu, 0.25 ± 0.01 Gy/h). In particular, we analysed the influence of radiation on DNA damage, cell cycle changes, and associated apoptosis. DNA damage was induced by doses as low as 0.05 Gy as observed by a significant increase in γH2AX-foci number 30 min after exposure in both cell lines. Foci number returned to baseline levels 24 h after exposure, indicating DNA repair. Changes in cell cycle 24 h after exposure, as seen by a G2 arrest, were limited to 5 Gy in both cell lines. A significant increase in apoptotic cells was observed to doses as low as 0.1 Gy for EA.hy926 cells and as low as 0.5 Gy for HUVECs 48 h after exposure.

Based on our results, it is concluded that DNA damage and apoptosis following radiation exposure is more pronounced in EA.hy926 cells compared to HUVECs. This indicates a larger radiation sensitivity of EA.hy926 cells for these investigated endpoints. In future research we will include gene expression profiling to get a better understanding of the underlying signalling pathways. In the context of radiation-related CVD assessment of ROS production, mitochondrial DNA damage and inflammatory response (adhesion of leukocytes and cell-cell integrity) will be performed as well.

Acknowledgements: This project is supported by the EU FP7 DoReMi network of excellence (*grant agreement: 249689*).

Maturating neurons exhibits a delay in neurite outgrowth upon exposure to low and moderate doses of ionising radiation

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In human, the period of fetal development (8 to 15 weeks of gestation) is highly sensitive to ionizing radiation. Epidemiological data have reported cases of mental retardation and decrease in school performance within victims of Hiroshima and Nagazaki A-bombing, irradiated *in utero* during the fetal period. Several studies have been performed to assess the risk of ionizing radiation on fetal and children health but a lot is still to be done in order to understand the radiation-induced brain damage, especially following exposure to low doses of radiation.

In this study, we aimed at observing morphological changes in neurons following exposure to low and moderate doses of X-rays during their early maturation period, followed by gene expression analysis. For this purpose we have used nearly pure primary cultures of neurons extracted from E17 mouse fetus in order to reveal specific response of neurons to ionizing radiation. This study revealed a reduction in neurite length, neurite number and branching points number following exposure to doses of 0.1, 0.2 and 0.5 Gy of X-rays. A 3 days follow-up of these cultures after irradiation showed a resuming of neurite outgrowth, which remained however insuficient to overcome the inhibition induced by radiation. Microarray analysis using GSEA indicated a significant downregulation of pathways involved in cytoskeleton remodeling and synaptogenesis. This study showed that a dose as low as 0.1 Gy was enough to induce neurite outgrowth inhibition which may ultimatly lead to a defect in neuronal network connection and to improper neuronal communication.

Acknowledgements: This work was supported by both EU projects; the DoReMi Network of Excellence (*GA 249689*) *and the CEREBRAD* (*GA 295552*).

Dose–responses from multi-model inference for the non-cancer disease mortality of atomic bomb survivors

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The non-cancer mortality data for cerebrovascular disease (CVD) and cardiovascular diseases without CVD from Report 13 on the atomic bomb survivors published by the Radiation Effects Research Foundation were analysed to investigate the dose–response for the influence of radiation on these detrimental health effects. Various parametric and categorical models (such as linear-no-threshold (LNT) and a number of threshold and step models) were analysed with a statistical selection protocol that rated the model description of the data. Instead of applying the usual approach of identifying one preferred model for each data set, a set of plausible models was applied, and a sub-set of non-nested models was identified that all fitted the data about equally well. Subsequently, this sub-set of non-nested models was used to perform multi-model inference (MMI), an innovative method of mathematically combining 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 CVD, MMI yielded a weak dose–response (with a risk estimate of about one-third of the LNT model) below a step at 0.6 Gy. Above the step at 0.6 Gy the dose–response continues in a linear fashion with statistically significant risk values. For mortalities related to cardiovascular diseases without CVD, an LNT-type dose–response was found up to a threshold dose at 2.2 Gy. At this dose a small step occurs in the dose–response for MMI. Above that step the dose–response continues in an essentially linear fashion with statistically significant risk values. For both endpoints the 90% confidence intervals related to the MMI include the value of zero below the threshold doses (Schöllnberger et al., 2012). The authors will also present results from their analyses of the latest non-cancer mortality data related to the atomic bomb survivors (Shimizu et al., 2010).

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Quantitative proteomic analysis of endothelial cells isolated from locally irradiated mouse hearts

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Radiation exposure is associated with a significantly increased risk of cardiac morbidity and mortality after a latency period of several decades. One of the main targets of radiation is the endothelial cell (EC) layer in the cardiac micro- and macrovasculature. 8-week old C57Bl/6 mice were locally irradiated on the heart using X-ray doses of 8 and 16 Gy and sacrificed after 16 weeks. After mechanic and enzymatic digestion of the heart tissue viable primary ECs were isolated using streptavidin-CD31 coated microbeads and tested for purity by FACS analysis. In order to quantify radiation-induced proteome alterations, the ECs were labelled with duplex Isotope Coded Protein Label (ICPL) methodology using three biological replicates isolated from control vs. exposed mice. To investigate the cross talk between the endothelium and cardiac tissue after radiation damage, we compared the whole heart lysates after similar exposures again using the ICPL labelling. All samples were analysed by ESI LC-MS/MS and Proteome Discoverer software. The response of the endothelium seemed to be distinct from that of the cardiac tissue. Ionising radiation induced significant alterations in the actin cytoskeleton and leukocyte extravasation signalling of the endothelium whereas in the cardiac tissue lipid metabolism and oxidative phosphorylation were the most affected pathways. Marked changes in the peroxisome proliferator-activated receptor (PPAR) regulation of fatty acid metabolism were found in both proteomes. The proteomics data were further analysed by bioinformatics tools and validated by immunoblotting.

This study will facilitate the identification of biomarkers associated with adverse cardiac effects of ionising radiation and the development of individualised radiotherapy.

Low/intermediate dose ionising radiation induces an anti-inflammatory phenotype of activated peritoneal macrophages of BALB/c mice

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Low and intermediate dose ionising radiation (LD-X-ray) is applied in treatment of painful and degenerative inflammatory diseases. The resulting anti-inflammatory effects are based on the one hand on alterations of chemokine profiles and adhesion molecules on immune cells. On the other hand, despite lowering the amount of inflammatory immune cells, a LD-X-ray induced change of the phenotype of activated immune cells is also assumed. Key players in regulation of inflammation are macrophages that are cells of the innate immunity. They secrete inflammatory cytokines such as Interleukin (IL)-1β or tumour necrosis factor (TNF)-α upon stimulation. In terms of medical application of LD-X-ray, but also of radiation protection issues, it is reasonable to expand our knowledge on how key immune cells involved in inflammation get modulated by LD-X-ray. Of special interest is how varying radiosensitivities due to the different genetic backgrounds influence this modulation.

We have previously shown that 0.5 or 0.7 Gy of LD-X-ray significantly reduces the amount of secreted IL-1β of human THP-1 macrophages that have been activated with lipopolysaccharide (LPS) and co-activated with monosodium urate crystals (MSU). Using now an *ex-vivo* model of peritoneal mouse macrophages obtained from mouse strains differing in their basal radiosensitivity, we also observed a significantly reduced secretion of IL-1β and a slight, but not significant, reduced secretion of TNF-α of activated (LPS plus MSU) macrophages of BALB/c mice after LD-X-ray treatment with 0.5 or 0.7 Gy. In contrast, the secretion of IL-1β and TNF-α of activated peritoneal macrophages from the less radiosensitive C57BL/6 mice was not influenced by LD-X-rays.

We then tested whether the secretion of cytokines by peritoneal macrophages of BALB/c mice that had only been activated with one stimulus (LPS before LD-X-ray) is also modulated by exposure to ionising irradiation. One day after irradiation, the IL-1β secretion was decreased after irradiation with 0.5, 0.7, 1.0 and 2.0 Gy. Of note is that also 0.01 Gy of X-ray led to decreased secretion of IL-1β while 0.05, 0.1, and 0.3 did not change the amount. Further, the amount

of secreted TNFα was slightly reduced by single doses of X-ray of 0.5, 0.7, 1.0 and even 2.0 Gy.

We conclude that only the inflammatory phenotype of more radiosensitive macrophages is reduced by LD-X-ray and that *ex vivo* and *in vivo* models with primary cells should be increasingly applied in the future to examine how the immune system is modulated by LD-X-ray. Future research will also reveal which inflammatory pathways such as the NFκB p65 and p-p38 MAPK get modulated by LD-X-rays.

Acknowledgements: This work is supported by the European Commissions (*DoReMi, European Network of Excellence, contract number 249689*).

How can the Eurados Network on Retrospective Dosimetry contribute to research in low doses?

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A Eurados (European Radiation Dosimetry Group) network of biological and physical dosimetrists was created (as Working Group 10) in 2008 with the aim to harmonize existing methods of retrospective dosimetry, to implement and validate new methods and to disseminate the scientific knowledge.

As a first action, WG10 has reviewed properties and drawbacks of retrospective dosimetry methods in low dose epidemiological studies and in emergency situations (Ainsbury et al., 2011) concluding with the identification of two needs: 1) to establish the usefulness and limitations of biological dosimetry in internal and mixed internal/external exposures and 2) to harmonize uncertainty analysis among the various methods. Two task groups have been created to explore these topics.

Moreover, within WG10, an inter-comparison to validate the EPR/OSL retrospective dosimetry method with portable devices is in progress and the Eurados School on Retrospective Dosimetry will be organized in Neuherberg, Germany, in October 2012, to train young scientists in different physical and biological assays for retrospective dose assessment.

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Cellular dosimetry of Sr-90 using Monte Carlo code MCNPX

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The aim of our study is to provide data that increases knowledge about possible health effects of exposure to Sr-90 contaminant in drinking water. The cellular localization in the mouse bone model plays an important complementary role with computational dosimetry in these investigations. This early research work is focused on using MCNPX and its validation at cellular level using three-dimensional voxelised geometry. S-values (absorbed dose per unit cumulated activity) calculations using Monte Carlo (MC) simulations are carried out. Cytoplasm (Cy), nucleus (N) and Sr-90 radiation source were simulated with MC code MCNPX. In the first step of this study, cells are assumed to be spherical with the radii of the cell and cell nucleus ranging from 2 to 10 µm. Different source to target combinations including nucleus to nucleus (N←N), cytoplasm to nucleus (N←Cy) are considered. The S-values (in Gy/Bq**.**s) were calculated for cell nucleus and cytoplasmic distribution of radioactivity.

A comparison of MC results with the MIRD values presented by Goddu et al., for the N←Cy configuration shows that deviations are less than 6% (5.5%). We generally find the largest deviations with MIRD for geometries where the target is at some distance from the source and, therefore, the results depend more strongly upon the penetration ability of electrons.

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Transcriptional dose-responses of radiation biomarkers in human and mouse blood samples *ex vivo*

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Modifications of gene expression following ionising radiation (IR) exposure of cells *in vitro* and *in vivo* have been well documented in the last few years. Recent studies have identified radiation responsive genes in human blood which can be potentially used for biological dosimetry. However, little is known about the dose-responses, especially at low doses. We have developed a multiplex quantitative reverse transcription polymerase chain reaction (MQRT-PCR) assay to accurately study modifications of gene expression following IR in human blood samples exposed *ex vivo*. Here we looked at dose responses in *ex vivo* irradiated human blood over a range of doses from 5 mGy up to 100 mGy and at two different time-points of 2 hr and 24 hr using a panel of 13 biomarkers of exposure (*BBC3*, *CDKN1A*, *CCNG1*, *DDB2*, *FAS*, *FDXR*, *GADD45*, *MDM2*, *MYC*, *PCNA*, *PHPT1*, *SESN1*, *TIGAR*). Linear responses were observed at low doses (5 mGy – 100 mGy) with an R^2 value of 0.939 for *FDXR* at 2 hrs. A linear response was also observed at low doses at 24 hrs with R^2 values reaching 0.984 for *DDB2* and 0.973 for *FDXR*. A dose estimation curve was developed from the low dose response of two donors and a further 8 donors were irradiated with a dose of 100 mGy at 24 hrs to assess individual response to low dose exposure. Using linear regression analysis, lower and upper 95% confidence intervals of 47 mGy and 128 mGy were obtained for *CCNG1*. Mouse blood (C57BL/6) irradiated at low doses *ex vivo* with a time-point of 2 hrs also produced a linear response. R^2 values of 0.94 and 0.91 were obtained for the genes *Bbc3* and *Cdkn1a*. These findings illustrate the linear response of specific genes in the low dose region and identify genes that fulfil some of the requirements of a good exposure biomarker, such as sensitivity and simple proportionality with dose.

Toxicokinetic of uranium nanoparticles after inhalation in rats

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The remediation and decommissioning of nuclear facilities lead to the use of processes that can generate nanoparticules (< 100 nm) containing uranium. The risk of accidental inhalation of nanoparticles by operators implementing such processes must be taken into account. For relevant dose calculation, it is strongly recommended in ICRP publication 66 to characterize in vivo or in vitro specific absorption rate for the aerosol of interest. Concerning uranium, absorption rate of inhaled ultrafine particles have never been assessed experimentally. To address this question, the present study considered, in vivo in rat, respiratory tract deposition, incorporation and biodistribution of uranium nanoparticles after inhalation. Rats were exposed, in a "nose only system", during 1 hour to an aerosol containing 10^7 particles of uranium/cm^3. The median diameter in number of these particles was 40 nm (δg = 2 nm). The quantities of uranium deposited in respiratory tract compartments and in target organs of uranium toxicity (skeleton and kidneys) were determined by ICP-MS 10 minutes, 4 hours and 24 hours after the end of inhalation. The results showed that 27% of the mass of inhaled uranium nanoparticles were deposited in the airways. 97% of the deposited mass of uranium were located in the deep lung. At the end of inhalation, 5.7% of the uranium deposited in the airways were transferred into the blood. 24 hours after inhalation, 3.1%, 4.6% and 77% of the incorporated uranium were located in skeleton, kidneys and airways, respectively. The rapid phase of lung clearance of UO_2 nanoparticles was similar to industrial UO_4 microparticles (Type F) and the slow one was slower than industrial UO_2 microparticles (Type S). As a conclusion, results of the present study demonstrated the specificity of uranium nanoparticles biokinetic and confirmed the necessity of experimental studies to better characterise rapid and slow phases of lung clearance of ultrafine UO_2 particles.

The novel approach for assessing organ doses from paediatric CT scans in EPI-CT

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The worldwide increasing use of paediatric computed tomography (CT) has led to increasing concerns regarding the subsequent effects from exposure to ionizing radiation. Several studies of CT use in children have been initiated, including recent study by Pearce et al. Availability of accurate and valid estimates of organ-specific doses is essential for quantifying the cancer induction rate per unit dose delivered to the organ. The absorbed organ dose from a CT scan depends on factors such as age, sex, examination type, and calendar period of scan. This information is available from Radiology Information System (RIS) and is used in the ongoing studies. However, individual dosimetry in CT scanning is more complex and a broad variety of technical parameters used to adapt the scanner settings to each specific scan has to be taken into account.

In the Epidemiological study to quantify risks for paediatric computerized tomography and to optimise doses (EPI-CT), the dosimetric data collection is split into 2 periods – before and after introduction of Picture Archiving Computerized System (PACS). For the distant past, only sparse information about scanner settings and technical parameters can be obtained. A patchwork approach will be used to retrieve information from a specially developed questionnaire, surveys, scientific publications, expert interviews and interpolations. For the recent years, scanner settings (such as mAs kVp, etc.) will be extracted from the Digital Imaging and Communications in Medicine (DICOM) headers. In the EPI-CT study, individual organ doses will be estimated for each child. Radiation fields will be simulated together with X-ray interactions with the body using a series of phantoms of various ages and Monte-Carlo software. Uncertainty analyses will be conducted and maximum likelihood functions will be derived from available data to provide distributions of doses.

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ProCardio: Cardiovascular risk from exposure to low dose and low dose rate radiation

Atkinson, Michael John; presenting on behalf of the ProCardio consortium

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ProCardio is a medium-scale collaborative project funded by EURATOM Fission. There are 12 participating institutions, representing 8 European countries and Russia.

Our project goals are:

- To understand the biological processes involved in the evolution of damage to the heart and blood vessels that follows exposure to low doses and low dose rates of ionizing radiation.
- To identify novel biomarkers of exposure tof the cardiovasculature.
- To apply these biomarkers in a large-scale molecular epidemiological study following cardiovascular status in a four-nation cohort of childhood cancer survivors.
- To construct biological-mathematical models of the cardiovascular effects of radiation, and to apply these models to the molecular epidemiologicaldata.

There are six scientific workpackages:

- Epidemiological and dosimetric studies on survivors of childhood cancer.
- Analysis of the effects of radiation quality on the cardiovascular system.
- Effects of low dose rates on development of cardiovascular disease.
- Tissue-level interactions in radiation-induced cardiovascular disease
- Identification of biomarkers.

Details of the consortium, the experimental strategy, and our latest results are available from the project website *www.procardio.eu*.

The annual scientific meeting of ProCardio will be held jointly with that of the CEREBRAD consortium, Tarragona, Spain, October 22–26, 2012. Please contact the management team (*www.gabo-mi.com*) or coordinator for details (*atkinson@helmholtz-muenchen.de*).

Acknowledgements: ProCardio is funded by the European Seventh Framework Programme under grant agreement No. 29523.

Department of Radiation Sciences at the Helmholtz Zentrum München

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The mission of the Department of Radiation Sciences is to undertake fundamental and applied research to "optimise the benefits from the applications of ionizing radiation, whilst protecting against possible damage".

Our research portfolio is based upon three columns:

- Environmental Radioecology and Dosimetry
- Radiation and Risk
- Medical applications of ionizing radiation.

The DRS is a member of MELODI, as well as a participant in the DoReMi network of excellence. We are partners in a number of EURATOM collaborative projects investigating risks at low doses, including EpiRadBio, MSCT, ProCardio and STORE.

Information on the activities of the DRS, a descriptive brochure and details of the individual scientists involved is available from the DRS public web site (*www.helmholtz-muenchen.de / en / drs / home / index.html*).

Epidemiological study on cancer and non-cancer incidence among Bulgarian medical radiogenic workers

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To contribute the cancer and non-cancer incidence estimate in relation to work history (years of first employment, duration of occupational exposure, radiation doses received for whole period) in a cohort of medical radiation workers.

A retrospective cohort study was carried out. The cancer incidence among 315 medical workers was compared with that of 320 other medical specialists who worked in hospitals on the territory in Sofia for period 1960–2000. Data concerning incident cancer and non-cancer occurrence was obtained from questionnaire. Data for individual doses were extracted from National System of Individual Dosimetric Control. Descriptive statistics, X^2, Fisher's exact test, ANOVA analyses were used.

Cancer incidence was more frequently diagnosed among radiation workers compared to other medical specialists. When analyzing by cancer type the results were the same. No statistically significant difference in the non – cancer incidences between cases and controls has been observed. No statistically significant relation was observed between cumulative radiation dose and cancer incidence, or non-cancer incidence. No statistical significance was found between the year of first employment and cancer development for cases and for controls as well as and non-cancer incidence. It was not established statistical correlation between work duration in an ionizing radiation environment and appearance of cancer or non-cancer.

The present study shows that cumulative dose obtained and the duration of work in an ionizing radiation environment does not substantially influence the arising of cancer and non-cancer. Although we find higher degree of cancer occurrence among cases compared to controls, those two factors do not give us ground to confirm that work in an ionizing radiation sphere increases the cancer incidence.

The EURADOS European Radiation Dosimetry Group Network

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EURADOS, the European Radiation Dosimetry Group (*www.eurados.org*), represents a network of more than 50 European institutions and 200 scientists. EURADOS promotes, as a non-profit organization, research and development and European cooperation in the field of the dosimetry of ionizing radiation, and maintains a network which includes experts from reference and research laboratories, regulatory bodies and dosimetry services. Main aims of EURADOS are coordination of research and dissemination of scientific knowledge.

EURADOS organizes Winterschools, Symposia and Workshops, Intercomparisons and Training Courses. Areas of activity include individual monitoring for external and internal exposure, retrospective dosimetry, environmental radiation monitoring, diagnostic and interventional radiology, nuclear medicine and radiation therapy, and computational dosimetry. The paper gives a brief overview on the EURADOS network. Special emphasis is placed on areas relevant for the MELODI low-dose radiation research including, for example, doses from incorporated radionuclides in Working Group WG7 with emphasis on studies of microdosimetry of internal emitters; computational methods applied to dosimetry in WG6; markers of exposure at low levels of dose in WG10; and quantification of out-of-field peripheral doses for second cancer risk estimates following radiotherapy (WG9).

The DoReMi Network of Excellence is supporting the creation of the MELODI platform

Salomaa, Sisko

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The aim of the DoReMi consortium is to promote the sustainable integration of low dose risk research in Europe. This will facilitate efforts to resolve the key policy questions identified by the 'High Level Expert Group (HLEG) on Low Dose Risk Research' (*www.hleg.de*). These are the shape/s of cancer dose-risk relationship/s, variation in risk between individuals, differences in tissue sensitivities for cancer, effects of radiation quality, risks from internal exposures and the risks of non-cancer effects. DoReMi provides an operational tool to continue the development of the MELODI platform (Multidisciplinary European Low Dose Risk Research Initiative) that represents the major national bodies and research programmes with a long term commitment to low dose risk research in Europe. Strategic planning of DoReMi activities is carried out in close collaboration with MELODI.

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. DoReMi continues 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. Engaging the broader scientific community via exploratory workshops has proven to be an efficient way in developing the research agendas. 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.

DoReMi has implemented research programs addressing the three key research areas: shape of dose-response curve for cancer, individual radiation sensitivity for cancer and non-cancer effects. The research activities are all performed at appropriately low doses. DoReMi defines these low doses as those of 100 mGy or less. Low dose rates are defined as 0.1 Gy/h or less for low LET radiations. For high-LET radiations, dose and dose-rates of interest are lower, e.g. for alpha radiation by an order of magnitude. Low dose studies are complemented by higher dose/dose-rate studies to inform judgments on extrapolation from moderate and high doses/dose-rates to low doses/dose-rates. All RTD activities address the cross-cutting issues of radiation quality, tissue

sensitivity and internal emitters. During the first 18 months, several workshops were convened to develop strategies that focus on the most promising lines of research for the three areas. Experimental programs have been launched in all three areas, including a number of feasibility studies preparing the field for large international collaborative efforts. 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 planned duration of DoReMi is 2010–2015. Despite the wide expertise available within DoReMi since its beginning, it has been quite clear that the emerging research needs also require competence and resources not available within the initial consortium. Therefore it was expected that DoReMi will be enlarged during the course of the project and additional beneficiaries join the consortium. To date, two competitive calls for new partners have been organised: first one in 2010, with ten new beneficiaries joining the consortium and the second one in 2012, again with ten new (potential) beneficiaries (expected to join 1 January 2013, subject to successful negotiations and EC approval). The competitive calls have enhanced the competence of the consortium in several key areas, by integrating research experts in biomarker identification, immunological/inflammatory pathways, the effects of chronic low dose exposures, vascular effects, lens opacities, epigenetics and novel approaches on studies on DNA lesions and their consequences.

List of DoReMi organisations in 2012

No	Beneficiary name	Beneficiary short name	Country	Date enter project	Date exit project
1	Radiation and Nuclear Safety Authority	STUK	Finland	1	72
2	Institut de Radioprotection et de Sûreté Nucléaire	IRSN	France	1	72
3	Helmholz Zentrum München	HMGU	Germany	1	72
4	Commissariat à l'Energie Atomique	CEA	France	1	72
5	Health Protection Agency	HPA	UK	1	72
6	University of Pavia	UNIPV	Italy	1	72
7	Istituto Superiore di Sanitá	ISS	Italy	1	72
8	Belgian Nuclear Research Centre	SCK·CEN	Belgium	1	72
9	Bundesamt für Strahlenschutz	BfS	Germany	1	72
10	University of Stockholm	SU	Sweden	1	72
11	Centre for Research in Environmental Epidemiology	CREAL	Spain	1	72
12	Institut Curie	IC	France	1	72
13	Universitaetsklinikum Erlangen	UKER	Germany	19	72
14	Johann Wolfgang Goethe Universitaet, Frankfurt am Main	GUF	Germany	19	72
15	Universitaet Rostock	UROS	Germany	19	72
16	Norwegian University of Life Sciences	UMB	Norway	19	72

No	Beneficiary name	Beneficiary short name	Country	Date enter project	Date exit project
17	Norwegian Radiation Protection Authority	NRPA	Norway	19	72
18	Nasjonalt Folkehelseinstitutt	NIPH	Norway	19	72
19	Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile	ENEA	Italy	19	72
20	Institute for Environmental Sciences	IES	Japan	19	72
21	Dublin Institute of Technology	DIT	Ireland	19	72
22	Erasmus Universitair Medisch Centrum Rotterdam	Erasmus MC	The Netherlands	19	72

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www.stuk.fi/julkaisut_maaraykset/en_GB/tutkimusjulkaisut/

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ISBN 978-952-478-739-0 (PDF)
ISSN 0781-1705
Painotalo Kyriiri Oy, Helsinki 2012