DoReMi -
Low Dose Research towards
Multidisciplinary Integration

TRA Statement + Annexes
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Contents

1. Purpose of DoReMi TRA statement

Since its establishment on January 1st, 2010 the DoReMi Network of Excellence (www.doremi-noe.net) has been focused on work on low dose health risk research in Europe following the principles and recommendations of the High Level and Expert Group (HLEG) (see HLEG report 2009 on www.hleg.de). With this, DoReMi has been directly contributing to the implementation of the Multidisciplinary European Low Dose Risk Research Initiative, the MELODI Association (www.melodi-online.eu). DoReMi has established its own short term Transitional Research Agenda (TRA) and has also been involved in setting up the Strategic Research Agendas for MELODI.

In order to foster research activities in this domain of research several short DoReMi statements specifying most urgent priorities have been established. In conjunction with the MELODI SRA, this helped to address the most important research needs within the setting of internal and competitive EU calls.

Recently, an even wider-ranging European initiative has been launched (within Horizon 2020) including the research platforms MELODI (together with OPERRA (http://www.melodi-online.eu/operra.html) as an operational tool) ALLIANCE (www.radioecology-exchange.org), EURADOS (http://www.eurados.org/), NERIS (http://www.eu-neris.net/) and Medical platforms in a combined European Joint Programme for the Integration of Radiation Protection Research, CONCERT (kick-off meeting in Munich 17-18 June 2015). In this context, it is important to state the main achievements of DoReMi in low dose health risk research and to emphasize the most urgent research needs and priorities in this domain that have to be considered next.

As before, this (last) DoReMi TRA statement aims (1) to identify most important future research lines, (2) to contribute to the establishment of future EU calls in the new highly integrative European research context and thus, to reinforce and further promote genuine research on radiation health risks and radiation protection.

This TRA statement is based on the previous DoReMi TRAs and is an extension of the previous TRA statements taking into account most recent scientific research achievements within and outside of DoReMi. It is the last DoReMi TRAstatement since the DoReMi project comes to its end in December 31, 2015.

2. Recent progress in DoReMi low dose health risk research

2.1. Development of DoReMi

The DoReMi TRA is based on the two main questions put forward by HLEG:

- How robust is the system of radiation protection and risk assessment?
- How can it be improved?

and also, on **the 9 key questions identified in DoReMi**:

1. What is the dependence on energy deposition?
2. What is the dependence on dose rate?
3. What are the tissue sensitivities?
4. What is the modification of risk by genetic and epigenetic factors and gender?
5. What is the effect of age on risk?
6. What is the effect of lifestyle and/or other exposures on risk?

7. What is the effect of physiological state?
8. Is there a hereditary component in risk?
9. What is the role of non-targeted effects in health risk?

Apart from the WP concerned with Management (WP1) and scientific integration strategies and Dissemination (WP2), DoReMi was structured around these questions focusing on dose responses for cancer (WP5), individual sensitivity (WP6) and non cancer effects (CDV, neurological effects and eye lens opacities) (WP7). In addition, aspects of Education & Training (to ensure human competences and sustainability) and infrastructures (irradiation facilities, epidemiological cohorts, bio-and data-banking facilities and analysis platforms ('omics', imaging, high throughput analysis, etc.) have been covered by WP3 and WP4, respectively.

With the Joint Programme for Integration (JPI) DoReMi helped establishing the MELODI association and its operational status, and attracted through 3 competitive calls 24 new partners to take part in the DoReMi project. With the Joint Programme for Research (JPR), DoReMi defined the three main areas of research: shape of dose response for cancer, individual sensitivity and non cancer effects, and established the TRA and TRA statements that helped initiating the internal and competitive calls and expanding the DoReMi Programme portfolio (from originally 22 to now 49 tasks with a total of 27 new tasks and/or subtasks. With this (JPR) DoReMi also supported the development of the MELODI Strategic Research Agenda (SRA). Furthermore, DoReMi developed a Joint Programme for Spreading Excellence (JPSE). This programme essentially supported capacity building by WP3 and WP4, i. e. development and keeping of competences (by WP3) by supporting 6-9 training courses per year in Europe and the development, availability and sustainability infrastructures (irradiation facilities, cohorts, bio-data banking, analysis platforms) by WP4. Dissemination activities were promoted through dedicated workshops, the DoReMi Website (www.doremi-noe.net) and publications.

Since epidemiological studies alone have been shown to be unable to assess radiation health risks at low doses (<100 mGy) and low< dose-rates (<1 mGy/ day) and therefore need to be backed up with mechanistic information on the biological effects involved at low doses and low dose rates, each DoReMi WP includes tasks concerning **mechanistic studies as well as epidemiological studies.** As can be seen in the list of DoReMi tasks (see Annex 1), in the course of the DoReMi project, a decent number of tasks were added (as outcomes from competitive and internal calls) taking into account most recent developments and needs in radiation research and radiation protection (see Annex I for the updated list of DoReMi tasks with their dates of inclusion).

During DoReMi, three competive and internal calls were run (in 2011, 2012 and 2013).

2.2. Aims and recent outcomes of DoReMi WPs

The scientific work of DoReMi falls into workpackages (WPs) 5-7 while capacity building has been promoted by WPs 3 and 4 of DoReMi. The results obtained so far are best reflected in the DoReMi publications (see Annex II).

Most studies in DoReMi have been feasibility studies. Because of the time allotted to the different tasks, at the present time, more results are already available from mechanistic studies than from epidemiological studies. Studies on cross-cutting issues, e.g. radiation quality, tissue sensitivity and internal emitter effects were included in the different WPs.

Work of the WPs focused on dose response relationships, the involvement of specific cell and tissue responses in cancers and non-cancer effects, non targeted and immunological effects, analysis of relevant metabolic pathways and regulatory systems (genetic and epigenetic controls), development of suitable molecular biomarkers for exposures, metabolic disturbance and defined pathological effects, possible contributions to molecular epidemiological studies, systems biology analyses, mechanistic and epidemiological modeling oriented towards low dose health risk evaluation. Here below, we briefly recall the aims of the DoReMi WPs and the answers obtained to the different key questions together with a short summary of the results.

The corresponding number of the DoReMi publication as included in Annex II is given in brackets.

2.2.1. WP5: Shape of the dose response and tissue sensitivities for cancer

WP5 aimed:

- to improve knowledge of low dose/dose-rate radiation cancer risk in humans and
- to improve low dose/dose-rate risk projection models based on knowledge of processes that drive carcinogenesis.

Response to key question 1: What is the dependence on energy deposition?

- In the two stage expansion model the risk increased with age, time of exposure and exposure rate (17)
- Telomeres are key players (76)
- Non linear responses for gene expression (29) and inflammatory immune reactions (59, 60, 61).
- Anti-inflammatory responses can be stimulated (non linear) (24, 25, 60, 61, 65, 79)
- Adaptive responses occur on DSB induction and cytokine excretion (13)
- Transcription factor PU.1 is turned on (78). Complex hemizygous deletions on mouse chromosome 2 (del2) were identified in a subset of neutron-induced AMLs, however, R235 point mutations in the Sfpi1/PU.1 DNA binding domain on the remaining chromosome 2 were not influenced by radiation quality (11)
- Non-linear up-regulation of p16 and induction of senescence in thyroid cancer cells (1).
- PARTRAC models show differences in RBE for DSB induction and DNA fragmentation at low and high LET (5, 6, 7). Furthermore, the release of IL-6 and DNA repair dynamics are LET dependent (41, 42). PARTRAC models can be applied to simulate light ion track structures and biological effcts for energies down to keV/u (75).
- A statistically significant relationship between low exposure to radon and cancer mortality, primarily due to lung cancer, was found in uranium millers (30).

Results:

- Demonstration of nonlinear responses in terms of gene expression and inflammatory and anti-inflammatory responses.
- Models confirm RBE>1 for high LET involving more DNA fragmentation
- In radiation-induced AML susceptible CBA mice, absence of radiation quality response on specific point mutations on chromosome 2 in the presence of complex deletions involving the Sfpi/PU.1 region of the other chromosome.

Response to key question 2: What is the dependence on dose rate?

- Lung cancer risk in Uranium miners is also dependent on exposure rate (17)
- In the two-stage expansion model the risk increased with age, time of exposure and exposure rate (17).
- Immune activating cytokines can be stimulated by the supernatant of fractionated RT irradiated tumour cells (31). Colorectal tumor cell death can be stimulated by fractionated RT (31).
- Protracted low dose can result in radioresistance, chronic exposure in immunosuppressive effects (40)

- Fractionated RT induces immunogenic cell death and Hsp70 release in p53 mutated glioblastoma cell lines (66). During split dose exposures, the initial exposure induces more γ-H2A foci than subsequent exposures (42)

Response to key question 3: What are the tissue sensitivities?

- No excess of kidney cancer risk in French and German uranium miners (15).
- IR induced sensitization of immunologically important cells types (24, 40).
- Persistent fractionated low dose radiation-induced DSB in spermatogonial stem cells in mice (27)

Results:

- Epi-study: no excess of kidney cancer in uranium miners (low sensitivity of the kidney?)
- IR can induce sensitization of immunologically important cell types
- Persistent DSB are induced by fractionated low dose irradiation of testicular tissue.

Response to key question 4: What is the modification of risk by genetic and epigenetic factors and gender?

- Cockayne patients' fibroblasts show high oxidative stress, perturbed oxidative metabolism and mitochondrial function (52).
- There is a genetic signature for IR induced AML in mice (Chromosome 2 deletion and SfPi1/Pu1 loss)(43, 44) and a signature for miRNAs for thyroid cancer cells (1, 2).
- PU.1 plays an important role in initiation and development of IR induced AML (78)

Results:

- Cockayne syndrome patients show oxidative stress, perturbation of oxidative metabolism and mitochondrial functions
- Chromosome 2 and SfPd/Pu1 loss are involved in IR induced AML in mice. Pu.1 is important for AML initiation.
- There is a genetic signature for thyroid cancer cells

Response to key question 5: What is the effect of age on risk?

- An epigenetic study reveals that cancer risk increases with age of exposure (17).

Response to key question 6: What is the effect of lifestyle and/or other exposures on risk?

- **No result yet**

Response to key question 7: What is the effect of physiological state?

- Metabolic changes occur in HUVEC cells after gamma-IR together with inflammatory responses

Response to key question 8: Is there a hereditary component in risk?

- **No result yet**

Response to key question 9: What is the role of non-targeted effects in health risk?

- A proposed cancer induction model includes NTE: NTE is involved in cancer induction and nonlinear IR responses
- Low dose radiation can transiently modulate the signaling processes underlying intercellular induction of apoptosis: depending on system parameters these perturbation act in an anti- or pro-carcinogenic way (34)
- ROS production and SOD activity are involved in low dose R responses.
- NTE can be observed *in vivo*

2.2.2. WP6 individual sensitivity

Response to key question 1: What is the dependence on energy deposition?

- Reduced Rb1 expression by common variants in regulatory regions can modify the risk for malignant transformation (osteosarcoma) in different mouse strains after alpha-ray exposure (64).

Results:

- Animal studies show efficient induction of RB1 dependent osteosarcoma in mice by alpha-irradiation in different predisposed mouse strains
- Involvement of changes in energy metabolism (mitochondrial functions)

Response to key question 2: What is the dependence on dose rate?

- Fractionated doses increase toxicity and probability to induce secondary malignancies in mice (19)

Results:

- In mice: fractionated IR may lead to higher probability of cancer
- Epidemiological study shows a dose rate effect on lung cancer risk in uranium miners (30)

Response to key question 3: What are the tissue sensitivities?

- Genetic DNA repair capacity determines the cumulative amount of DNA damage induced by repetitive low doses (10x 10 mGy) (6 MV photons). Lung, heart and brain cells show different susceptibility (73).

Response to key question 4: What is the modification of risk by genetic and epigenetic factors and gender?

- Inter-individual differences in sensitivities are greater than the differences in individual proteomic profiles after 10Gy (27). Differences include changes in energy metabolism and mitochondrial functions.
- Lead perturbs telomere replication and causes neurotoxicity and problems in brain development (56)
- Polymorphisms in p53 affect cancer induction (breast and colorectal) (55, 67, 68).
- Raman spectroscopy can differentiate different IR –induced lymphocyte donor responses (38, 39)

Results:

- Proteomic profiles reveal individual differences after 10 Gy: changes involve changes in energy metabolism and mitochondrial functions.
- Telomere functions are involved in individual responses
- Gene polymorphisms play an important role in breast and colorectal cancer susceptibilities
- ATM+/- heterozygotes can cope with cumulative low dose induced damage (73)
- Raman spectroscopy profiles reveal individual IR responses

Response to key question 5: What is the effect of age on risk?

- In the two-stage expansion model the risk increased with age, time of exposure and exposure rate (17).

Response to key question 6: What is the effect of lifestyle and/or other exposures on risk?

- **No result yet**

Response to key question7: What is the effect of physiological state?

- There is nonlinear regulation of ROS production and SOD activity in a specific endothelial cell line (EAhy926 HUVEC derived cells) contributing to nonlinear DNA damage

response (34)
- ROS have a crucial role in the effects of low dose IR (including the release of the pro-inflammatory cytokine, Il-6) (41)

Response to key question 8: Is there a hereditary component in risk?
- **No result yet**

Response to key question 9: What is the role of non-targeted effects in health risk?
- A cancer induction model suggests the involvement of cell communication and non-targeted effects appear to be involved in cancer induction (11)
- Antitumor immunity (abscopal) can be induced in cancer therapy (20, 22, 23, 24, 25, 31, 61, 66, 73, 79)
- A mechanistic model suggests intercellular induction of apoptosis (by stopping proliferation of transformed cells by signals from normal neighboring cells (33).

Results:
- Cell communication and non-targeted effects appear to be involved in cancer induction. Immunological responses are modulated by low dose and low dose rate (fractionated)(40) exposures.
- Low dose exposure of normal cells can affect cell growth of transformed cells.

2.2.3. WP7 Non-cancer effects

Response to key question 1: What is the dependence on energy deposition?
- Low ray doses modulate Il-beta activated macrophage responses in mice. (0.5-0.7 Gy produce an anti-inflammatory phenotype by reducing NF-kB dependent IL-1beta secretion (37).
- Several painful human syndromes (elbow, shoulder, calcaneodynia, etc.) can be managed by low dose RT < 1 Gy (45, 46, 47, 48, 49, 50, 51) and inflammatory immune reactions can be modulated in endothelial, mononuclear and polynuclear cells by low doses (0.5-0.7 Gy) (59) and IR can contribute to induction of antitumor immunity (65).

Results:
- Medium doses 0.5-0.7 Gy can modulate painful human syndromes, manage inflammatory responses and contribute to antitumor immunity

Response to key question 2: What is the dependence on dose rate?
- Chronic gamma-irradiation induces dose-rate dependent pro-inflammatory response and associated loss of function in human umbilical vein endothelial cells (16).
- Low dose rate exposure results in premature senescence (63).
- Chronic low dose gamma IR induces premature senescence, the p53/p21 pathway is induced affecting the replicative potential of the HUVEC cells (80)

Results:
- Nonlinear dependency on dose rate is observed (1.4 mGy/h differs from 4.1 mGy/h) (81)
- Clear nonlinear dependency of senescence induction by low dose rate gamma-ray exposures in HUVEC cells.

Response to key question 3: What are the tissue sensitivities?
- IR is a source of stress to neurons. N-methyl-D aspartate receptors are involved in neural apoptosis (72)

Results:
- Low dose IR induces stress in neurons involving NMDA receptors

Response to key question 4: What is the modification of risk by genetic and epigenetic

factors and gender?

- Gene expression at low dose rate in endothelial cells involves the insulin-like growth actor binding protein 5 (IGFBP5) (63)

Results:

- Altered gene expression IGFBP5 is involved in the IR response of HUVEC cells

Response to key question 5: What is the effect of age on risk?

- **No results yet**

Response to key question 6: What is the effect of lifestyle and/or other exposures on risk?

- **No results yet**

Response to key question7: What is the effect of physiological state?

- Metabolic changes are observed in HUVEC cells after chronic gamma-IR together with an inflammatory response (16)

Response to key question 8: Is there a hereditary component in risk?

- **No results yet**

Response to key question 9: What is the role of non-targeted effects in health risk?

- Low dose induced immune reactions can be considered as being non targeted IR effects. Some of the already mentioned effects observed on the immune responses (37, 59) may well fall into this category: they modulate macrophage activity (37), antitumor immunity (59) as well as many non cancer inflammatory conditions (45, 46, 47, 48, 49, 50, 51) and endothelial cells involved in CVD (59).

2.2.4. WP3 Education and Training

Education & Training constitutes an essential part of DoReMi activities aiming to maintain and expand the competences in radiation research and radiation protection and to build and strengthen an important driving force in career building in this domain of research. With this, Education & Training also holds an important position in MELODI, OPERRA and Horizon 2020 (CONCERT) activities. Therefore, WP3 launched a wide range of courses and yearly training events and continued the promotion of a sustainable Integrated and Education Network (ITEN) in Europe. Up to now, many DoReMi courses were held yearly that covered as much as possible most low dose health risk research and radiation protection issues related to the DoReMi key questions (see main achievements here below).

Response to key question 1: What is the dependence on energy deposition?

- Course: Modelling radiation effects from initial physical events, UNIPV, Pavia, Italy.

Response to key question 2: What is the dependence on dose rate?

- Course: Cellular effects of low doses and low dose-rates with focus on DNA damage and stress response. SU, Stockholm, Sweden.
- Course: Molecular consequences of low dose and low dose-rate exposures: impact of individual susceptibility on outcome and biomarker development. IC/CEA, Paris, France

Response to key question 3: What are the tissue sensitivities?

- Course: Radiation-induced effects with particular emphasis on genetics, development, teratology, cognition as well as space-related health issues. SCK-CEN, Mol, Belgium.
- Course: TIETO Non-cancer effects of low dose radiation. HMGU, Neuherburg Germany

Response to key question 4: What is the modification of risk by genetic and epigenetic factors and gender?

- Radiation-induced effects with particular emphasis on genetics, development, teratology, cognition as well as space-related health issues. SCK-CEN, Mol, Belgium.
- Molecular radiation carcinogenesis. HMGU, Neuherberg, Germany.
- Molecular Consequences of low dose and low dose-rate exposures: impact of individual susceptibility on outcome and biomarker development. IC/CEA, Paris, France

Response to key question 5: What is the effect of age on risk?

- **No specifically dedicated course yet**

Response to key question 6: What is the effect of lifestyle and/or other exposures on risk?

- **No specifically dedicated course yet**

Response to key question7: What is the effect of physiological state?

- **No specifically dedicated course yet**

Response to key question 8: Is there a hereditary component in risk?

- Course: Molecular Consequences of low dose and low dose-rate exposures: impact of individual susceptibility on outcome and biomarker development. IC/CEA, Paris, France
- Course: Molecular radiation carcinogenesis. HMGU, Neuherberg, Germany

Response to key question 9: What is the role of non-targeted effects in health risk?

- **No specifically dedicated course yet**

Responses to all the above questions have been considered in the 6 - 9 courses/ year organized by DoReMi WP3. However, additional aspects are now going to be included concerning the setting up and planning of animal and human studies (epidemiological studies, molecular studies on *in vivo* or biobank stored biosamples, including ethical and related countries' legislatory aspects.

2.2.5. WP4 Infrastructures

WP4 of DoReMi has been involved in facilitating research related to the DoReMi key questions by promoting the access to and development of suitable **irradiation facilities** (for low dose and/or dose rate exposures, microbeam and heavy ion facilities etc.), **epidemiological cohorts** (some of which suitable for contributions from molecular studies), **data-and biobanks** for studies on rare (historical or recent) biological samples and **analysis platforms** allowing analyses of biological samples using most recent technologies (including high throughput analyses).

The following DoReMi WP4 activities facilitated to tackle specific DoReMi questions (see relevant key questions below):

- Identification of accessibility and availability of external and internal radiation facilities
    - 1. What is the dependence on energy deposition?
    - 2. What is the dependence on dose rate?
- Assessment of suitable data-and biobanks for optimized radiation research
    - 1. What is the dependence on energy deposition?
    - 2. What is the dependence on dose rate?
    - 3. What are the tissue sensitivities?
    - 4. What is the modification of risk by genetic and epigenetic factors and gender?
    - 5. What is the effect of age on risk?
    - 6. What is the effect of lifestyle and/or other exposures on risk?

  - 7. What is the effect of physiological state?
  - 8. Is there a hereditary component in risk?
  - 9. What is the role of non-targeted effects in health risk?
- Identification and/or set up of suitable epidemiological cohorts for classical and molecular studies
  - 1. What is the dependence on energy deposition?
  - 2. What is the dependence on dose rate?
  - 3. What are the tissue sensitivities?
  - 4. What is the modification of risk by genetic and epigenetic factors and gender?
  - 5. What is the effect of age on risk?
  - 6. What is the effect of lifestyle and/or other exposures on risk?
  - 7. What is the effect of physiological state?
  - 8. Is there a hereditary component in risk?
  - 9. What is the role of non-targeted effects in health risk?

- Identification and promotion of suitable platforms for high throughput analysis
  - 1. What is the dependence on energy deposition?
  - 2. What is the dependence on dose rate?
  - 4. What is the modification of risk by genetic and epigenetic factors and gender?

3. Main outcomes from the DoReMi project (September 2015)

3.1. Challenging the LNT hypothesis

DoReMi low dose research challenges the general validity of the LNT hypothesis:
Currently, the LNT hypothesis is applied in radiation protection when considering low dose and low dose rate effects. The results obtained in mechanistic DoReMi studies show the existence of non-linear responses in terms of dose and dose-rate. The dose dependent effects concern in particular gene expression (29), inflammatory immune reactions (59, 60, 61) and anti-inflammatory responses (24, 25, 37, 60, 61, 65, 79), upregulation of p16 at 62.5 mGy, induction of senescence in thyroid cancer cells (0.5 Gy) (1). Of particular interest, have been results showing that inflammatory immune reactions in endothelial (mononuclear and polynuclear) cells can be modulated at low doses (0.5-0.7 Gy) (24, 59). The dose-rate dependent effects have been observed for the induction of senescence by gamma-ray exposures in human epithelial cells (81).

3.2. Studies on Cancer induction

The uniqueness of the radiation-mutation model for cancer induction is challenged by the findings on the involvement of stem cells in cancer induction as cancer stem cells or as actors of repopulation and replacement of damaged parts of tissues and the involvement of cell-cell communication (11). Modifications in telomere lengths may play an important role in the process of carcinogenesis. (56, 75). → research on the radiation sensitivity and behavior of tissue specific stem cells is of utmost importance!

The classical notion of DNA damage being the initiator of carcinogenesis has been further examined and extended by modeling the induction of DNA damage by radiation of different qualities (41, 42). The LET dependent DNA fragmentation (5, 6, 7) has been confirmed and shown to affect not only DNA repair dynamics but also the induction of ROS associated with the release of pro-inflammatory cytokines such as IL-6 (41). Modeling also supported RBE values higher than 1 for high LET radiations. Interestingly, cytokine secretion GCSF, IL-6, IL-8, TGFbeta) are also stimulated in conditions of adaptive responses but do not seem to underly adaptive responses that clearly affect DNA repair kinetics of radiation-induced DSB (13, 42). PARTRAC models are useful for simulating light ion track structures and biological effects of energies down to keV/u (relevant for Hadrooin therapy) (75). Very importantly, modelling of cell-cell interactions showed that low dose radiation can modulate the signaling processes underlying the intercellular induction of apoptosis; depending on system pararmeters these perturbationscan act in an anti-or pro- carcinogenic way (34) → research on the LET dependence of NTE and immune reactions appears to be important.

Progress in research on gene expression following low dose exposures clearly revealed an important role for the transcription factor PU.1 in the initiation and development of IR-induced AML (78). In fact, deletion of chromosome 2 (del2) and Sfri/1/ Pu.1 loss constitutes a molecular signature of radiation induced acute myloid leukaemia (AML) (43, 44). The complexity of del2 in radiation-induced AML in mice showed a clear LET-dependence without impact upon the sfpi1/PU.1 deletion and/or point mutation frequency on the other chromosome 2 (11). Also, evidence has been obtained for the involvement of dose and time dependent miRNA expression in IR responses (29). Indeed, micro RNAs such as miRNAs *miR-34a-5p (a target of TP53 with strong pro-apoptotic and anti-proliferative properties), miR-182-5p (with likewise oncogene and tumor suppressor related features)* were responsive in a time and dose dependent manner (29). Thyroid cancer cells for example were radiation dose responsive, and a miRNA signature could be observed (2) → thus, from gene expression studies new biomarkers are expected to come up and await their validation.

Studies in animals (mice) showed clear mouse strain dependent differences in the DNA damage and repair responses in bronchiolar and alveolar epithelial lung cells (19). Fractionated exposures (100 mGy) are suspected to cause an increase in possible secondary malignancies (19). The genetically controlled DNA repair capacity determines the amount of cumulative DNA damage induced by repetitive low dose 6 MV photon radiation, lung, heart and brain cells show different susceptibilities to the induction of such DNA damage. ATM heterozygotes apparently can cope with DNA damage from repetitive low dose exposures (Schanz S et al. 2014). On the other hand, fractionated low dose irradiation of testicular mouse tissue induced persistent DSB in spermatogonial stem cells (27). Furthermore, genetic variations in regulatory regions of the RB1 gene were shown to modify the risk for malignant transformation of bone cells after R exposure (64).

Altogether these findings provide further evidence for the existence of individual radiation sensitivities and their regulation.

3.3. Epidemiological studies

In a sub group of 4054 men of the German uranium minor cohort (1946-1989) in milling facilities an excess mortality from lung cancer due to radon exposure, and from solid cancers due to external gamma radiation was observed but not statistically significant. Apparently, at low absorbed organ doses uranium was not associated with any cause of death (30). Likewise, no excess of kidney cancer induction by radon was found in the French and German cohort of uranium miners (15, 36). Mathematical model analysis of the ELDORADO cohort concerning lung cancer in uranium minors clearly that the cancer risk increased with obtained age, time of exposure and exposure rate, however, in the low dose range large uncertainties remain (17). → This again underlines the importance of associating molecular biomarker studies with epidemiological studies at low doses and low dose rates of ionizing radiation (53, 54).

3.4. Biomarker studies

From the start of DoReMi, the TRA put a lot of emphasis on research on suitable biomarkers. In fact, a long list of classical and recent molecular biomarkers was established (53) together with possible modes of use and application (53, 54). Several new biomarkers, the suitability of γH2AX fand BP53p or the detection of radiation induced DSBs and related repair capacities, Chromosome 2 deletion, Pu.1 loss for radiation induced AML, several transcriptional (example: CDKN1A) and epigenetic signatures (example: miR-34a-p5 and miR-182-5p) were found (29) and await now further validation. Moreover, Raman spectroscopic analysis revealed characteristic donor specific changes in human lymphocytes after low doses of IR (38, 39) in parallel to changes in the extent of induced DNA damage (gH2AX). Furthermore, the involvement of specific polymorphisms in breast, colorectal and lung cancer were reported (55, 67, 68). The fact that gamma ray exposure induces individual differences in proteomic profiles in human lymphoblastoid cell lines points to the possibility to develop proteomic biomarkers for individual IR responses (27).

A recent very exciting finding from Atkinson's group in Munich is that among long non-coding RNAs a biomarker for low dose IR responses (PARTICLE) could be established that regulates locus-specific methylation (activation of certain genes)(V. B. O'Leary et al. 2015, Cell Reports 11,1-12, 2015).

Considering low dose effects on the vascular cell system it is important to note that the insulin-like growth factor binding protein 5 IGFB5 has been shown to be specifically involved in low dose rate induced premature senescence in human endothelial cells (63).

→Biomarkers can be considered to be extremely useful for defining IR exposure, metabolic changes and pathological changes (different types of cancers and non cancers).

4. Main tendencies noted from DoReMi achievements

- **From low dose IR (<100 mGy) to medium and high dose IR including environmental and medical options.**

- **From DNA oriented work towards RNA, stem cell, immunological and multi-exposure oriented work?**

- **From better understanding of most relevant metabolic pathways in the induction of cancers and non cancer effects towards Systems biology, Modeling and assessment of IR health risks.**

- **From classical epidemiological work towards molecular Epidemiology, integrated and individual health risk assessment**

- The work on **suitable infrastructures (WP4)** has opened up new possibilities for well controlled and scientifically sound research on radiation health risks and radiation protection in Europe and thus consolidated the basis for future research in the context of **Horizon 2020 (OPERRA, CONCERT…).** The tendency here is to further stimulate and harmonize integrative research in most suitable conditions.

- The **development of courses (by WP3 Education & Training)** specifically dedicated to the maintenance and further development of expertise and competences in this domain of research on health risks and radiation protection provides a solid framework for widening the scope of the courses including not only low dose research health risk aspects but also new dosimetric, ecological, biological, medical and interventional (urgency) and societal aspects (within the context of Horizon 2020).

5. Priorities for future research (with justification)

Together with MELODI the DoReMi project focused so far, nearly exclusively, on low dose and low dose rate effects. The results mentioned above clearly demonstrate that "there is really something to see at low IR doses" ,and the strict emphasis of the DoReMi NoE on low dose effects was fully justified. In fact, it has stimulated the development and application of many new mechanistic and modeling approaches to detect hitherto unknown low dose effects with improved precision. Cells and tissues are responding to IR exposures in a complex, but highly integrated and interactive network.

It will be essential to intensify the **work on specific pathways** involved and on 'decisive' metabolic nodes affected by IR which drastically diminish specific metabolic functions (for example, energy metabolism and mitochondrial functions) and may initiate pathological (cancer or non cancer) effects in the most susceptible, and, possibly, pre-damaged (stressed) cells and tissues. In this regard, **a systems biology approach** appears to be most appropriate because it can provide a global understanding of the dose response relationships.

1. **Metabolic pathway analysis** is most useful for identifying biomarkers for application in epidemiological studies and health risk modeling and assessment including systems biology approaches.
    - *Further research in this area is needed to identify new biomarkers from 'omics' studies and epigenetic profiling (coding and non coding RNAs) in order to define IR exposure, metabolic insults and initiation of pathologies (cancer, non cancer) and individual IR responses.*
    - *Systems biology approaches facilitating health risk evaluations need to be favored*

2. **Research on the mechanisms and biological impact of different radiation quality need to be extended**
    In spite of big efforts to clarify the impact of radiation of different LET in terms of the induced cellular damage (especially, simple and complex DNA damage) this line of research has not been able to explain the differences obtained in biological effectiveness of radiations in the induction of cancers and non cancers. Apparently, the mixture of varying types of DNA lesions induced by each type of radiation elicits general but sometimes very specific responses in some cells and tissues. Thus, effects of radiations of different energies and LET need further analysis.

3. **Dose-rate effects (fractionated exposures) need to be further explored.**
    Tissue specific and individual effects involving the management of IR-induced radicals, intercellular communication signals (cytokine release) and specific metabolic states of tissues have been observed. The DDREF needs to be better defined. However, it may be dependent on many parameters and associated with cell and tissue specific pathological effects (cancers and non cancers). In addition, knowing that fractionated low dose exposures on testicular tissue in mice yield persistent DSBs in spermatogonial stem cells it appears of interest to pursue the research onspecific tissue sensitivities by also including germ cells. Also, the DNA repair capacity of different tissues after fractionated low dose exposures merits to be explored further.

4. **The effects of different internal emitters have to be further analyzed.**
    Recent results suggest that at equal doses internal and external exposures may exert quite comparable biological effects. This means that more emphasis has to be put on specific emitters, better dosimetry assessments, specific localized effects eliciting metabolic

disturbances and/ or pathological effects in specific cells and tissues.

5. **Research on Cancer induction in specific tissues.**
The evaluation of specific stem cell or progenitor cell IR sensitivities will be important to explain the preferential induction of cancers in specific tissues. Similar to chemically induced cancers the specificity of effects may lie in preferential exposure conditions but in the case of IR exposure may be related to radiation quality, specific tissue sensibility (susceptibility) (hematopoietic system, lung, breast, skin, brain) and the individual metabolic, genetic and epigenetic context. Adequate modelling of the mechanisms involved should facilitate health risk assessments.

6. **Research on Non cancer effects.**
For this, the mechanisms involving radiation-induced alterations of specific cells and tissues are to be further explored. Results obtained within DoReMi clearly show the involvement of processes such as senescence, interactions and communication (signaling) between different cell types, inflammatory (immune) reactions and IR-induced radical reactions. Some of them show non-linear IR responses. Also, the assessments of individual variations of responses are likely to be of great importance in health risk evaluations in patients exposed to medical radiations. Modelling of non cancer effects is important for health risk estimations.

7. **Analysis of Non targeted effects (NTE) and immunological effects in vivo.**
The importance of this research has been strengthened by recent findings suggesting that NTE may be fully integrated into the system of immunological responses with tight links to important cellular protection mechanisms such as energy metabolism, anti-radical defenses and DNA repair. Moreover, the fact that low dose induced intercellular apoptosis (involving NTE) may represent an important anti-carcinogenic mechanism depending on system parameters asks for more experimental studies along these lines.

8. **Research on IR impacts on energy metabolism and mitochondrial functions.**
Several studies of DoReMi pointed to the involvement of changes in energy metabolism and mitochondrial functions after low IR doses (25, 52, 41, 27). This does not seem to be without consequences when this happens in cell immunity competent cells. Recently, it has been shown that the protein called lymphocyte expansion molecule (LEM) promotes CD8+ T cell immunity through effects on mitochondrial respiratory functions. LEM controls oxidative phosphorylation complexes and respiration (through interaction with the CR6 interaction Factor 1 (CRIF1)) leading to the production of pro-proliferative mitochondrial reactive oxygen species. LEM thus links immune activation to the expansion of protective CD8+ T cells (see Okoye I et al. Science 2015 , 348(2015) 995-1001). IR effects on LEM may well affect individual IR responses.

9. **Epidemiological studies taking into account possible contributions from molecular studies**
Projects putting together physical (dosimetry), biological and epidemiological efforts should be favored (example: the DoReMi project CURE on the health risk of internal emitters).
Epidemiological projects associated with molecular marker based studies are clearly most promising because recent research has shown that some specific DNA repair pathway genes are involved in specific cancers (APC and MLH1 in colon cancers (The Cancer Genome Atlas Network, Nature 487 (2012) 30-33), Kaiser JC et al. 2014 PLOS One 0 (2014) e111024), CLIP2 in thyroid cancer (Selmansberger M. et al, Carcinogenesis 2015, May 8).

10. **Dose and dose-rate dependency of IR induced cancer and non cancer effects.**
As far as the dose levels are considered, it has become evident from the above DoReMi studies that very much can be learned also from studies on IR responses at medium and high doses. Some mechanisms are acting over all these dose ranges, others are relatively sensitive to

specific dose levels (energy metabolism, mitochondrial functions versus enzymatic pathways: DNA repair, membrane structures, specific cell functions). Mechanistic studies at low, medium and high doses should be useful to get a better understanding of IR responses and the health risks involved.

11. **Research on the effects of mixed exposures.**
    Human beings are rarely just exposed to a single type of stressor. It is thus essential to undertake some pilot studies in order to analyze the effects of mixed exposures and combined exposures to different stressors (chemical + IR, IR + chemical, IR1 + IR2). As an example, one might cite the interaction of human exposures to Radon and cigarette smoking which are known to be particularly efficient for producing lung cancer in humans (Darby et al. 2009). Moreover, in anticancer treatments using chemotherapy plus RT the increase of efficacy is well documented. What happens at very low exposures (synergistic or additive effects) is, however, largely unknown.

12. **Transgenerational IR effects.**
    Epigenetic profile changes may be transmitted to the next generation and lead to genomic instability (see Filkowski J et al. Carcinogenesis 2010). Thus, it seems wise to study, wherever possible, such possible transgenerational effects in more detail in suitable cohorts (see for example in the Mayak and Chernobyl cohorts where several generations can be studied).

6. Recommendations

The research performed in DoReMi has been focused on low dose radiation health risk research. It was highly integrated from the start and thus benefited from the inclusion of a wide range of scientific disciplines. This has created a well -founded dynamic of research and widened the list of essential parameters to be taken into account in low dose health risk research (see regular updates of the DoReMi TRA and the MELODI SRA). On these grounds, DoReMi has been nicely progressing in this very difficult field of research. From this, it is clear that further integration of new research activities is needed to define common future research strategies in this domain capable of incorporating dosimetric, environmental, medical and urgency aspects.

1. **Further analyses of dose rate effects:**
   Are there differences between low dose rate, chronic and fractionated dose exposures? There is some confusion between environmental chronic exposures and fractionated exposures in medical applications (mammography, Colins C et al. 2012, and RT (66, 40, 31). And what about ultrahigh dose rates?
   *Depending on the type of radiation the results are quite different.***.** *Acute and protracted exposures are also important for the induction of non-cancer pathologies and should be considered in studies on lens opacities, CDV and neurological effects.*

2. **IR effects on regulatory control systems:**
   Genetic and epigenetic control systems of metabolic pathways affected by IR should be studied. It has become clear that the induction of damage is important but for the final biological outcome of the radiation insult most important are the regulatory systems that activate or inactivate certain metabolic pathways involved in the IR response (example: the DDR 'DNA Damage Response (regulatory) pathway) which is fully integrated in the IR cellular metabolic response network).

3. **Possible transgenerational effects:**
   Transmission of IR induced alterations (epigenetic factors) affecting the fitness (susceptibility to cancer and non cancers) and IR resistance of the next generation should be analysed in

suitable cohorts (Mayak, Chernobyl, Fukushima).

4. **Accurate dose responses for specific cancers and non cancer diseases:**
Urgently needed is the analysis of processes involved using suitable animal models as well as suitable human cohorts (for example: CT scans in children) and validated biomarkers for IR exposure and the specific pathology.

5. **Launching of molecular epidemiological studies:**
Classical epidemiological studies may be supported by molecular studies in order to reduce uncertainties in the low dose range. Such studies may include studies on genetic (transcriptional) and epigenetic profile changes.

6. **Launching of systems biology studies on specific metabolic pathways:**
Important is the mechanistic analysis of IR induced pathologies in order to estimate the probability of IR exposures that will give rise to specific diseases.

7. **Reinforcement of dosimetry and biological studies on the effects of internal contamination exposures.**
This research should help to get a better precision in local dose distribution in relation to pathological outcomes.

8. **Research aiming at a better mechanistic understanding of IR induced non cancer effects**
More emphasis has to be put on this type of research in order to get an understanding of the mechanisms involved and their dose and dose-rate dependeny, low and medium dose and dose rate responses (especially important, in medical applications), definition of the metabolic pathways affected and their regulation, the radio-sensitivity of the different cell types involved and their mode of interaction.

9. **Clarification of social context and ethical concepts underlying this research:**
The projected research involves studies on animals and humans. Thus, it is necessary to define the social context and the ethical concepts and rules that drive this research.

Recommendations for Education & Training and Infrastructures:

Although these activities have already been taken up by the **OPERRA** project and the future, wide ranging project **CONCERT**, we are listing here some aspects that should be reinforced taking into account the experiences from DoReMi.

Education& Training (recommendation from WP3):

- Incorporation of E&T activities and development of sustainability through MELODI, OPERRA and CONCERT in the framework of Horizon 2020.
- Adaptation of courses to the next generation of researchers
- Dissemination of new E&T work to the research community (Further development of Website support)
- Inclusion of new areas of expertise, technologies, disciplines (omics, bioinformatics, . . .) in E&T activities
- Development of awareness and use of infrastructures (Irradiation facilities, epidemiological cohorts,

Infrastructures (recommendations from WP4)

- Promotion of the accessibility and use of available infrastructures among researchers *(through dissemination activities such as Education& Training, Website information and* *workshops)*

- Promotion of efforts for harmonization and integrative research through the use of available infrastructure *(through dissemination activities such as Education& Training, Website information and workshops)*
- Identification of as yet missing infrastructures that need to be developed

7. Concluding remarks

This statement has been written before all results of the DoReMi project are known. Obviously, the results obtained in the various tasks and subtasks of DoReMi that started in very recent years are likely to be published several months after the end of the DoReMi project. From this work, major inputs are expected that will change our present vision and promote new strategies of research in radiation induced health risk evaluation and radiation protection.

Annex 1: DoReMi tables of tasks and published results (by September 2015)

Table 1: WP3 Education and Training program

Task	Work	Starting
3.1	Preliminary investigative work prior to identify the format of the ITEN	2010
3.2	Set up a new low-dose risk ITEN following the recommendations from the previous task	2010
3.3	Develop sustainable funding in collaboration with WP2	2010
3.4	Oversee management of ITEN during the period of transition to sustainable funding	2010
3.5	Funding training activities	2010

The four tables below show the enlargement of the WP's:

Table 2: WP4 Infrastructures program enlargement

Task	Work	Starting
4.1	Survey of existing facilities for low dose risk research	2010
4.2	Characterization of infrastructure needs and roadmap of implementation	2010
4.3	Implementation of DoReMi support activities for shared infrastructures	2010
4.4	Development and implementation of access to Infrastructure	2010
4.5	**Open Access to the UMB low dose irradiation facility (FIGARO)**	**2011**
4.6	**Dose/Dose-rate Radiation Effects in Brain Cancer Risk (DDRE-BrainCancer)** • Tanori M et al. 2013 4.6 Developmental and oncogenic radiation effects on neural cells and their differentiating progeny in the mouse cerebellum. Irradiation of newborn Ptc11/2 mice dramatically increases the frequency and shortens the latency of MB.	**2011**

4.7	**Low dose/dose rate gamma irradiation facility for in vitro biological systems (LIBIS)**	**2012**
4.8	**Integration of STORE into DoReMi as a trustable and viable database and/or pointer to biobanks and ascertain sustainability**	**2012**
4.9	**Provision of ion microbeam irradiation facility SNAKE (MicroRAD)** • Drexler GA et al. 2015 4.9 Live cell imaging and damage to subcellular structures	**2013**
4.10	**Laboratory infrastructure for retrospective radon and thoron dosimetry (RETRODOS)** • Pressyanov, D et al. 2015 4.10 and 5.5 Optimization of etching conditions for CD's/DVDs used as detectors for 222Rn.	**2014**

Table 3: WP5 Shape of dose response program enlargement

Task	**Work**	**Starting**
5.1	Phase – shifts in responses and processes at high/low doses and dose rates • Shim G et al. 2014 5.1 and 6.2 Review : Cross-talk between telomere maintenance and radiation effects. Telomers are key players in the process of IR-induced carcinogenesis. • Large M et al. 2014 5.1 Non linear regulation of ROS production and SOD activity in endothelial cells (EA.hy926 HUVEC derived cells) contribute to non-linear discontinuous dose-response relationship of gH2AX (DSB) foci detection. • Kabacik S et al. 2015 5.1 and 5.2 ATM status dependent miRNA expression after IR. Different expression of protein-coding genes in health and AT donors at 2h, but not 24h after IR (2-5 Gy). There is a linear dose response of the genes at 24 h. Some miRNAs are responsive to IR in a dose and time dependent manner. There is upregulation of FAS-AS1 lncRNA by IR in an ATM dependent manner.	2010
5.1.1	**Low dose Gene Expression signature (LoGiC)**	**2011**
5.2	Assessing the relative contribution of targeted (DNA), non-targeted and systemic processes to radiation carcinogenesis • Acheva A et al. 2014 5.2 3-D models are relevant for cancer, non cancer, tissue sensitivity studies and modeling purposes • Babini G et al. 2015 5.2 Nonlinear interaction between inflammatory and signalling pathways in the dose range of 0.5-2 Gy.	2010

	• Campa A et al. 2013 5.2 Cancer model focusing on cell communication and non targeted effects • Kabacik S et al. 2015 5.1 and 5.2 ATM status dependent miRNA expression after IR. Different expression of protein-coding genes in health and AT donors at 2h, but not 24h after IR (2-5 Gy). There is a linear dose response of the genes at 24 h. Some miRNAs are responsive to IR in a dose and time dependent manner. There is upregulation of FAS-AS1 lncRNA by IR in an ATM dependent manner. • Rödel F et al. 2012a 5.2.1, 5.2 and 7.6 Modulation of inflammatory immune reactions by low doses of IR 0.5-0.7 Gy in endothelial, mononuclear and polynuclear cells. Non linear biphasic responses are seen.	
5.2.1	**Modulation of Inflammation by low and moderate dose Ionising Radiation (ModInIR)** • Dieriks B et al. 2011 5.2.1 Adaptive responses on DSB induction and cytokine secretion after 0 .1- 0.5 Gy and 24h show upregulation of cytokines GM-CSF, IL6, IL8, TGF beta. However, IL6 and TGFbeta are not responsible for adaptive response. The adaptive response alters the gH2AX spot size and DSB repair kinetics. • Frey B et al. 2012 5.2.1 Abscopal antitumor immunity and immunogenic tumor cell death in cancer therapy. • Frey B et al. 2012 5.2.1 Modulation of RT effects by hyperthermia activation of natural killer cells and phagocytes. • Frey B et al. 2012 5.2.1 Induction of immunogenic potential in colorectal tumors by CT and RT. • Frey B et al. 2014 5.2.1 Induction of immunogenic cell death by IR. IR induced systemic antitumor responses can be boosted by additional immune therapy. • Frischholz B et l. 2013 5.2.1. Low dose RT (0.5 or 07 Gy) in mice reduces the inflammatory phenotype in the more radiosensitive macrophages of Balb/c mice. • Gaipl US et al. 2014 5.2.1 RT induced non targeted effects, induction of immune modulating danger signals (hsp70, ATP, HMGB1) by X-rays. Antitumor and immunogenic effects are induced by IR. • Kulzer L. et al. 2014 5.2.1. RT induces immunostimulatory forms of tumor cell death. The supernatant of fractionated RT irradiated tumor cells resulted in a significant increased secretion of immune activating cytokines (IL12p70, IL8, IL6, TNFalpha) in comparison to single dose treated tumor cells. Norm and fractionated RT induces fast human colorectal tumor cell death with immunogenic potential (triggering DC maturation and activation in vitro). All this may help to improve RT. • Manda K et al. 2012 5.2.1 Protracted low dose IR can result in radio-resistance, but immunosuppressive effects of chronic low dose IR are also reported, with sensitization of certain cell types (see IR effects on the interaction of DC and T cells).	**2011**

	• Rödel F et al. 2012a 5.2.1, 5.2 and 7.6 Modulation of inflammatory immune reactions by low doses of IR 0.5-0.7 Gy in endothelial, mononuclear and polynuclear cells. Non linear biphasic responses are seen. • Rödel F et al. 2012b 5.2.1 and 7.6 Review on the immunomodulatory properties of low dose RT, with a maximum effect at 0.5 -0 .7 Gy. • Rödel F et al. 2013 5.2.1 and 7.6 Review on anti-inflammatory activities at <1Gy, and induction of harmful side effects, IR induced immune modulation or induction of anti-tumor immune responses at higher doses. • Rubner Y et al. 2012 5.2.1. 7.6 IR contributes to the induction of anti-tumor immunity • Rubner Y et al. 2014 5.2.1. Fractionated RT induces immunogenic cell death and Hsp70 release in p53 mutated glioblastoma cell lines. • Schauer C et al. 2014 5.2.1 Neutrophils recruited to sites of inflammation undergo oxidative burst and form neutrophil extracellular traps (NNETs). NETs promote the resolution of neutrophilic inflammation by degrading cytokine and chemokines and disrupting neutrophil recruitment and activation. • Wunderlich R et al. 2014 5.2.1. Low and moderate doses of IR up to 2 Gy modulate transmigration and chemotaxis of activated macrophages, produces an anti-inflammatory cytokine milieu, but does not affect viability and phagocytic function of mouse macrophages.	
5.3	The dynamics of pre-neoplastic change and clonal development • Abou-El-Ardat K et al. 2011 5.3 In thyroid cancer cells 62.5 mGy upregulate p16. 0.5 mGy cause senescence without involvement of p16, p21 • Abou-El-Ardat K et al. 2012 5.3 Thyroid cancer cells are radiation responsive and dose responsive. There is a signature of miRNAs. Low doses induce proliferation in normal thyroid cells • Brown, N et al. 2015 5.3 Influence of radiation quality on mouse chromosome 2 deletions inradiation-induced acute myeloid leukaemia. Influence of radiation quality on mouse chromosome 2 deletions in radiation-induced acute myeloid leukaemia. • Olme CH et al. 2013a 5.3 Radiation induced acute myeloid leukaemia (AML) involves chromosome 2 deletion and Sfpi1/Pu1 loss. • Olme CH 2013b 5.3 There is a signature for IR induced AML : the chromosome 2 deletion detected in half of the irradiated mice after one year does not provide an advantage for growth and in vivo repopulation in the bone marrow of the mice. • Pascucci B et al. 2012 5.3 Primay fibroblasts from Cockayne syndrome (CS) patients share high levels of oxidative stress, perturbed oxidative energy metabolism and mitochondrial	2010

	functions. Oxidative DNA damage is causative for the observed pathophysiology (CS cells are slightly X-ray sensitive). • Raj K et al. 2012 5.3 Workshop report on Stem cells and Radiation sensitivity. • Verbiest T et al. 2015 5.3 The transcription factor PU.1 is down regulated in IR induce AML. PU.1 plays a role in the initiation and development of IR induced AML.	
5.4	Mathematical models to link experimental findings and epidemiological data • Eidemüller M et al. 2012 5.4 Lung cancer in uranium miners. In the two stage expansion model (TSCE) the risk increased with obtained age, time of exposure and exposure rate. Large uncertainties remain for small exposures. • Kundrat P et al. 2011 (2012) 5.4 Mechanistic model of triggering apoptosis in transformed cells under coculture conditions. The model predicts that intercellular induction of apoptosis is balancing the proliferation of transformed cells • Kundrat P and Friedland W. 2015 5.4 Impact of intercellular induction of apoptosis on low-dose radiation carcinogenesis.	2010
5.5	Assessing the risk from internal exposures • Drubay D et al 2014 5.5 Kidney cancer induction by Radon ? No excess of such cancers is found in the French and German cohort among uranium miners. • Laurier D et al. 5.5 Review : Cancer risks from internal contamination research opportunities. • Kreuzer M et al. 2015 5.5 and 5.8 In a cohort of Uranium millers, there is a statistically significant excess mortality from total cancer due to radon exposure and no significant excess from solid cancers due to external gamma radiation (at low organ doses). • Pressyanov, D et al. 2015 4.10 and 5.5 Optimization of etching conditions for CD's/DVDs used as detectors for 222Rn.	2010
5.5.1	**Internal Emitters in Uranium Miners (INTEMITUM)**	**2013**
5.5.2	**Assembly of internal radiation dose for UKAEA and AWE epidemiology cohorts (AIRDoseUK)**	**2013**
5.6	**Track structures and initial events: an integrated approach to assess the issue of radiation quality dependence (INITIUM)** • Alloni D. et al. 2012 5.6 Monte Carlo codes, PARTRAC code for radiation-induced DNA damage.The RBE is >1 for high LET IR. • Alloni D et al. 2011 5.6 PARTRAC code models DNA fragmentation in diploid human fibroblasts at different LETs and reveals the importance of small DNA fragments • Alloni D et al. 2013 5.6 PARTRAC code model : DNA	**2012**

	fragmentation after gamma and nitrogen heavy ions: indication of an RBE of > 1 and a higher amount of short DNA fragments induced for high LET nitrogen ions. • Mariotti LG et al. 2012 5.6 Crucial role of ROS in transducing the effect of initial IR and subsequent release of IL-6. The effect is LET dependent. • Mariotti LG et al. 2013 5.6 DNA repair dynamiccs after low and high LET exposures. Full recovery is observed after 12 hours. Split dose experimental results show that initial IR exposure induces more gH2AX foci than subsequent exposures. • Schmitt E et al. 2015 5.6 Cross-section scaling for track structure simulations of low energy ions in liquid water.	
5.7	**Induction and facilitation of chromothripsis by low dose ionizing radiation (In-FaCT-IR)**	**2013**
5.8	**Concerted Action for an Integrated (biology-dosimetry-epidemiology) Research project on Occupational Uranium Exposure (CURE)** • Kreuzer M et al. 2015 5.5 and 5.8 In a cohort of Uranium millers, there is a statistically significant excess mortality from total cancer due to radon exposure and no significant excess from solid cancers due to external gamma radiation (at low organ doses).	**2013**
5.9	**Low dose radiation-induced non-targeter effects in vivo: the role of microvesicles in signal transduction (Rad-Mvivo)**	**2014**
5.10	**Effects of Chronic LOw-dose Gamma Irradiation on GAstrointestinal Tumorigenesis (CLOGICAT)**	**2014**

Table 4: WP6 Individual sensitivities program enlargement

Task	Work	Starting
6.1	**Molecular epidemiological studies to address the role of individual genetic variation in determining susceptibility to low doses** • Flockerzi E et al. 2014 6.1 DNA repair and repair capacity in lung in different mouse strains : differences in 53BP1 induction in bronchiolar and alveolar epithelial cells. Fractionated doses 100 mGy daily increase toxicity and probability to induce secondary malignancies. • Grewenig, A et al. 2015 6.1 and 6.10 Persistent DNA Damage in Spermatogonial Stem Cells After Fractionated Low-Dose Irradiation of Testicular Tissue. • Schanz, S and Flockerzi, E et al. 2014 6.1 and 6.10 Genetically-Defined DNA Repair Capacity Determines the Extent of DNA	2010

	Damage Accumulation in Healthy Mouse Tissues after Very Low Doses of Ionizing Radiation.	
6.2	**Identification of genetic modifiers of individual cancer susceptibility and their mechanisms of action** • Gürtler A et al. 2014 6.2 10 Gy of gamma rays induced individual differences in proteomic profiles are low compared with inter-individual differences seen in lymphoblastoids cell lines. • Pottier G et al 2013 6.2 Lead exposure induces telomere instability in human cells by perturbing telomere replication on the lagging strand. This is important in brain development and neurotoxicity. • Rosemann M et al. 2014 6.2. Alpha-ray induced osteosarcoma and different tumor susceptibility genes in mouse strains : Reduced Rb1 expression by common variants in regulatory regions can modify the risk for malignant transformation of bone cells after IR exposure. • Shim G et al. 2014 5.1 and 6.2 Review : Cross-talk between telomere maintenance and radiation effects. Telomeres are key players in the process of IR-induced carcinogenesis.	2010
6.3	**Modelling of the effects on risk prediction models due to changes in biological processes influenced by genetic variability**	2010
6.4	**The effect of genetic modifiers on carcinogenesis following low dose <u>rate</u> exposure** • Perriaud L et al. 2014 6.4 Intronic TP53 polymorphisms affect G4 formation and expression of isoform specific transcripts of the TP53 gene. • Sagne C et al. 2013 6.4 Meta-analysis of cancer risk associated with a specific rS17878362 polymorphism of the TP53 tumor suppressor gene. The cancer is increased for homozygous A2A2 carriers for breast and colorectal but not for lung cancer. rs17878362 is associated with increased cancer risk with a population and tumor specific effects. • Sagne C et al. 2014 6.4 Age effect on cancer onset in Li-Fraumeni/Li-Fraumeni-like syndrome. Dependency on G4 polymorphisms in haplotypes of the WT TP53 allele.	2010
6.5	**Contribution of genetic and epigenetic mechanisms that indirectly influence susceptibility to radiation-induced cancer**	2010
6.6	**Implementation of the DoReMi strategy for a large scale molecular epidemiological study to quantify genetic contribution to individual susceptibility** • Pernot E. et al. 2012 6.6 Biomarkers suitable for epidemiological studies	2010

	• Pernot E et al. 2014 6.6 Saliva samples are potentially useful for epidemiological studies with biomaker, particularly in children.	
6.7	**Planning expansion of research portfolio**	2010
6.8	**Predicting individual radiation sensitivity with Raman microspectroscopy (PRISM)** • Maguire A et al.2015a 6.8 Classification of leukocyte subtypes by Raman spectroscopy. • Maguire A et al. 2015b 6.8 Detection of IR induced damage in lymphocytes after doses of 0.05 and 0.5 Gy by Raman spectroscopy in parallel to DNA damage (gH2AX). Differences are observed with different donors.	**2011**
6.9	**Integrating radiation biomarker into epidemiology of post-Chernobyl thyroid cancer from Belarus (INT-Thyr)**	**2012**
6.10	**Characterization of DNA lesions in the nuclear ultrastructure of differentiated and tissue-specific stem cells after protracted low-dose radiation (Zif-TEM)** • Grewenig, A et al. 2015 6.1 and 6.10 Persistent DNA Damage in Spermatogonial Stem Cells After Fractionated Low-Dose Irradiation of Testicular Tissue. • Schanz, S and Flockerzi, E et al. 2014 6.1 and 6.10 Genetically-Defined DNA Repair Capacity Determines the Extent of DNA Damage Accumulation in Healthy Mouse Tissues after Very Low Doses of Ionizing Radiation.	**2013**
6.11	**Mechanism of low dose response to ionizing radiation and its significance in radiation protection (RADSENS)**	**2013**

Table 5: WP7 Non-cancer effects program enlargement

Task	**Work**	**Starting**
7.1	Structuring the research effort on non-cancer effects according to the HLEG roadmap: organisation of consultation/exploratory meetings and funding integrative RTD projects	2010
7.2	Preparation of a pilot study to conduct molecular epidemiology studies in vascular radiation damage • Kreuzer M at al. 2015 7.2 Low-dose ionising radiation and cardiovascular diseases – Strategies for molecular epidemiological	2010

	studies in Europe	
7.3	Feasibility study towards a systems biology approach of radiation response of the endothelium • Ebrahimian T. et al. 2015 7.3 Chronic gamma-irradiation induces a dose-rate-dependent pro-inflammatory response and associated loss of function in human umbilical vein endothelial cells. • Gerard A.C et al. 2012 7.3 Iodine deficiency induces a long lasting angiogenic phenotype in thyroid cancer cells leading to uncontrolled growth. • Rombouts C et al. 2013 7.3 Low dose X-rays induce DNA damage and apoptosis in endothelial cells (HUVEC and EA.hy926). • Rombouts C et al. 2014 7.3 Low dose rate (4.1 mGy/h) results in premature senescence in endothelial cells. Gene expression analysis shows that the insulin-like growth factor binding protein 5 IGFBP5 is involved. • Yentrapalli R et al. 2013a 7.3 Induction of premature senescence in human umbelical endothelial cells exposed to chronic low dose rate gamma ray exposure (4.1 mGy/h). Such chronic exposure results in induction of the p53/p21 pathway in HUVEC cells affecting the replicative potential of these cells and leading to premature senescence. • Yentrapalli R et al. 2013b 7.3 A non linear response to dose rate is observed. Responses at 1.4 mGy/h differ from those at 4.1 mGy/h.	2010
7.4	Pilot epidemiological study of lens opacities among a cohort of interventional • Farah J et al. 2013 7.4 Estimation of cumulative eye lens doses in intervention settings of cardiologists radiologists and cardiologists	2010
7.4.1	**Lens opacities: Methodology implementation (ELDO)**	**2012**
7.5	Pilot study of external irradiation versus internal contamination effects on neurogenesis • Samari N et al. 2013 7.5 IR is a source of stress for immature neurons. N-methyl D-aspartate (NMDA) receptors are involved in RI induced neural death by apoptosis.	2010
7.6	**Study on contribution of low dose X-radiation in induction of anti-inflammation** • Lödermann B et al. 2012 7.6 Modulation of IL-1beta activated macrophages by low X-ray doses : Doses of 0.5 and 0.7 Gy in mice produce an anti-inflammatory phenotype of activated macrophages by reducing NFkB dependent IL-1beta secretion in mice. • Ott OJ et al. 2012 7.6 Management of benign painful elbow syndrome by < 3Gy RT.	**2011**

	- Ott OJ et al. 2012 7.6 Management of benign painful shoulder syndrome by low dose RT 0.5-1 Gy single dose, not exceeding 3-6 Gy in total. - Ott OJ et al. 2013 7.6 Treatment of achillodynia is possible with 0.5 or 1 Gy not exceeding 3-6 Gy in total. - Ott OJ et al. 2013 7.6 RT and calcaneodynia management. - Ott OJ et al. 2014 7.6 RT for benign calcaneodynia : long term results. - Ott OJ et al. 2014 7.6 Optimization of low dose RT of benign painful elbow syndrome - Ott OJ et al 2014 7.6 Optimization of low dose RT of benign painful elbow syndrome : no significant differences between 0.5 and 1 Gy dose schedules. - Rödel F et al. 2012a 5.2.1, 5.2 and 7.6 Modulation of inflammatory immune reactions by low doses of IR 0.5-0.7 Gy in endothelial, mononuclear and polynuclear cells. Non linear biphasic responses are seen - Rödel F et al. 2012b 5.2.1 and 7.6 Review on the immunomodulatory properties of low dose RT, with a maximum effect at 0.5 -0 .7 Gy. - Rödel F et al. 2013 5.2.1 and 7.6 Review on anti-inflammatory activities at <1Gy, and induction of harmful side effects, IR induced immune modulation or induction of anti-tumor immune responses at higher doses. - Rubner Y et al. 2012 5.2.1 and 7.6 IR contributes to the induction of anti-tumor immunity.	
7.7	**Low dose Gene Expression signature and its impact on Cardiovascular disease (LoGiC)**	**2011**
7.8	**Study on contribution of low dose X-radiation in induction of cataractogenesis and influencing genetic and cell communication factors (LDR-OPTI-GEN)**	**2013**
7.9	**Low and moderate dose radiation effects on brain microvascular pericytes: epigenetic mechanisms and functional consequences (PERIRAD)**	**2013**
7.10	**Influence of a chronic LD and LDR exposure onto the development of Parkinson symptoms in genetically predisposed Pitx3-EYL/EYL Ogg1-/- mouse mutant (OSTINATO)**	**2013**
7.11	**Epidemiological pilot study on radiation-induced cataract in interventional cardiology (EVAMET)**	**2014**
7.12	**Effect of low doses of low-LET radiation on impaired vascular endothelium (ELDORENDO)**	**2014**
7.13	**Low-dose ionizing radiation-induced cataracts in the mouse: in vivo**	**2014**

	and studies (RadCat)	

Annex 2: DoReMi Peer-reviewed publications and abstracts (by September 2015)

The DoReMi project started in January 2010, and it will end in December 2015. In order to illustrate the scientific progress of DoReMi achieved, the peer reviewed DoReMi publications have been categorized by the following key words:

1. Cancer
2. Non-cancer
3. Individual sensitivity
4. Radiation quality
5. Tissue sensitivity;
6. Internal emitters (contamination)
7. Epidemiology
8. Modelling
9. Non-targeted effects (bystander)

In addition to key word, the publications have also been categorized according to different tasks in work packages: WP5 Shape of dose response, WP6 Individual sensitivities and WP7 Non-cancer effects (for description of scientific content of each WP, see here).

In the list below, you can find the DoReMi publications listed in alphabetical order according to first author (collected by September 2015) as well as their abstracts, and indication on:
Key words: in red
DoReMi task number and institution: in green.

Please note that the numbering of the publications is for calculation purposes and is subject to change when new publications are added.

For simple listing of the DoReMi publications, with links to abstracts and the possibility to refine the list by key word, please see:
http://www.doremi-noe.net/doremi_scientific_information_center.html.

1. **Abou-El-Ardat**, K, Derradji H, de Vos W, de Meyer T, Bekaert S, van Criekinge W, Baatout S. : Response to low-dose X-irradiation is p53-dependent in a papillary thyroid carcinoma model system. Int J Oncol 2011, 39(6): 1429-1441. (Task 5.3; SCK-CEN)
Cancer

The link between high doses of radiation and thyroid cancer has been well established in various studies, as opposed to the effects of low doses. In this study, we investigated the effects of low-dose X-ray irradiation in a papillary thyroid carcinoma model with wild-type and mutated p53. A low dose of 62.5 mGy was enough to cause an upregulation of p16 and a decrease in the number of TPC-1 cells in the S phase, but not in the number of BCPAP p53-mutant cells. At a dose of 0.5 Gy, visible signs of senescence appeared only in the TPC-1 cells. We conclude that low doses of X-rays are enough to cause a change in cell cycle distribution, possibly p53-dependent p16 activation, but no significant apoptosis. Senescence requires higher doses of X-irradiation via a mechanism involving both p16 and p21.

2. **Abou-El-Ardat**, K, Monsieurs P, Anastasov N, Atkinson M, Derradji H, De Meyer T, Bekaert S, Van Criekinge W, Baatout S. : Low dose irradiation of thyroid cells reveals a unique transcriptomic and epigenetic signature in RET/PETC-positive cells.
Mutat Res . 2012, 731(1-2): 27-40. (Task 5.3; SCK-CEN)
Cancer

The high doses of radiation received in the wake of the Chernobyl incident and the atomic bombing of Hiroshima and Nagasaki have been linked to the increased appearance of thyroid cancer in the children living in the vicinity of the site. However, the data gathered on the effect of low doses of radiation on the thyroid remain limited. We have examined the genome wide transcriptional response of a culture of TPC-1 human cell line of papillary thyroid carcinoma origin with a RET/PTC1 translocation to various doses (0.0625, 0.5, and 4 Gy) of X-rays and compared it to response of thyroids with a RET/PTC3 translocation and against wild-type mouse thyroids irradiated with the same doses using Affymetrix microarrays. We have found considerable overlap at a high dose of 4 Gy in both RET/PTC-positive systems but no common genes at 62.5 mGy. In addition, the response of RET/PTC-positive system at all doses was distinct from the response of wild-type thyroids with both systems signaling down different pathways. Analysis of the response of microRNAs in TPC-1 cells revealed a radiation-responsive signature of microRNAs in addition to dose-responsive microRNAs. Our results point to the fact that a low dose of X-rays seems to have a significant proliferative effect on normal thyroids. This observation should be studied further as opposed to its effect on RET/PTC-positive thyroids which was subtle, anti-proliferative and system-dependent.

3. **Acheva A.,** Aerts A., Rombouts Ch., Baatout S., Salomaa S., Manda K., Hildebrandt G., Kämäräinen M. : Human 3-D tissue models in radiation biology: current status and future perspectives. Int.J. Radiat. Res., April 2014; 12(2):81-98. (Task 5.2; SCK-CEN, UROS, STUK)
Cancer
Non-cancer
Tissue sensitivity
Modeling

In this review, we discuss the use of a variety of 3-D models (particularly skin, lung, breast and endothelial) in radiobiological research and highlight the differences in responses compared to 2-D culturing conditions (monolayers). We review the characteristics of existing 3-D models and aim to point out the substantial advantages 3-DN cultures provide for modern radiobiology. In particular, they may facilitate the shift from the classical DNA damage and repair studies mainly carried out in monolayer cultures to the investigation of more generalized responses through pathway analysis and a systems biology approach. 3-D models are expected to be very informative for investigations on radiotherapy responses in addressing the low dose risk. However, the 3-D model systems are not easy to propagate and to standardize as monolayer cultures. Therefore, we discuss the problems and limitations of 3-D models and propose ways to overcome some of the problems.

4. **Aerts, A,** Impens, N, Baatout, S, Benotmane, M A, Camps, J, Dabin, J M, Derradji, H, Grosche, B, Horemans, N, Jourdain, J-R, Moreels, M, Perko, T, Quintens, R, Repussard, J, Rühm, W, Schneider, T, Struelens, L, Hardeman, F. : Joint research towards a better radiation protection - highlights of the Fifth MELODI Workshop. J. Radiol. Prot. 34 (2014) 931-956. (WP1-7; SCK-CEN, BfS, IRSN and HMGU)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Tissue sensitivity

Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

MELODI is the European platform dedicated to low-dose radiation risk research. From 7 October through 10 October 2013 the Fifth MELODI Workshop took place in Brussels, Belgium. The workshop offered the opportunity to 221 unique participants originating from 22 countries worldwide to update their knowledge and discuss radiation research issues through 118 oral and 44 poster presentations. In addition, the MELODI 2013 workshop was reaching out to the broader radiation protection community, rather than only the low-dose community, with contributions from the fields of radioecology, emergency and recovery preparedness, and dosimetry. In this review, we summarise the major scientific conclusions of the workshop, which are important to keep the MELODI strategic research agenda up-to-date and which will serve to establish a joint radiation protection research roadmap for the future.

5. **Alloni, D**, Campa A, Friedland W, Mariotti L, Ottolenghi A. : Track structure, radiation quality and initial radiobiological events: considerations based on the PARTRAC code experience. Int J Radiat Biol. 2012, 88 (1-2): 77-86. (Task 5.6; UNIPV, ISS and HMGU)
Cancer
Radiation quality
Modeling
Non-targeted effects (bystander)

Purpose: The role of track structures for understanding the biological effects of radiation has been the subject of research activities for decades. The physics that describes such processes is the core Monte Carlo codes, such as the biophysical PARTRAC (PARticle TRACks) code described in this review, which follow the mechanisms of radiation-matter interaction from the early stage. In this paper, a review of the track structure theory (and of its possible extension concerning non-DNA targets) is presented.
Materials and methods: The role of radiation quality and track structure is analyzed starting from the heavy ions results obtained with the biophysical Monte Carlo code PARTRAC (PARticles TRACks). PARTRAC calculates DNA damage in human cells based on the superposition of simulated track structures in liquid water to an 'atom-by-atom' model of human DNA.
Results: Calculation for DNA fragmentation compared with experimental data for different radiation qualities are illustrated. As an example, the strong dependence of the complexity of DNA damage on radiation track structure, and the very large production of very small DNA fragments (lower than 1 kbp (kilo base pairs) usually not detected experimentally) after high LET (high-Linear Energy Transfer) irradiation is shown. Furthermore, the possible importance of non-nuclear/non-DNA targets is discussed in case of cellular membrane and mitochondria.
Conclusions: The importance of the track structure is underlined, in particular the dependence of a given late cellular effect on the spatial distribution of DNA double-strand breaks (DSB) along the radiation track. These results show that the relative biological effectiveness (RBE) for DSB production can be significantly larger than 1. Moreover the cluster properties of high LET radiation may determine specific initial targets and damage evolution.

6. **Alloni, D**, Campa A, Belli M, Esposito G, Mariotti L, Liotta M, Friedland W, Paretzke H, Ottolenghi A. : Monte Carlo evaluation of DNA fragmentation spectra induced by different radiation qualities. Radiat Prot Dosimetry 2011, 143(2-4), 226-231. (Task 5.6; UNIPV, ISS and HMGU)
Cancer
Radiation quality
Modeling
Non-targeted effects (bystander)

The PARTRAC code has been developed constantly in the last several years. It is a Monte Carlo code based on an event-by-event description of the interactions taking place between the ionising radiation and liquid water, and in the present version simulates the transport of photons, electrons, protons, helium and heavier ions. This is combined with an atom-by-atom representation of the biological target, i.e. the DNA target model of a diploid human fibroblast in its interphase (genome of 6 Gigabase pairs). DNA damage is produced by the events of energy depositions, either directly, if they occur in the volume occupied by the sugar-phosphate backbone, or indirectly, if this volume is reached by radiation-induced radicals. This requires the determination of the probabilities of occurrence of DNA damage. Experimental data are essential for this determination. However, after the adjustment of the relevant parameters through the comparison of the simulation data with the DNA fragmentation induced by photon irradiation, the code has been used without further parameter adjustments, and the comparison with the fragmentation induced by charged particle beams has validated the code. In this paper, the results obtained for the DNA fragmentation induced by gamma rays and by charged particle beams of various LET are shown, with a particular attention to the production of very small fragments that are not detected in experiments.

7. **Alloni, D**, Campa A, Friedland W, Mariotti L, Ottolenghi A. : Integration of Monte Carlo Simulation with PFGE Experimental Data Yields Constant RBE of 2.3 for DNA Double-Strand Break Induction by Nitrogen Ions between 125 and 225 keV/μm LET. Radiat Res. 2013, 179 (6): 690-697. (Task 5.6; UNIPV, ISS and HMGU)
Cancer
Radiation quality
Modeling
Non-targeted effects (bystander)

The number of small radiation-induced DNA fragments can be heavily underestimated when determined from measurements of DNA mass fractions by gel electrophoresis, leading to a consequent underestimation of the initial DNA damage induction. In this study we reanalyzed the experimental results for DNA fragmentation and DNA double-strand break (DSB) yields in human fibroblasts irradiated with γ rays and nitrogen ion beams with linear energy transfer (LET) equal to 80, 125, 175 and 225 keV/μm, originally measured by Höglund et al. (Radiat Res 155, 818-825, 2001 and Int J Radiat Biol 76, 539-547, 2000). In that study the authors converted the measured distributions of fragment masses into DNA fragment distributions using mid-range values of the measured fragment length intervals, in particular they assumed fragments with lengths in the interval of 0-48 kbp had the mid-range value of 24 kbp. However, our recent detailed simulations with the Monte Carlo code PARTRAC, while reasonably in agreement with the mass distributions, indicate significantly increased yields of very short fragments by high-LET radiation, so that the actual average fragment lengths, in the interval 0-48 kbp, 2.4 kbp for 225 keV/μm nitrogen ions were much shorter than the assumed mid-range value of 24 kbp. When the measured distributions of fragment masses are converted into fragment distributions using the average fragment lengths calculated by PARTRAC, significantly higher yields of DSB related to short fragments were obtained and resulted in a constant relative biological effectiveness (RBE) for DSB induction yield of 2.3 for nitrogen ions at 125-225 keV/μm LET. The previously reported downward trend of the RBE values over this LET range for DSB induction appears to be an artifact of an inadequate average fragment length in the smallest interval.

8. **Babini G.,** Ugolini M, Morini J., Baiocco G., Mariotti L., Tabarelli de Fatis P., Liotta M., Ottolenghi A.: Investigation of radiation-induced multilayered signalling response of the inflammatory pathway. Rdiation Prot. Dosim. April 15, 2015, pp.1-4 (Task 5.2, UNIPV)
Cancer
Non-targeted effects (bystander)

Ionising radiation exposure of cells might induce the perturbation of cell functions and, in particular, the activation or inhibition of several important pathways. This perturbation can cause the deregulation of both intra- and extra-cellular signalling cascades (such as the inflammatory pathway) and alter not only the behaviour of directly exposed cells but also the neighbouring non-irradiated ones, through the so-called bystander effect. The aim of the present work was to investigate the complex nonlinear interactions between the inflammatory pathway and other strictly interlaced signalling pathways, such as Erk1/2 and Akt/PKB, focusing on the radiation-induced perturbation of such pathways in the dose range of 0-2 Gy. The results show how radiation affects these interconnected pathways and how confounding factors, such as the change of culture medium, can hide radiation-induced perturbations.

9. **Belli, M**, Salomaa S, Ottolenghi A. : MELODI: the 'Multidisciplinary European Low-Dose Initiative'. Radiat Prot. Dosimetry 2011, 143(2-4): 330-334. (WP1-7; ISS, STUK and UNIPV)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Tissue sensitivity
Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

The importance of research to reduce uncertainties in risk assessment of low and protracted exposures is now recognised globally. In Europe a new initiative, called 'Multidisciplinary European LOw Dose Initiative' (MELODI), has been proposed by a 'European High Level and Expert Group on low-dose risk research' (www.hleg.de), aimed at integrating national and EC (Euratom) efforts. Five national organisations: BfS (DE), CEA (FR), IRSN (FR), ISS (IT) and STUK (FI), with the support of the EC, have initiated the creation of MELODI by signing a letter of intent. In the forthcoming years,
MELODI will integrate in a step-by-step approach EU institutions with significant programmes in the field and will be open to other scientific organisations and stakeholders. A key role of MELODI is to develop and maintain over time a strategic research agenda (SRA) and a road map of scientific priorities within a multidisciplinary approach, and to transfer the results for the radiation protection system. Under the coordination of STUK a network has been proposed in the 2009 Euratom Programme, called DoReMi (Low-Dose Research towards Multidisciplinary Integration), which can help the integration process within the MELODI platform. DoReMi and the First MELODI Open Workshop, organised by BfS in September 2009, are now important inputs for the European SRA.

10. **Belli, M**, Tabocchini, M.A., Jourdain, J-R, Salomaa, S and Repussard, J.: The European Initiative on low-dose risk research: From the HLEG to MELODI. Radiat Prot Dosimetry (2015) doi: 10.1093/rpd/ncv136 (early online). (WP1-7; ISS, IRSN and STUK)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Tissue sensitivity
Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

The importance of low-dose risk research for radiation protection is now widely recognised. The

European Commission (EC) and five European Union (EU) Member States involved in the Euratom Programme set up in 2008 a 'High Level and Expert Group on European Low Dose Risk Research' (HLEG) aimed at identifying research needs and proposing a better integration of European efforts in the field. The HLEG revised the research challenges and proposed a European research strategy based on a 'Multidisciplinary European LOw Dose Initiative' (MELODI). In April 2009, five national organisations, with the support of the EC, created the initial core of MELODI (http://www.melodi-online.eu) with a view to integrate the EU institutions with significant programmes in the field, while being open to other scientific organisations and stakeholders, and to develop an agreed strategic research agenda (SRA) and roadmap. Since then, open workshops have been organised yearly, exploring ideas for SRA implementation. As of October 2014, 31 institutions have been included as members of MELODI. HLEG recommendations and MELODI SRA have become important reference points in the radiation protection part of the Euratom Research Programme. MELODI has established close interactions through Memorandum of Understanding with other European platforms involved in radiation protection (Alliance, NERIS and EURADOS) and, together with EURADOS, with the relevant medical European Associations. The role of Joint Programming in priority setting, foreseen in the forthcoming EU Horizon 2020, calls for keeping MELODI an open, inclusive and transparent initiative, able to avoid redundancies and possible conflicts of interest, while promoting common initiatives in radiation protection research. An important issue is the establishment of a proper methodology for managing these initiatives, and this includes the set-up of an independent MELODI Scientific Committee recently extended to Alliance, NERIS and EURADOS, with the aim of identifying research priorities to suggest for the forthcoming Euratom research calls.

11. **Brown, N,** Finnon, R, Manning, G, Bouffler, S and Badie, C.: Influence of radiation quality on mouse chromosome 2 deletions inradiation-induced acute myeloid leukaemia. Influence of radiation quality on mouse chromosome 2 deletions in radiation-induced acute myeloid leukaemia. Mutat. Res.: Genet. Toxicol. Environ. Mutagen. (2015). (Task 5.3; DH-PHE).
Cancer
Radiation quality

Leukaemia is the prevailing neoplastic disorder of the hematopoietic system. Epidemiological analy-ses of the survivors of the Japanese atomic bombings show that exposure to ionising radiation (IR) cancause leukaemia. Although a clear association between radiation exposure and leukaemia developmentis acknowledged, the underlying mechanisms remain incompletely understood. A hemizygous deletionon mouse chromosome 2 (del2) is a common feature in several mouse strains susceptible to radiation-induced acute myeloid leukaemia (rAML). The deletion is an early event detectable 24 h after exposurein bone marrow cells. Ultimately, 15–25% of exposed animals develop AML with 80–90% of cases carry-ing del2. Molecular mapping of leukaemic cell genomes identified a minimal deleted region (MDR) onchromosome 2 (chr2) in which a tumour suppressor gene, Sfpi1 is located, encoding the transcriptionfactor PU.1, essential in haematopoiesis. The remaining copy of Sfpi1 has a point mutation in the codingsequence for the DNA-binding domain of the protein in 70% of rAML, which alters a single CpG sequencein the codon for arginine residue R235. In order to identify chr2 deletions and Sfpi.1/PU.1 loss, we per-formed array comparative genomic hybridization (aCGH) on a unique panel of 79rAMLs. Using a custommade CGH array specifically designed for mouse chr2, we analysed at unprecedentedly high resolution(1.4M array- 148 bp resolution) the size of the MDR in low LET and high-LET induced rAMLs (32 X-ray-and 47 neutron-induced). Sequencing of Sfpi1/PU.1DNA binding domain identified the presence of R235point mutations, showing no influence of radiation quality on R235 type or frequency. We identified for the first time rAML cases with complex del2 in a subset of neutron-induced AMLs. This study allowed us to re-define the MDR to a much smaller 5.5Mb region (still including Sfpi1/PU.1), identical regardless ofradiation quality.

12. **Campa, A**, Balduzzi M, Dini V, Esposito G, Tabocchini MA.: The complex interactions between radiation induced non-targeted effects and cancer. Cancer Letters 2013, Oct 1,: pages 1-11. (Task 5.2; ISS)
Cancer
Radiation quality
Non-targeted effects (bystander)

Radiation induced non-targeted effects have been widely investigated in the last two decades for their potential impact on low dose radiation risk. In this paper we will give an overview of the most relevant aspects related to these effects, starting from the definition of the low dose scenarios. We will underline the role of radiation quality, both in terms of mechanisms of interaction with the biological matter and for the importance of charged particles as powerful tools for low dose effects investigation. We will focus on cell communication, representing a common feature of non-targeted effects, giving also an overview of cancer models that have explicitly considered such effects.

13. **Dieriks, B**, De Vos W, Baatout S, Van Oostveldt P.: Repeated exposure of human fibroblasts to ionizing radiation reveals and adaptive response that is not mediated by interleukin-6 or TGF-beta. Mutat. Res . 2011,Oct 1, 715(1-2): 19-24. (Task 5.2.1; SCK-CEN)
Cancer
Non-targeted effects (bystander)

Exposing cells to a low dose can protect them against a subsequent higher exposure. This phenomenon is known as adaptive response and is frequently observed in a variety of cells. Even though similarities are suspected with other non-targeted effects, such as bystander effects, the exact mechanism behind adaptive response is not fully clarified.
In this study human primary fibroblasts were tested for their response to ionizing radiation (IR) after administrating a low priming dose (0.1–0.5 Gy). Both the abundance of γH2AX as a marker for double-stranded breaks and the levels of cytokines, secreted in the medium, were monitored in time. Upon challenge, IR-primed cells showed modified γH2AX spot size distributions and altered repair kinetics, consistent with an adaptive response. In addition, 24 h after priming with IR, four cytokines were significantly upregulated in the medium – GM-CSF (1.33×); IL6 (4.24×); IL8 (1.33×); TGF-β (1.46×). In order to mimic the protective effect of IR priming, we primed the cells with either IL6 or TGF-β. This did not elicit an altered γH2AX response as observed in IR-primed cells, indicating that the adaptive response in these primary fibroblasts is regulated in an IL-6 and TGF-β independent manner.

14. **Drexler, G A,** Siebenwirth, C, Drexler, S E, Girst, S, Greubel, C, Dollinger, G and Friedl, A A.: Live cell imaging at the Munich ion microbeam SNAKE – a status report. Radiation Oncology (2015) 10:42. (Task 4.9; LMU)
Cancer
Radiation quality

Ion microbeams are important tools in radiobiological research. Still, the worldwide number of ion microbeam facilities where biological experiments can be performed is limited. Even fewer facilities combine ion microirradiation with live-cell imaging to allow microscopic observation of cellular response reactions starting very fast after irradiation and continuing for many hours. At SNAKE, the ion microbeam facility at the Munich 14 MV tandem accelerator, a large variety of biological experiments are performed on a regular basis. Here, recent developments and ongoing research projects at the ion microbeam SNAKE are presented with specific emphasis on live-cell imaging experiments. An overview of the technical details of the setup is given, including examples of suitable biological samples. By ion beam focusing to submicrometer beam spot size and single ion detection it is possible to target subcellular structures with defined numbers of ions. Focusing of high numbers of ions to single spots allows studying the influence of high local damage density on recruitment of damage response proteins.

15. **Drubay D,** Ancelet S, Acker A, Kreuzer M, Laurier D, Rage E.: Kidney cancer mortality and ionizing radiation among French and German uranium miners. Radiat Environ Biophys. 2014 Aug;53(3):505-13. (Task 5.5; IRSN and BfS)
Cancer
Individual sensitivity
Radiation quality
Epidemiology
Modeling

The investigation of potential adverse health effects of occupational exposures to ionizing radiation, on uranium miners, is an important area of research. Radon is a well-known carcinogen for lung, but the link between radiation exposure and other diseases remains controversial, particularly for kidney cancer. The aims of this study were therefore to perform external kidney cancer mortality analyses and to assess the relationship between occupational radiation exposure and kidney cancer mortality, using competing risks methodology, from two uranium miners cohorts. The French (n = 3,377) and German (n = 58,986) cohorts of uranium miners included 11 and 174 deaths from kidney cancer. For each cohort, the excess of kidney cancer mortality has been assessed by standardized mortality ratio (SMR) corrected for the probability of known causes of death. The associations between cumulative occupational radiation exposures (radon, external gamma radiation and long-lived radionuclides) or kidney equivalent doses and both the cause-specific hazard and the probability of occurrence of kidney cancer death have been estimated with Cox and Fine and Gray models adjusted to date of birth and considering the attained age as the timescale. No significant excess of kidney cancer mortality has been observed neither in the French cohort (SMR = 1.49, 95 % confidence interval [0.73; 2.67]) nor in the German cohort (SMR = 0.91 [0.77; 1.06]). Moreover, no significant association between kidney cancer mortality and any type of occupational radiation exposure or kidney equivalent dose has been observed. Future analyses based on further follow-up updates and/or large pooled cohorts should allow us to confirm or not the absence of association.

16. **Ebrahimian T,** Le Gallic C, Stefani J, Dublineau I, Yentrapalli R, Harms-Ringdahl M, Haghdoost S.: Chronic Gamma-Irradiation Induces a Dose-Rate-Dependent Pro-inflammatory Response and Associated Loss of Function in Human Umbilical Vein Endothelial Cells. Radiat. Res. 2015, April 183 (4) 447-454. (Task 7.3; IRSN, HMGU and SU)
Non-cancer
Radiation quality
Tissue sensitivity

A central question in radiation protection research is dose and dose-rate relationship for radiation-induced cardiovascular diseases. The response of endothelial cells to different low dose rates may contribute to help estimate risks for cardiovascular diseases by providing mechanistic understanding. In this study we investigated whether chronic low-dose-rate radiation exposure had an effect on the inflammatory response of endothelial cells and their function. Human umbilical vein endothelial cells (HUVECs) were chronically exposed to radiation at a dose of 1.4 mGy/h or 4.1 mGy/h for 1, 3, 6 or 10 weeks. We determined the pro-inflammatory profile of HUVECs before and during radiation exposure, and investigated the functional consequences of this radiation exposure by measuring their capacity to form vascular networks in matrigel. Expression levels of adhesion molecules such as E-selectin, ICAM-1 and VCAM-1, and the release of pro-inflammatory cytokines such as MCP-1, IL-6 and TNF-α were analyzed. When a total dose of 2 Gy was given at a rate of 4.1 mGy/h, we observed an increase in IL-6 and MCP-1 release into the cell culture media, but this was not observed at 1.4 mGy/h. The increase in the inflammatory profile induced at the dose rate of 4.1 mGy/h was also correlated with a decrease in the capacity of the HUVECs to form a vascular network in matrigel. Our results suggest that dose rate is an important parameter in the alteration of HUVEC inflammatory profile and function

17. **Eidemüller, M**, Jacob P, Lane RS, Frost SE, Zablotska LB.: Lung cancer mortality (1950-1999) among Eldorado uranium workers: A comparison of models of carcinogenesis and empirical excess risk models. PLoS One, 2012, 7 (8): e4131. (Task 5.4; HMGU)
Cancer
Epidemiology
Modeling

Lung cancer mortality after exposure to radon decay products (RDP) among 16,236 male Eldorado uranium workers was analyzed. Male workers from the Beaverlodge and Port Radium uranium mines and the Port Hope radium and uranium refinery and processing facility who were first employed between 1932 and 1980 were followed up from 1950 to 1999. A total of 618 lung cancer deaths were observed. The analysis compared the results of the biologically-based two-stage clonal expansion (TSCE) model to the empirical excess risk model. The spontaneous clonal expansion rate of pre-malignant cells was reduced at older ages under the assumptions of the TSCE model. Exposure to RDP was associated with increase in the clonal expansion rate during exposure but not afterwards. The increase was stronger for lower exposure rates. A radiation induced bystander effect could be a possible explanation for such an exposure response. Results on excess risks were compared to a linear dose-response parametric excess risk model with attained age, time since exposure and dose rate as effect modifiers. In all models the excess relative risk decreased with increasing attained age, increasing time since exposure and increasing exposure rate. Large model uncertainties
were found in particular for small exposure rates.

18. **Farah, J**, Struelens L, Dabin J, Koukorava C, Donadille L, Jacob S, Schnelzer M, Auvinen A, Vanhavere F, Clairand I.: A correlation study of eye lens dose and personal dose equivalent for interventional cardiologists. Radiat Prot Dosimetry 2013, Dec, 157(4):561-569. (Task 7.4; IRSN, SCK-CEN and STUK)
Non-cancer

This paper presents the dosimetry part of the European ELDO project, funded by the DoReMi Network of Excellence, in which a method was developed to estimate cumulative eye lens doses for past practices based on personal dose equivalent values, H(p)(10), measured above the lead apron at several positions at the collar, chest and waist levels. Measurement campaigns on anthropomorphic phantoms were carried out in typical interventional settings considering different tube projections and configurations, beam energies and filtration, operator positions and access routes and using both mono-tube and biplane X-ray systems. Measurements showed that eye lens dose correlates best with H(p)(10) measured on the left side of the phantom at the level of the collar, although this correlation implicates high spreads (41 %). Nonetheless, for retrospective dose assessment, H(p)(10) records are often the only option for eye dose estimates and the typically used chest left whole-body dose measurement remains useful.

19. **Flockerzi, E.,** Schanz S, Rübe CE.: Even low doses of radiation lead to DNA damage accumulation in lung tissue according to the genetically-defined DNA repair capacity. Radiotherapy and Oncology, 2014, May, 111(2): 212-218. (Task 6.1; USAAR)
Individual sensitivity
Tissue sensitivity

BACKGROUND AND PURPOSE: Intensity-modulated radiation therapy for thoracic malignancies increases the exposure of healthy lung tissue to low-dose radiation. The biological impact of repetitive low-dose radiation on the radiosensitive lung is unclear. MATERIALS AND METHODS:

In the present study, using mouse strains with different genetic DNA repair capacities, we monitored the extent of DNA damage in lung parenchyma after 2, 4, 6, 8, and 10weeks of daily low-dose 100-mGy radiation. RESULTS: Using 53BP1 as a marker for double-strand breaks, we observed DNA damage accumulation during fractionated low-dose radiation with increasing cumulative doses. The amount of radiation-induced 53BP1 varied significantly between bronchiolar and alveolar epithelial cells, suggesting that different cell populations in the lung parenchyma had varying vulnerabilities to ionizing radiation. The genetic background of DNA repair determined the extent of cumulative low-dose radiation damage. Moreover, increased DNA damage during fractionated low-dose radiation affected replication, and apoptosis in the lung parenchyma, which may influence overall lung function. CONCLUSION: Collectively, our results suggest that low, yet damaging, doses of radiation increase the risk of toxicity to normal lung tissue and the probability of developing secondary malignancies.

20. **Frey B**, Rubner Y, Wunderlich R, Weiss EM, Pockley AG, Fietkau R, Gaipl US.: Induction of abscopal anti-tumor immunity and immunogenic tumor cell death by ionizing irradiation-implications for cancer therapies. Curr. Medicinal Chemistry 2012, 19 (12), 1751-1764. (Task 5.2.1; UKER)
Cancer
Non-targeted effects (bystander)

Although cancer progression is primarily driven by the expansion of tumor cells, the tumor microenvironment and anti-tumor immunity also play important roles. Herein we consider how tumors can become established by escaping immune surveillance and also how cancer cells can be rendered visible to the immune system by standard therapies such as radiotherapy or chemotherapy, either alone or in combination with additional immune stimulators.
Although local radiotherapy results in DNA damage (targeted effects), it is also capable of inducing immunogenic forms of tumor cell death which are associated with a release of immune activating danger signals (non-targeted effects), such as necrosis. Necrotic tumor cells may result from continued exposure to death stimuli and/or an impaired phosphatidylserine (PS) dependent clearance of the dying tumor cells. In such circumstances, mature dendritic cells take up tumor antigen and mediate the induction of adaptive and innate anti-tumor immunity. Locally-triggered, systemic immune activation can also lead to a spontaneous regression of tumors or metastases that are outside the radiation field - an effect which is termed abscopal. Preclinical studies have demonstrated that combining radiotherapy with immune stimulation can induce anti-tumor immunity. Given that it takes time for immunity to develop following exposure to immunogenic tumor cells, we propose practical combination therapies that should be considered as a basis for future research and clinical practice. It is essential that radiation oncologists become more aware of the importance of the immune system to the success of cancer therapy.

21. **Frey, B**, Weiss EM, Rubner Y, Wunderlich R, Ott OJ, Sauer R, Fietkau R, Gaipl US.: Old and new facts about hyperthermia-induced modulation of the immune system. Int J Hyperthermia 2012, 28(6): 528—542. (Task 5.2.1; UKER)
Cancer
Non-targeted effects (bystander)

Hyperthermia (HT) is a potent sensitiser for radiotherapy (RT) and chemotherapy (CT) and has been proven to modulate directly or indirectly cells of the innate and adaptive immune system. We will focus in this article on how anti-tumour immunity can be induced by HT. In contrast to some in vitro assays, in vivo examinations showed that natural killer cells and phagocytes like granulocytes are directly activated against the tumour by HT. Since heat also activates dendritic cells (DCs), HT should be combined with further death stimuli (RT, CT or immune therapy) to allocate tumour antigen, derived from, for example, necrotic tumour cells, for uptake by DCs. We will outline thatinduction of immunogenic tumour cells and direct tumour cell killing by HT in combination with other therapies contributes to immune activation against the tumour. Studies will be presented showing that non-beneficial effects of HT on immune cells are mostly timely restricted. A special focus is set on immune activation mediated by extracellular present heat shock proteins (HSPs) carrying tumour antigens and further danger signals released by dying tumour cells. Local HT treatment in addition to further stress stimuli exerts abscopal effects and might be considered as*in situ* tumour vaccination. An increased natural killer (NK) cell activity, lymphocyte infiltration and HSP-mediated induction of immunogenic tumour cells have been observed in patients. Treatments with the addition of HT therefore can be considered as a personalised cancer treatment approach by specifically activating the immune system the individual unique tumour.

22. **Frey, B**, Stache C, Rubner Y, Werthmöller N, Schulz K, Sieber R, Semrau S, Rödel F, Fietkau R, Gaipl US.: Combined treatment of human colorectal tumor cell lines with chemotherapeutic agents and ionizing irradiation can in vitro induce tumor cell death forms with immunogenic potential. J. Immunotoxicology 2012, 9(3): 301-313. (Task 5.2.1; UKER and GUF)
Cancer
Non-targeted effects (bystander)

Chemotherapeutic agents (CT) and ionizing radiation (X-ray) induce DNA damage and primarily aim to stop the proliferation of tumor cells. However, multimodal anti-cancer therapies should finally result in tumor cell death and, best, in the induction of systemic anti-tumor immunity. Since distinct therapy-induced tumor cell death forms may create an immune activating tumor microenvironment, this study examined whether sole treatment with CT that are used in the therapy for colorectal cancer or in combination with X-ray result in colorectal tumor cell death with immunogenic potential. 5-Fluorouracil (5-FU), Oxaliplatin (Oxp), or Irinotecan (Irino) in combination with X-ray were all potent inhibitors of colorectal tumor cell colony formation. This study then examined the forms of cell death with AnnexinA5-FITC/Propidium Iodide staining. Necrosis was the prominent form of cell death induced by CT and/or X-ray. While only a combination of Irino with X-ray leads to death induction already 1 day after treatment, also the combinations of Oxp or 5-FU with X-ray and X-ray alone resulted in high necrosis rates at later time points after treatment. Inhibition of apoptosis increased the amount of necrotic tumor cells, suggesting that a programmed form of necrosis can be induced by CT + X-ray. 5-FU and Oxp alone or in combination with X-ray and Irino plus X-ray were most effective in increasing the expression of RIP, IRF-5, and p53, proteins involved in necrotic and apoptotic cell death pathways. All treatments further resulted in the release of the immune activating danger signals high-mobility group box 1 (HMGB1) and heat shock protein 70 (HSP70). The supernatants of the treated tumor cells induced maturation of dendritic cells. It is, therefore, concluded that combination of CT with X-ray is capable of inducing *in vitro* cell death forms of colorectal tumors with immunogenic potential.

23. **Frey, B**, Rubner Y, Kulzer L, Werthmöller N, Weiss EM, Fietkau R, Gaipl US.: Antitumor immune responses induced by ionizing irradiation and further immune stimulation. Cancer Immunol Immunother 2014, 63(1): 29-36. (Task 5.2.1; UKER)
Cancer
Non-targeted effects (bystander)

The therapy of cancer emerged as multimodal treatment strategy. The major mode of action of locally applied radiotherapy (RT) is the induction of DNA damage that triggers a network of events that finally leads to tumor cell cycle arrest and cell death. Along with this, RT modifies the phenotype of the tumor cells and their microenvironment. Either may contribute to the induction of specific and systemic antitumor immune responses. The latter are boosted when additional immune therapy (IT) is applied at distinct time points during RT. We will focus on therapy-induced necrotic tumor cell death that is immunogenic due to the release of damage-associated molecular patterns. Immune-mediated distant bystander (abscopal) effects of RT when combined with dendritic cell-based IT and the role of fractionation of radiation in the induction of immunogenic tumor cell death will be discussed. Autologous whole-tumor-cell-based vaccines generated by high hydrostatic pressure technology will be introduced and the influence of cytokines and the immune modulator AnnexinA5 on the ex vivo generated or in situ therapy-induced vaccine efficacy will be outlined. RT should be regarded as immune adjuvant for metastatic disease and as a tool for the generation of an in situ vaccine when applied at distinct fractionation doses or especially in combination with IT to generate immune memory against the tumor. To identify the most beneficial combination and chronology of RT with IT is presumably one of the biggest challenges of innovative tumor research and therapies.

24. **Frischholz, B**, Wunderlich R., Rühle PF, Schorn C., Rödel F., Keilholz L., Fietkau R., Gaipl US, Frey B.: Reduced secretion of the inflammatory cytokine IL-1β by stimulated peritoneal macrophages of radiosensitive Balb/c mice after exposure to 0.5 or 0.7 Gy of ionizing radiation. Autoimmunity 2013, 46(5): 323-328. (Tasks 5.2.1 and 7.6; UKER and GUF)
Cancer
Non-targeted effects (bystander)

Since the beginning of the 20th century, low dose radiotherapy (LD-RT) has been practiced and established as therapy of inflammatory diseases. Several clinical studies already have proven the anti-inflammatory effect of low doses of ionizing irradiation (LDR). However, further research is inevitable to reveal the underlying immune- biological mechanisms. Focus has been set on the modulation of activated macrophages by LDR, since they participate in both, initiation and resolution of inflammation. Here we examined with an *ex vivo* peritoneal mouse macrophage model how LDR modulates the secretion of the inflammatory cytokines IL-1β and TNF-α by activated macrophages and whether the basal radiosensitivity of the immune cells has influence on it. Peritoneal macrophages of Balb/c mice responded to exposure of 0.5 or 0.7 Gy of ionizing irradiation (X-ray) with significant decreased release of IL-1β and slightly, but not significantly, reduced release of TNF-α. Macrophages of the less radiosensitive C57BL/6 mice did not show this anti-inflammatory reaction. This was observed in both wild type and human TNF-α transgenic animals with C57BL/6 background. We conclude that only the inflammatory phenotype of more radiosensitive macrophages is reduced by LDR and that *ex vivo* and *in vivo* models with primary cells should be applied to examine how the immune system is modulated by LDR.

25. **Gaipl US,** Multhoff G, Scheithauer H, Laubor K, Hehlgans S, Frey B, Rödel F.: Kill and spread the word: stimulation of antitumor immune responses in the context of radiotherapy. Immunotherapy 2014, 6(5): 597-610. (Task 5.2.1; UKER and GUF)
Cancer
Non-targeted effects (bystander)

Besides the direct, targeted effects of ionizing irradiation (x-ray) on cancer cells, namely DNA damage and cell death induction, indirect, non-targeted ones exist, which are mediated in large part by the immune system. Immunogenic forms of tumor cell death induced by x-ray, including immune modulating danger signals like the heat shock protein 70, adenosine triphosphate, and high-mobility group box 1 protein are presented. Further, antitumor effects exerted by cells of the innate (natural killer cells) as well as adaptive immune system (T cells activated by dendritic cells) are outlined. Tumor cell death inhibiting molecules such as survivin are introduced as suitable target for molecularly tailored therapies in combination with x-ray. Finally, reasonable combinations of immune therapies with radiotherapy are discussed.

26. **Gerard, AC,** Humblet K, Wilvers C, Poncin S, Derradji H, de Ville de Goyet C, Abou-el-Ardat K, Baatout S, Sonveaux P, Denef JF, Colin IM.: Iodine-deficiency-induced long lasting

angiogenic reaction in thyroid cancers occurs via vascular endothelial growth factor-hypoxia inducible factor-1-dependent, but not a reactive oxygen species-dependent pathway. Thyroid 2012, 22(7), 699-708. (Task 7.3; SCK-CEN)
Cancer
Non-cancer

Background: In the thyroid, iodine deficiency (ID) induces angiogenesis via a tightly controlled reactive oxygen species (ROS)–hypoxia inducible factor-1 (HIF-1)–vascular endothelial growth factor (VEGF) dependent pathway (ROS-HIF-VEGF). Deficient iodine intake may be associated with increased thyroid cancer incidence. The hypothesis of this work is to test whether ID affects the angiogenic processes in thyroid malignant cells by altering the ROS-HIF-VEGF pathway. ***Methods:*** Goiters were obtained in RET/PTC3 transgenic and wild-type (wt) mice and ID was induced in three thyroid carcinoma cell lines (TPC-1, 8305c, and R082-w1). Thyroid blood flow, VEGF mRNA and protein, and HIF-1α protein expression were measured. The role of HIF-1 and of ROS was assessed using echinomycin and N-acetylcysteine (NAC), respectively. ***Results:*** The goitrogen treatment increased the thyroid blood flow in wt and RET/PTC3 mice. Compared with wt mice, basal VEGF expression was higher in RET/PTC3 mice and increased with goitrogen treatment. In the three cell lines, ID induced marked increases in VEGF mRNA, and moderate increases in HIF-1α protein expression that were not transient as in normal cells. ID-induced VEGF mRNA expression was fully (8305c), partially (TPC-1), or not (R082-w1) blocked by echinomycin. NAC had no effect on ID-induced VEGF mRNA and HIF-1α protein expression in the three cell lines. ***Conclusions:*** ID induces a long lasting angiogenic phenotype in thyroid cancer cells that occurs through VEGF induction via a pathway partially mediated by HIF-1, but not by ROS. These results suggest that, in contrast with normal cells, ID-induced angiogenesis in cancer cells occurs via alternative and likely less controlled routes, thereby leading to uncontrolled growth.

27. **Grewenig, A,** Schuler, N, Rübe, CE.: Persistent DNA Damage in Spermatogonial Stem Cells After Fractionated Low-Dose Irradiation of Testicular Tissue. Int J Radiat Oncol Biol Phys. 2015 Aug 1;92(5):1123-31. (Task 6.1 and 6.10; USAAR)
Tissue sensitivity

PURPOSE: Testicular spermatogenesis is extremely sensitive to radiation-induced damage, and even low scattered doses to testis from radiation therapy may pose reproductive risks with potential treatment-related infertility. Radiation-induced DNA double-strand breaks (DSBs) represent the greatest threat to the genomic integrity of spermatogonial stem cells (SSCs), which are essential to maintain spermatogenesis and prevent reproduction failure. METHODS AND MATERIALS: During daily low-dose radiation with 100 mGy or 10 mGy, radiation-induced DSBs were monitored in mouse testis by quantifying 53 binding protein 1 (53BP-1) foci in SSCs within their stem cell niche. The accumulation of DSBs was correlated with proliferation, differentiation, and apoptosis of testicular germ cell populations. RESULTS: Even very low doses of ionizing radiation arrested spermatogenesis, primarily by inducing apoptosis in spermatogonia. Eventual recovery of spermatogenesis depended on the survival of SSCs and their functional ability to proliferate and differentiate to provide adequate numbers of differentiating spermatogonia. Importantly, apoptosis-resistant SSCs resulted in increased 53BP-1 foci levels during, and even several months after, fractionated low-dose radiation, suggesting that surviving SSCs have accumulated an increased load of DNA damage. CONCLUSIONS: SSCs revealed elevated levels of DSBs for weeks after radiation, and if these DSBs persist through differentiation to spermatozoa, this may have severe consequences for the genomic integrity of the fertilizing sperm.

28. **Guertler, A,** Hauptmann, M. Pautz, S, Kulka, U, Friedl, A A, Lehr, S, Hornhardt, S, M. Gomolka, M.: The inter-individual variability outperforms the intra-individual variability of differentially expressed proteins prior and post irradiation in lymphoblastoid cell lines. Arch

Physiol Biochem December 2014, Vol. 120, No. 5, 198-207. (Task 6.2; BfS and LMU)
Cancer
Individual sensitivity
Radiation quality

CONTEXT: Radio-sensitivity in normal tissue is characterized by heterogeneity throughout the population and the absence of pre-diagnostic biomarkers. OBJECTIVE: We conducted a proteomic approach to search for radiation characteristic protein regulation. MATERIALS AND METHODS: Cell lines were 10 Gy irradiated and analysed by 2D-DIGE after 24 h. RESULTS were analysed intra- and inter-individually. The principal component analysis and hierarchical clustering was applied to all datasets. RESULTS: Differences in intra-individual spot abundance prior and post irradiation exactly show the separation of sample classes in two groups: sham-irradiated and irradiated. The inter-individual datasets clustered according to the cell line. The intra-individual differences on protein level after gamma-irradiation are very low, compared with the inter-individual differences among cell lines derived from the same tissue. CONCLUSION: The application of 2-D DIGE may offer a realistic chance for a better molecular characterization of radio-sensitivity and for the discovery of candidate biomarkers.

29. **Kabacik, S,** Manning, G, Raffy, C, Bouffler, S and Badie, C.: Time, Dose and Ataxia Telangiectasia Mutated (ATM) Status Dependency of Coding and Noncoding RNA Expression after Ionizing Radiation Exposure. Radiation Research, 183(3):325-337. (Tasks 5.1 and 5.2; DH-PHE)
Cancer
Individual sensitivity

Studies of gene expression have proved important in defining the molecular mechanisms of radiation action and identifying biomarkers of ionizing radiation exposure and susceptibility. The full transcriptional response to radiation is very complex since it also involves epigenetic mechanisms triggered by radiation exposure such as modifications of expression of noncoding RNA such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) that have not been fully characterized. To improve our understanding of the transcriptional response to radiation, we simultaneously monitored the expression of ten protein-coding genes, as well as 19 miRNAs and 3 lncRNAs in a time- and dose-dependent manner in stimulated human T lymphocytes obtained from two healthy donors (C1 and C2) and one patient with ataxia telangiectasia (AT), which is a well characterized radiosensitivity disorder. After 2 Gy X irradiation, expression levels were monitored at time points ranging from 15 min up to 24 h postirradiation. The majority of genes investigated responded rapidly to radiation exposure, with the peak up-regulation (CDKN1A, SESN1, ATF3, MDM2, PUMA and GADD45A) or down-regulation (CCNB1) occurring 2-3 h postirradiation, while DDB2, FDXR and CCNG1 responded with slower kinetics reaching a peak of expression between 5 and 24 h. A significant modification of expression after radiation exposure was observed for miR-34a-5p and miR-182-5p, with an up-regulation occurring at late time points reaching two to threefold at 24 h. Differences between two donors in miR-182-5p response to radiation were detected: for C2, up-regulation reached a plateau-phase around 5 Gy, while for C1, up-regulation was at its maximum around 3 Gy and then decreased at higher doses. Among the three lncRNAs studied, TP53TG1 demonstrated a weak up-regulation, reaching a maximum of 1.5-fold at 24 h after radiation exposure. Conversely, FAS-AS1 was up-regulated up to fivefold by 5 Gy irradiation. Our results indicate that expression of the majority of protein-coding genes allows discrimination of the AT from healthy donors when analyzed at 2 h. However, differences in expression between AT and healthy donors are no longer detectable 24 h postirradiation although, interestingly, linear dose responses for some of the genes studied are obtained at this time point. Furthermore, our study shows that miRNAs miR-34a-5p and miR-182-5p are responsive to radiation exposure in a dose- and time-dependent manner. Additionally, to the best of our knowledge, this is the first study to report that FAS-AS1 lncRNA is up-regulated by radiation exposure in an ATM-dependent fashion in human T lymphocytes.

30. **Kreuzer M**, Dufey F, Laurier D, Nowak D, Marsh JW, Schnelzer M, Sogl M, Walsh L.: Mortality from internal and external radiation exposure in a cohort of male German uranium millers, 1946-2008. Int Arch Occup Environ Health, 2015 May 88 (4) 431-441. (Tasks 5.5 and 5.8; BfS, IRSN and DH-PHE)
Cancer
Non-cancer
Radiation quality
Internal emitters (contamination)
Epidemiology

PURPOSE: To examine exposure-response relationships between ionizing radiation and several mortality outcomes in a subgroup of 4,054 men of the German uranium miner cohort study, who worked between 1946 and 1989 in milling facilities, but never underground or in open pit mines.METHODS: Mortality follow-up was from 1946 to 2008, accumulating 158,383 person-years at risk. Cumulative exposure to radon progeny in working level months (WLM) (mean = 8, max = 127), long-lived radionuclides from uranium ore dust in kBqh/m(3) (mean = 3.9, max = 132), external gamma radiation in mSv (mean = 26, max = 667) and silica dust was estimated by a comprehensive job-exposure matrix. Internal Poisson regression models were applied to estimate the linear excess relative risk (ERR) per unit of cumulative exposure. RESULTS: Overall, a total of 457, 717 and 111 deaths occurred from malignant cancer, cardiovascular diseases and non-malignant respiratory diseases, respectively. Uranium ore dust and silica dust were not associated with mortality from any of these disease groups. A statistically significant relationship between cumulative radon exposure and mortality from all cancers (ERR/100 WLM = 1.71; p = 0.02), primarily due to lung cancer (n = 159; ERR/100 WLM = 3.39; p = 0.05), was found. With respect to cumulative external gamma radiation, an excess of mortality of solid cancers (n = 434; ERR/Sv = 1.86; p = 0.06), primarily due to stomach cancer (n = 49, ERR/Sv = 10.0; p = 0.12), was present. CONCLUSION: The present findings show an excess mortality from lung cancer due to radon exposure and from solid cancers due to external gamma radiation in uranium millers that was not statistically significant. Exposure to uranium was not associated with any cause of death, but absorbed organ doses were estimated to be low

31. **Kreuzer, M.,** Auvinen, A., Cardis, E., Hall, J., Jourdain, J-R., Laurier, D., Little, M.P., Peters. A., Raj, K., Russell, N.S., Tapio, S., Zhang, W., Gomolka, M.: Low-dose ionising radiation and cardiovascular diseases – Strategies for molecular epidemiological studies in Europe. Mutation Research 764 (2015) 90–100. (Task 7.2 ; BfS, STUK, CREAL, IC, IRSN, DH-PHE and HMGU)
Non-cancer
Epidemiology

It is well established that high-dose ionising radiation causes cardiovascular diseases. In contrast, the evidence for a causal relationship between long-term risk of cardiovascular diseases after moderate doses (0.5–5 Gy) is suggestive and weak after low doses (<0.5 Gy). However, evidence is emerging that doses under 0.5 Gy may also increase long-term risk of cardiovascular disease. This would have major implications for radiation protection with respect to medical use of radiation for diagnostic purposes and occupational or environmental radiation exposure. Therefore, it is of great importance to gain information about the presence and possible magnitude of radiation-related cardiovascular disease risk at doses of less than 0.5 Gy. The biological mechanisms implicated in any such effects are unclear and results from epidemiological studies are inconsistent. Molecular epidemiological studies can improve the understanding of the pathogenesis and the risk estimation of radiation-induced circulatory disease at low doses. Within the European DoReMi (Low Dose Research towards Multidisciplinary Integration) project, strategies to conduct molecular epidemiological studies in this field have been developed and evaluated. Key potentially useful European cohorts are the Mayak workers, other nuclear workers, uranium miners, Chernobyl liquidators, the Techa river residents and several diagnostic or low-dose radiotherapy patient cohorts. Criteria for informative studies are given and biomarkers to be investigated suggested. A close collaboration between epidemiology, biology and dosimetry is recommended, not only among experts in the radiation field, but also those in cardiovascular diseases.

32. **Kulzer L**, Rubner Y, Deloch L, Allgäuer A, Frey B, Fietkau R, Dörrie J, Schaft N, Gaipl US. Norm- and hypo-fractionated radiotherapy is capable of activating human dendritic cells. J. Immunotoxicol 2014, Feb 10, pages 1-9. (Task 5.2.1; UKER)
Cancer
Non-targeted effects (bystander)

Despite the transient immunosuppressive properties of local radiotherapy (RT), this classical treatment modality of solid tumors is capable of inducing immunostimulatory forms of tumor-cell death. The resulting 'immunotoxicity' in the tumor, but not in healthy tissues, may finally lead to immune-mediated destruction of the tumor. However, little is known about the best irradiation scheme in this setting. This study examines the immunological effects of differently irradiated human colorectal tumor cells on human monocyte-derived dendritic cells (DC). Human SW480 tumor cells were irradiated with a norm-fractionation scheme (5×2Gy), a hypo-fractionated protocol (3×5Gy), and with a high single irradiation dose (radiosurgery; 1×15Gy). Subsequently, human immature DC (iDC) were co-incubated with supernatants (SN) of these differently treated tumor cells. Afterwards, DC were analyzed regarding the expression of maturation markers, the release of cytokines, and the potential to stimulate $CD4^+$ T-cells. The co-incubation of iDC with SN of tumor cells exposed to norm- or hypo-fractionated RT resulted in a significantly increased secretion of the immune activating cytokines IL-12p70, IL-8, IL-6, and TNFα, compared to iDC co-incubated with SN of tumor cells that received a high single irradiation dose or were not irradiated. In addition, DC-maturation markers CD80, CD83, and CD25 were also exclusively elevated after co-incubation with the SN of fractionated irradiated tumor cells. Furthermore, the SN of tumor cells that were irradiated with norm- or hypo-fractionated RT triggered iDC to stimulate $CD4^+$ T-cells not only in an allogenic, but also in an antigen-specific manner like mature DC. Collectively, these results demonstrate that norm- and hypo-fractionated RT induces a fast human colorectal tumor-cell death with immunogenic potential that can trigger DC maturation and activation in vitro. Such findings may contribute to the improvement of irradiation protocols for the most beneficial induction of anti-tumor immunity.

33. **Kundrat P,** Bauer G, Jacob P, Friedland W.: Mechanistic modelling suggests that the size of preneoplastic lesions is limited by intercellular induction of apoptosis in oncogenically transformed cells. Carcinogenesis 2011, 33(2): 253-259. (Task 5.4; HMGU)
Cancer
Modeling
Non-targeted effects (bystander)

Selective removal of oncogenically transformed cells by apoptosis induced via signalling by surrounding cells has been suggested to represent a natural anticarcinogenic process. To investigate its potential effect in detail, a mechanistic model of this process is proposed. The model is calibrated against in vitro data on apoptosis triggered in transformed cells by defined external inducers as well as through signalling by normal cells under coculture conditions. The model predicts that intercellular induction of apoptosis is capable of balancing the proliferation of oncogenically transformed cells and limiting the size of their populations over long times, even if their proliferation per se were unlimited. Experimental research is desired to verify whether the predicted stable population of transformed cells to a kind of dormancy during early-stage carcinogenesis (dormant preneoplastic lesions), and how this process relates to other anticarcinogenic mechanisms taking place under in vivo conditions.

34. **Kundrat P** and Friedland W.: Impact of intercellular induction of apoptosis on low-dose radiation carcinogenesis. Radiation Protection Dosimetry, 2015. (Task 5.4; HMGU)
Cancer

In vitro data indicate that selective removal of oncogenic transformed cells by apoptosis induced via signalling by neighbouring cells may represent an important anti-carcinogenic process. Mechanistic modelling supports this concept and predicts that the phenomenon can stop the growth of a transformed cell population, forming a dormant pre-neoplastic lesion, or even remove the transformed clone completely. Radiation has been shown to enhance the underpinning signalling and increase the extent and rate of apoptosis induction in precancerous cells. Implications for low-dose radiation carcinogenesis are discussed based on in vitro data and mechanistic modelling. The possibility is outlined for radiation to act in a pro-carcinogenic manner, i.e. to reduce rather than enhance the removal of transformed cells by apoptosis. The effects of radiation exposure during early or late carcinogenesis are discussed.

35. **Large M**, Reichert S, Hehlgans S, Fournier C, Rödel C, Rödel F.: A non-linear detection of phospho-histone H2AX in EA.hy926 endothelial cells following low dose x-irradiation is modulated by reactive oxygen species. Radiat Oncol 2014, Mar 22, 9: 80 (Task 5.1; GUF)
Cancer
Non-cancer

BACKGROUND: A discontinuous dose response relationship is a major characteristic of the anti-inflammatory effects of low-dose X-irradiation therapy. Although recent data indicate an involvement of a variety of molecular mechanisms in these characteristics, the impact of reactive oxygen species (ROS) production to give rise or contribute to these phenomena in endothelial cells (EC) remains elusive.
MATERIAL AND METHODS: HUVEC derived immortalized EA.hy926 cells were stimulated by tumor necrosis factor-α (TNF-α, 20 ng/ml) 4 h before irradiation with doses ranging from 0.3 to 1 Gy. To analyse DNA repair capacity, phospho-histone H2AX foci were assayed at 1 h, 4 h and 24 h after irradiation. ROS production and superoxide dismutase (SOD) activity were analysed by fluorometric 2',7'-dichlorodihydrofluorescein-diacetate (H2DCFDA) and colorimetric assays. A functional impact of ROS on γH2AX production was analysed by treatment with the scavenger N-acetyl-L-cysteine (NAC).
RESULTS: Irrespective of stimulation by TNF-α, EA.hy926 cells revealed a linear dose response characteristic of γH2AX foci detection at 1 h and 4 h after irradiation. By contrast, we observed a discontinuity in residual γH2AX foci detection at 24 h after irradiation with locally elevated values following a 0.5 Gy exposure that was abolished by inhibition of ROS by NAC. Moreover, SOD protein expression was significantly decreased at doses of 0.5 Gy and 0.7 Gy concomitant with a reduced SOD activity.
CONCLUSION: These data implicate a non-linear regulation of ROS production and SOD activity in EA.hy926 EC following irradiation with doses < 1 Gy that may contribute to a discontinuous dose-response relationship of residual γH2AX foci detection.

36. **Laurier D.,** Guseva Canu I, Baatout S, Bertho J-M, Blanchardon E., Bouffler S, Cardis E., Gomolka M, Hall J, Kesminiene A, Kreuzer M, Rage E.: DoReMi workshop on multidisciplinary approaches to evaluating cancer risks associated with low-dose internal contamination. Radioprotection 2012, 47(1): 119-148. (Task 5.5; IRSN, SCK-CEN, DH-PHE, CREAL, BfS and IC)
Cancer
Internal emitters (contamination)
Epidemiology
Modeling

A workshop dedicated to cancer risks associated with low-dose internal contamination was organized in March 2011, in Paris, in the framework of the DoReMi (Low Dose Research towards

Multidisciplinary Integration) European Network of Excellence. The aim was to identify the best epidemiological studies that provide an opportunity to develop a multidisciplinary approach to improve the evaluation of the cancer risk associated with internal contamination. This workshop provided an opportunity for in-depth discussions between researchers working in different fields including (but not limited to) epidemiology, dosimetry, biology and toxicology. Discussions confirmed the importance of research on the health effects of internal contamination. Several existing epidemiological studies provide a real possibility to improve the quantification of cancer risk associated with internal emitters. Areas for future multidisciplinary collaborations were identified, that should allow feasibility studies to be carried out in the near future. The goal of this paper is to present an overview of the presentations and discussions that took place during the workshop.

37. **Lödermann B.,** Wunderlich R, Frey S, Schorn C, Stangl S, Rödel F, Keilholz L, Fietkau R, Gaipl US, Frey B.: Low dose ionizing radiation leads to a *NF-kB* dependent decreased secretion of active IL-1ß by activated macrophages with a discontinuous dose-dependency. Int J Rad Biol. 2012, 88 (10) 727-734. (Task 7.6; UKER and GUF)
Non-cancer
Non-targeted effects (bystander)

Purpose: Therapy with low doses of ionising radiation (X-rays) exerts anti-inflammatory effects. Little is known about whether and how low doses of X-ray treatment modulate the inflammatory phenotype of macrophages, especially the secretion of Interleukin-1beta (IL-1 β). *Materials and methods:* Macrophages were differentiated from human THP-1 monocytes, activated with lipopolysaccharide (LPS), treated with distinct low doses of X-rays, and co-activated with monosodium urate crystals (MSU) to induce inflammasome activation. Secretion of IL-1 β was analysed by an enzyme-linked immunosorbent assay (ELISA) and Western blot. Furthermore, we analysed the intracellular amounts of the serine/threonine protein kinase B (named: Akt), mitogen-activated protein kinase p38 (p38), the v-rel reticuloendotheliosis viral oncogene homolog A (RelA), and pro- and cleaved IL-1 β. *Results:* Low dose X-rays led to decreased secretion of active IL-1 β in a manner discontinuous with dose which was most pronounced after 0.5 or 0.7 Gy. Passiv release of lactate dehydrogenase (LDH) was not influenced by X-rays. The decreased secretion of IL-1 β correlated with reduced translocation of RelA, being part of the nuclear factor kappa light- chain-enhancer of activated B cells (NF- κB) complex, into the nucleus. After 0.5 or 0.7 Gy of X-rays, the intracellular protein amounts of up (p38) and downstream molecules (Akt) of NF- κ B were reduced in activated macrophages, as were the pro- and cleaved forms of IL-1 β. *Conclusions:* Distinct low doses of X-rays induce an anti-inflammatory phenotype of activated macrophages by lowering the amount of secreted IL-1 β in a NF- κ B dependent manner.

38. **Maguire, A,** Vega-Carrascal, I, Bryant, J, White, L, Howe, O, Lyng, F M, Meade, A D.: Competitive Evaluation of Data Mining Algorithms for Use in Cassification of Leukocyte Subtypes with Raman Microspectroscopy. Analyst, 2015, 140, 2473-2481. (Task 6.8; DIT)
Individual sensitivity
Tissue sensitivity
Modeling

Raman microspectroscopy has been investigated for some time for use in label-free cell sorting devices. These approaches require coupling of the Raman spectrometer to complex data mining algorithms for identification of cellular subtypes such as the leukocyte subpopulations of lymphocytes and monocytes. In this study, three distinct multivariate classification approaches, (PCA-LDA, SVMs and Random Forests) are developed and tested on their ability to classify the cellular subtype in extracted peripheral blood mononuclear cells (T-cell lymphocytes from myeloid cells), and are evaluated in terms of their respective classification performance. A strategy for optimisation of each of the classification algorithm is presented with emphasis on reduction of model complexity in each of the algorithms. The relative classification performance and performance characteristics are highlighted, overall suggesting the radial basis function SVM as a robust option for classification of leukocytes with Raman microspectroscopy.

39. **Maguire A,** Vegacarrascal I, White L, McClean B, Howe O, Lyng FM, Meade AD.: Analyses of Ionizing Radiation Effects In Vitro in Peripheral Blood Lymphocytes with Raman Spectroscopy. Radiat Res. 2015 Apr 6. [Epub ahead of print] (Task 6.8; DIT)
Individual sensitivity
Tissue sensitivity
Modeling

The use of Raman spectroscopy to measure the biochemical profile of healthy and diseased cells and tissues may be a potential solution to many diagnostic problems in the clinic. Although extensively used to identify changes in the biochemical profiles of cancerous cells and tissue, Raman spectroscopy has been used less often for analyzing changes to the cellular environment by external factors such as ionizing radiation. In tandem with this, the biological impact of low doses of ionizing radiation remains poorly understood. Extensive studies have been performed on the radiobiological effects associated with radiation doses above 0.1 Gy, and are well characterized, but recent studies on low-dose radiation exposure have revealed complex and highly variable responses. We report here the novel finding that demonstrate the capability of Raman spectroscopy to detect radiation-induced damage responses in isolated lymphocytes irradiated with doses of 0.05 and 0.5 Gy. Lymphocytes were isolated from peripheral blood in a cohort of volunteers, cultured ex vivo and then irradiated. Within 1 h after irradiation spectral effects were observed with Raman microspectroscopy and principal component analysis and linear discriminant analysis at both doses relative to the sham-irradiated control (0 Gy). Cellular DNA damage was confirmed using parallel γ-H2AX fluorescence measurements on the extracted lymphocytes per donor and per dose. DNA damage measurements exhibited interindividual variability among both donors and dose, which matched that seen in the spectral variability in the lymphocyte cohort. Further evidence of links between spectral features and DNA damage was also observed, which may potentially allow noninvasive insight into the DNA remodeling that occurs after exposure to ionizing radiation.

40. **Manda K.,** Glasow A, Paape D, Hildebrandt G.: Effects of ionizing radiation on the immune system with special emphasis on the interaction of dendritic and T cells. Front Oncology 2012, Aug 24, 2, 102; p.1-9. (Task 5.2.1; UROS)
Cancer
Non-targeted effects (bystander)

Dendritic cells (DCs), as professional antigen presenting cells, are members of the innate immune system and function as key players during the induction phase of adaptive immune responses. Uptake, processing, and presentation of antigens direct the outcome toward either tolerance or immunity. The cells of the immune system are among the most highly radiosensitive cells in the body. For high doses of ionizing radiation (HD-IR) both immune-suppressive effects after whole body irradiation and possible immune activation during tumor therapy were observed. On the other hand, the effects of low doses of ionizing radiation (LD-IR) on the immune system are controversial and seem to show high variability among different individuals and species. There are reports revealing that protracted LD-IR can result in radioresistance. But immune-suppressive effects of chronic LD-IR are also reported, including the killing or sensitizing of certain cell types. This articles hall review the current knowledge of radiation-induced effects on the immune system, paying special attention to the interaction of DCs and T cells.

41. **Mariotti LG,** Bertolotti A, Ranza E, Babini G, Ottolenghi A.: Investigation of the mechanisms underpinninjg IL-6 cytokine release in bystander responses. The roles of radiation

dose, radiation quality and specific ROS/RNS scavengers. Int J Radiat. Biol. 2012, Oct 88 (10): 751-762. (Task 5.6; UNIPV)
Cancer
Radiation quality
Modeling
Non-targeted effects (bystander)

Purpose : To investigate the mechanisms regulating the pathways of the bystander transmission in vitro, focusing on the radiation perturbed signaling (via Interleukin 6, IL-6) of the irradiated cells after exposure to low doses of different radiation types. *Materials and methods :* An integrated ' systems radiation biology ' approach was adopted. Experimentally the level of the secreted cytokine from human fibroblasts was detected with ELISA (Enzyme-Linked ImmunoSorbent Assay) method and subsequently the data were analyzed and coupled with a phenomenological model based on differential equations to evaluate the single-cell release mechanisms. *Results:* The data confirmed the important effect of radiation on the IL-6 pathway, clearly showing a crucial role of the ROS (Reactive Oxygen Species) in transducing the effect of initial radiation exposure and the subsequent long-term release of IL-6. Furthermore, a systematic investigation of radiation dose/radiation quality dependence seems to indicate an increasing efficiency of high LET (Linear Energy Transfer) irradiation in the release of the cytokine. Basic hypotheses were tested, on the correlation between direct radiobiological damage and signal release and on the radiation target for this endpoint (secretion of IL-6). *Conclusions:* The results demonstrate the role of reactive oxygen and nitrogen species in the signaling pathways of IL-6. Furthermore the systems radiation biology approach here adopted, allowed us to test and verify hypotheses on the behavior of the single cell in the release of cytokine, after the exposure to different doses and different qualities of ionizing radiation.

42. **Mariotti LG,** Pirovano G, Savage KI, Ghita M, Ottolenghi A, Prise KM, Schettino G.: Use of the γ-H2AX assay to investigate DNA repair dynamics following multiple radiation exposures. PLoS One 2013,Nov 29, 8(11): e79541. (Task 5.6; UNIPV)
Cancer
Radiation quality
Modeling
Non-targeted effects (bystander)

Radiation therapy is one of the most common and effective strategies used to treat cancer. The irradiation is usually performed with a fractionated scheme, where the dose required to kill tumour cells is given in several sessions, spaced by specific time intervals, to allow healthy tissue recovery. In this work, we examined the DNA repair dynamics of cells exposed to radiation delivered in fractions, by assessing the response of histone-2AX (H2AX) phosphorylation (γ-H2AX), a marker of DNA double strand breaks. γ-H2AX foci induction and disappearance were monitored following split dose irradiation experiments in which time interval between exposure and dose were varied. Experimental data have been coupled to an analytical theoretical model, in order to quantify key parameters involved in the foci induction process. Induction of γ-H2AX foci was found to be affected by the initial radiation exposure with a smaller number of foci induced by subsequent exposures. This was compared to chromatin relaxation and cell survival. The time needed for full recovery of γ-H2AX foci induction was quantified (12 hours) and the 1:1 relationship between radiation induced DNA double strand breaks and foci numbers was critically assessed in the multiple irradiation scenarios

43. **Olme C-H.,** Finnon R, Brown N, Kabacik S, Bouffler SD, Badie C.: Live cell detection of chromosome 2 deletion and Sfpi1/PU1 loss in radiation-induced mouse acute leukaemia. Leuk Res 2013, Oct 37(10): 1374-1382. (Task 5.3; DH-PHE)
Cancer
Tissue sensitivity

The CBA/H mouse model of radiation-induced acute myeloid leukaemia (rAML) has been studied for decades to bring to light the molecular mechanisms associated with multistage carcinogenesis. A specific interstitial deletion of chromosome 2 found in a high proportion of rAML recognised as the initiating event. The deletion leads to the loss of Sfpi, a gene essential for haematopoietic development. Its product, the transcription factor PU.1 acts as a tumour suppressor in this model. Although the deletion can be detected early following ionising radiation exposure by cytogenetic techniques, precise characterization of the haematopoietic cells carrying the deletion and the study of their fate in vivo cannot be achieved. Here, using a genetically engineered C57BL/6 mouse model expressing the GFP fluorescent molecule under the control of the Sfpi1 promoter, which we have bred onto the rAML-susceptible CBA/H strain, we demonstrate that GFP expression did not interfere with X-ray induced leukaemia incidence and that GFP fluorescence in live leukaemic cells is a surrogate marker of radiation-induced chromosome 2 deletions with or without point mutations on the remaining allele of the Sfpi1 gene. This study presents the first experimental evidence for the detection of this leukaemia initiating event in live leukemic cells.

44. **Olme CH.,** Brown N, Finnon R, Bouffler SD, Badie C.: Frequency of acute myeloid leukaemia-associated mouse deletions chromosome 2 in X-ray exposed immature haematopoietic progenitors and stem cells. Mutat Res Genetic Toxicology and Environmental 2013, Aug 30, 756(1-2): 119-126. (Task 5.3; DH-PHE)
Cancer
Tissue sensitivity

Exposure to ionising radiation can lead to an increased risk of cancer, particularly leukaemia. In radiation-induced acute myeloid leukaemia (rAML), a partial hemizygous deletion of mouse chromosome 2 is a common feature in several susceptible strains. The deletion is an early event detectable 24 h after expo-sure in bone marrow cells using cytogenetic techniques. Expanding clones of bone marrow cells with chromosome 2 deletions can be detected less than a year after exposure to ionising radiation in around half of the irradiated mice. Ultimately, 15–25% of exposed animals develop AML. It is generally assumed that leukaemia originates in an early progenitor cell or haematopoietic stem cell, but it is unknown whether the original chromosome damage occurs at a similar frequency in committed progenitors and stem cells. In this study, we monitored the frequency of chromosome 2 deletions in immature bone marrow cells (Lin−) and haematopoietic stem cells/multipotent progenitor cells (LSK) by several techniques, fluorescent in situ hybridisation (FISH) and through use of a reporter model, flow cytometry and colony forming units in spleen (CFU-S) following ex vivo or in vivo exposure. We showed that partial chromosome 2 deletions are present in the LSK subpopulation, but cannot be detected in Lin−cells andCFU-S12 cells. Furthermore, we transplanted irradiated Lin−or LSK cells into host animals to determine whether specific irradiated cell populations acquire an increased proliferative advantage compared to unirradiated cells. Interestingly, the irradiated LSK subpopulation containing cells carrying chromosome2 deletions does not appear to repopulate as well as the unirradiated population, suggesting that the chromosomal deletion does not provide an advantage for growth and in vivo repopulation, at least at early stages following occurrence.

45. **Ott, OJ,** Hertel S, Gaipl US, Frey B, Schmidt M, Fietkau R.: Benign painful elbow syndrome. First results of a single-center prospective randomized radiotherapy dose optimization trial. Strahlenther Onkol 2012, Okct, 188(10): 873-877. (Task 7.6; UKER)
Non-cancer
Tissue sensitivity

Background and purpose: The goal of the present study was to evaluate the efficacy of two different dose-fractionation schedules for radiotherapy (RT) of patients with painful elbow syndrome. Patients and methods: Between February 2006 and February 2010, 199 consecutive evaluable patients were recruited for this prospective randomized trial. All patients received RT in orthovoltage technique. One RT course consisted of 6 single fractions/3 weeks. In case of insufficient remission of pain after 6 weeks a second radiation series was performed. Patients were randomly assigned to receive either single doses of 0.5 or 1.0 Gy. Endpoint was pain reduction. Pain was measured before, right after, and 6 weeks after RT by a visual analogue scale (VAS) and a comprehensive pain score (CPS). Results: The overall response rate for all patients was 80% direct after and 91% 6 weeks after RT. The mean VAS values before, after and 6 weeks after treatment for the 0.5 and 1.0 Gy groups were 59.6 ± 20.2 and 55.7 ± 18.0 (p=0.463), 32.1 ± 24.5 and 34.4 ± 22.5 (p=0.256), and 27.0 ± 27.7 and 23.5 ± 21.6 (p=0.818). The mean CPS before, after, and 6 weeks after treatment was 8.7 ± 2.9 and 8.1 ± 3.1 (p=0.207), 4.5 ± 3.2 and 5.0 ± 3.4 (p=0.507), 3.9 ± 3.6 and2.8 ± 2.8 (p=0.186), respectively. No statistically significant differences between the two single dose trial arms for early (p=0.103) and delayed response (p=0.246) were found. Conclusion: RT is an effective treatment option for the management of benign painful elbow syndrome. For radiation protection reasons the dose for a RT series is recommended not to exceed 3.0 Gy.

46. **Ott, OJ** Hertel S, Gaipl US, Frey B, Schmidt M, Fietkau R.: Benign painful shoulder syndrome: initial results of a single-center prospective randomized radiotherapy dose-optimization trial. Strahlenther Onkol 2012, 188(12), 1108- 1113. (Task 7.6; UKER)
Non-cancer
Tissue sensitivity

BACKGROUND AND PURPOSE: To compare the efficacy of two different dose-fractionation schedules for radiotherapy of patients with benign painful shoulder syndrome. PATIENTS AND METHODS: Between February 2006 and February 2010, 312 consecutive evaluable patients were recruited for this prospective randomized trial. All patients received radiotherapy with an orthovoltage technique. One radiotherapy course consisted of 6 single fractions in 3 weeks. In case of insufficient remission of pain after 6 weeks, a second radiation series was performed. Patients were randomly assigned to receive either single doses of 0.5 or 1.0 Gy. The endpoint was pain reduction. Pain was measured before, right after, and 6 weeks after radiotherapy using a visual analogue scale (VAS) and a comprehensive pain score (CPS). RESULTS: The overall response rate for all patients was 83% directly after and 85% 6 weeks after radiotherapy. The mean VAS values before, directly after, and 6 weeks after treatment for the 0.5 and 1.0 Gy groups were 56.8 ± 23.7 and 53.2 ± 21.8 (p = 0.158), 38.2 ± 26.1 and 34.0 ± 24.5 (p = 0.189), and 33.0 ± 27.2 and 23.7 ± 22.7 (p = 0.044), respectively. The mean CPS before, directly after, and 6 weeks after treatment was 9.7 ± 3.0 and 9.5 ± 2.7 (p = 0.309), 6.1 ± 3.6 and 5.4 ± 3.6 (p = 0.096), 5.3 ± 3.7 and 4.1 ± 3.7 (p = 0.052), respectively. Despite a slight advantage in the VAS analysis for the 1.0 Gy group for delayed response, the CPS analysis revealed no statistically significant differences between the two single-dose trial arms for early (p = 0.652) and delayed response quality (p = 0.380). CONCLUSION: Radiotherapy is an effective treatment option for the management of benign painful shoulder syndrome. Concerning radiation protection, the dose for a radiotherapy series is recommended not to exceed 3-6 Gy.

47. **Ott, OJ,** Jeremias C, Gaipl US, Frey B, Schmidt M, Fietkau R.: Radiotherapy for achillodynia: results of a single-center prospective randomized dose-optimization trial. Strahlenther Onkol 2013,Feb, 189(2), 142-146. (Task 7.6; UKER)
Non-cancer
Tissue sensitivity

BACKGROUND AND PURPOSE: The aim of this study was to compare the efficacy of two different dose-fractionation schedules for radiotherapy of patients with achillodynia. PATIENTS AND METHODS: Between February 2006 and February 2010, 112 consecutive evaluable patients were recruited for this prospective randomized trial. All patients underwent radiotherapy with an orthovoltage technique. One radiotherapy course consisted of 6 single fractions over 3 weeks. In case of insufficient remission of pain after 6 weeks, a second radiation series was performed. Patients were randomly assigned to receive either single doses of 0.5 or 1.0 Gy. The endpoint was pain reduction. Pain was measured before, right after, and 6 weeks after radiotherapy with a visual analogue scale (VAS) and a comprehensive pain score (CPS).RESULTS: The overall response rate for all patients was 84% directly after and 88% 6 weeks after radiotherapy. The mean VAS values before, directly after, and 6 weeks after treatment for the 0.5 and 1.0 Gy groups were 55.7 ± 21.0 and 58.2 ± 23.5 (p = 0.526), 38.0 ± 23.2 and 30.4 ± 22.6 (p = 0.076), and 35.4 ± 25.9 and 30.9 ± 25.4 (p = 0.521), respectively. The mean CPS before, directly after, and 6 weeks after treatment was 8.2 ± 3.0 and 8.9 ± 3.3 (p = 0.239), 5.6 ± 3.1 and 5.4 ± 3.3 (p = 0.756), 4.4 ± 2.6 and 5.3 ± 3.8 (p = 0.577), respectively. No statistically significant differences were found between the two single-dose trial arms for early (p = 0.366) and delayed response (p = 0.287). CONCLUSION: Radiotherapy is an effective treatment option for the management of achillodynia. For radiation protection, the dose of a radiotherapy series is recommended not to exceed 3-6 Gy.

48. **Ott OJ,** Jeremias C., Gaipl US, Frey B., Schmidt M., Fietkau R.: Radiotherapy for calcaneodynia. Results of a single center prospective randomized dose optimization trial. Strahlenther Onkol. 2013, 189(4), 329-334. (Task 7.6; UKER)
Non-cancer
Tissue sensitivity

Ionizing radiation is a known human carcinogen that can induce a variety of biological effects depending on the physical nature, duration, doses and dose-rates of exposure. However, the magnitude of health risks at low doses and dose-rates (below 100mSv and/or 0.1mSvmin(-1)) 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 epidemiology studies incorporating biomarkers and bioassays. A number of these have been used to investigate exposure, effects and susceptibility to ionizing radiation, albeit often at higher doses and dose rates, with each reflecting time-limited cellular or physiological alterations. This review summarises the multidisciplinary work undertaken in the framework of the European project DoReMi (Low Dose Research towards Multidisciplinary Integration) to identify the most appropriate biomarkers for use in population studies. In addition to logistical and ethical considerations for conducting large-scale epidemiological studies, we discuss the relevance of their use for assessing the effects of low dose ionizing radiation exposure at the cellular and physiological level. We also propose a temporal classification of biomarkers that may be relevant for molecular epidemiology studies which need to take into account the time elapsed since exposure. Finally, the integration of biology with epidemiology requires careful planning and 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 appropriate population to be investigated (occupationally, environmentally or medically exposed), and study design. The consideration of the logistics of biological sample collection, processing and storing and the choice of biomarker or bioassay, as well as awareness of potential confounding factors, are also essential.

49. **Ott OJ,** Jeremias C., Gaipl US, Frey B., Schmidt M., Fietkau R.: Radiotherapy for benign calcaneodynia: Long-term results of the Erlangen Dose Optimization (EDO) trial. Strahlenther Onkol. 2014, Jul, 190 (7), 671-675. (Task 7.6; UKER)
Non-cancer
Tissue sensitivity

BACKGROUND AND PURPOSE: The goal of this work was to evaluate the long-term efficacy of two dose-fractionation schedules for radiotherapy of calcaneodynia. PATIENTS AND METHODS: Between February 2006 and February 2010, 457 evaluable patients were recruited for this prospective trial. All patients received orthovoltage radiotherapy. One course consisted of 6 fractions/3 weeks. In case of insufficient remission of pain after 6 weeks a second series was performed. Patients were randomly assigned to receive either single doses of 0.5 or 1.0 Gy. Endpoint was pain reduction. Pain was measured before, right after (early response), 6 weeks (delayed response), and approximately 2.5 years after radiotherapy (long-term response) with a questionnaire-based visual analogue scale (VAS) and a comprehensive pain score (CPS). RESULTS: The median follow-up was 32 months (range 9-57 months). The overall early, delayed, and long-term response rates for all patients were 87, 88, and 95%. The mean VAS values before treatment, for early, delayed, and long-term response for the 0.5 and 1.0 Gy groups were 65.5±22.1 and 64.0±20.5 (p=0.19), 34.8±24.7 and 39.0±26.3 (p=0.12), 25.1±26.8 and 28.9±26.8 (p=0.16), and 16.3±24.3 and 14.1±19.7 (p=0.68). The mean CPS values before treatment, for early, delayed, and log-term response were 10.1±2.7 and 10.0±3.0 (p=0.78), 5.6±3.7 and 6.0±3.9 (p=0.34), 4.0±4.1 and 4.3±3.6 (p=0.26), and 2.1±3.3 and 2.3±3.2 (p=0.34), respectively. No significant differences in long-term response quality between the two arms were found (p=0.50). CONCLUSION: Radiotherapy is a very effective treatment for the management of benign calcaneodynia. For radiation protection reasons, the dose for a RT series should not exceed 3.0 Gy.

50. **Ott OJ,** Hertel S, Gaipl US, Frey B, Schmidt M, Fietkau R.: The Erlangen Dose Optimization trial for low-dose radiotherapy of benign painful elbow syndrome. Strahlenther Onkol 2014, Mar, 190(3), 293-297. Erratum in: Strahlenther Onkol. 2014 Jun;190(6):604. (Task 7.6; UKER)
Non-cancer
Tissue sensitivity

BACKGROUND AND PURPOSE: To evaluate the long-term efficacy of pain reduction by two dose fractionation schedules used for low-dose radiotherapy of painful elbow syndrome. PATIENTS AND METHODS: Between February 2006 and February 2010, 199 evaluable patients were recruited for this prospective trial. All patients received low-dose orthovoltage radiotherapy. One course consisted of 6 fractions in 3 weeks. In the case of insufficient pain remission after 6 weeks, a second course was administered. Patients were randomly assigned to one of two groups to receive single doses of either 0.5 or 1.0 Gy. Endpoint was pain reduction. Pain was measured before radiotherapy, as well as immediately after (early response), 6 weeks after (delayed response) and approximately 3 years after (long-term response) completion of radiotherapy using a questionnaire-based visual analogue scale (VAS) and a comprehensive pain score (CPS). RESULTS: Median follow-up was 35 months (range 9-57 months). The overall early, delayed and long-term response rates for all patients were 80, 90 and 94%, respectively. The mean VAS scores before treatment and those for early, delayed and long-term response in the 0.5- and 1.0-Gy groups were 59.6±20.2 and 55.7±18.0 (p=0.46); 32.1±24.5 and 34.4±22.5 (p=0.26); 27.0±27.7 and 23.5±21.6 (p=0.82) and 10.7±15.0 and 21.5 ± 26.9 (p=0.12), respectively. The mean CPS values before treatment and those for early, delayed and long-term response were 8.7±2.9 and 8.1±3.1 (p=0.21); 4.5±3.2 and 5.0±3.4 (p=0.51); 3.9±3.6 and 2.8±2.8 (p=0.19) and 1.5±2.3 and 2.4±3.5 (p=0.27), respectively. No significant differences in the quality of the long-term response were found between the 0.5- and 1.0-Gy arms (p=0.28). CONCLUSION: Low-dose radiotherapy is an effective treatment for the management of benign painful elbow syndrome. For radiation protection reasons, the dose for a radiotherapy series should not exceed 3.0 Gy.

51. **Ott OJ,** Hertel S, Gaipl US, Frey B, Schmidt M, Fietkau R.: The Erlangen Dose Optimization trial for radiotherapy of benign painful shoulder syndrome. Long term results. Strahlenther Onkol. 2014, Apr, 190(3), 394-398. Erratum in: Strahlenther Onkol. 2014 Jun;190(6):605. (Task 7.6; UKER)
Non-cancer

Tissue sensitivity

Background and purpose. To evaluate the long-term efficacy of pain reduction by two dose-fractionation schedules for radiotherapy of painful shoulder syndrome. Patients and methods. Between February 2006 and February 2010, 312 evaluable patients were recruited for this prospective trial. All patients received low-dose orthovoltage radiotherapy. One course consisted of 6 fractions in 3 weeks. In the case of insufficient pain remission after 6 weeks, a second course was administered. Patients were randomly assigned to one of two groups to receive single doses of either 0.5 or 1.0 Gy. Endpoint was pain reduction. Pain was measured before radiotherapy, as well as immediately after (early response), 6 weeks after (delayed response) and approximately 3 years after (long-term response) completion of radiotherapy using a questionnaire-based visual analogue scale (VAS) and a comprehensive pain score (CPS). Results. Median follow-up was 35 months (range 11–57). The overall early, delayed and long-term response rates for all patients were 83, 85 and 82%, respectively. The mean VAS scores before treatment and those for early, delayed and long-term response in the 0.5- and 1.0-Gy groups were 56.8±23.7 and 53.2±21.8 (p=0.16); 38.2±36.1 and 34.0±24.5 (p=0.19); 33.0±27.2 and 23.7±22.7 (p=0.04) and 27.9±25.8 and 32.1±26.9 (p=0.25), respectively. The mean CPS values before treatment and those for early, delayed and long-term response were 9.7±3.0 and 9.5±2.7 (p=0.31); 6.1±3.6 and 5.4±3.6 (p=0.10); 5.3±3.7 and 4.1±3.7 (p=0.05) and 4.0±3.9 and 5.3±4.4 (p=0.05), respectively. No significant differences in the quality of the long-term response were found between the 0.5- and 1.0-Gy arms (p=0.28). Conclusion. Radiotherapy is an effective treatment for the management of benign painful shoulder syndrome. For radiation protection reasons, the dose for a radiotherapy series should not exceed 3.0 Gy.

52. **Pascucci B,** Lemma T, Iorio E, Giovannini S, Vaz B, Iavarone I, Calcagnile A, Narciso L, Degan P, Podo F, Roginskya V, Janjic BM, Van Houten B, Stefanini M, Dogliotti E, D'Errico M.: An altered redox balance mediates the hypersensitivity of Cockayne syndrome primary fibroblasts to oxidative stress. Aging Cell 2012, Jun, 11(3), 520-529. (Task 5.3; ISS)
Cancer
Tissue sensitivity

Cockayne syndrome (CS) is a rare hereditary multisystem disease characterized by neurological and cells are hypersensitive to oxidative stress, but the molecular mechanisms involved remain unresolved. Here we provide the first evidence that primary fibroblasts derived from patients with CS-A and CS-B present an altered redox balance with increased steady-state levels of intracellular reactive oxygen species (ROS) and basal and induced DNA oxidative damage, loss of the mitochondrial membrane potential, and a significant decrease in the rate of basal oxidative phosphorylation. The Na / K-ATPase, a relevant target of oxidative stress, is also affected with reduced transcription in CS fibroblasts and normal protein levels restored upon complementation with wild-type genes. High-resolution magnetic resonance spectroscopy revealed a significantly perturbed metabolic profile in CS-A and CS-B primary fibroblasts compared with normal cells in agreement with increased oxidative stress and alterations in cell bioenergetics. The affected processes include oxidative metabolism, glycolysis, choline phospholipid metabolism, and osmoregulation. The alterations in intracellular ROS content, oxidative DNA damage, and metabolic profile were partially rescued by the addition of an antioxidant in the culture medium suggesting that the continuous oxidative stress that characterizes CS cells plays a causative role in the underlying pathophysiology. The changes of oxidative and energy metabolism offer a clue for the clinical features of patients with CS and provide novel tools valuable for both diagnosis and therapy.

53. **Pernot, E.,** , Hall J, Baatout S, Benotmane MA, Blanchardon E, Bouffler S, El Saghire H, Gomolka M, Guertler A, Harms-Ringdahl M, Jeggo P, Kreuzer M, Laurier D, Lindholm C, Mkacher R, Quintens R, Rothkamm K, Sabatier L, Tapio S, de Vathaire F, Cardis E.: Ionizing radiation biomarkers for potential use in epidemiological studies. Mutat Res. 2012, Oct-Dec 751(2), 258-

286. (WP1-7; CREAL, IC, SCK-CEN, IRSN, DH-PHE, BfS, SU, STUK, CEA and HMGU)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Tissue sensitivity
Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

Ionizing radiation is a known human carcinogen that can induce a variety of biological effects depending on the physical nature, duration, doses and dose-rates of exposure. However, the magnitude of health risks at low doses and dose-rates (below 100mSv and/or 0.1mSvmin(-1)) 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 epidemiology studies incorporating biomarkers and bioassays. A number of these have been used to investigate exposure, effects and susceptibility to ionizing radiation, albeit often at higher doses and dose rates, with each reflecting time-limited cellular or physiological alterations. This review summarises the multidisciplinary work undertaken in the framework of the European project DoReMi (Low Dose Research towards Multidisciplinary Integration) to identify the most appropriate biomarkers for use in population studies. In addition to logistical and ethical considerations for conducting large-scale epidemiological studies, we discuss the relevance of their use for assessing the effects of low dose ionizing radiation exposure at the cellular and physiological level. We also propose a temporal classification of biomarkers that may be relevant for molecular epidemiology studies which need to take into account the time elapsed since exposure. Finally, the integration of biology with epidemiology requires careful planning and 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 appropriate population to be investigated (occupationally, environmentally or medically exposed), and study design. The consideration of the logistics of biological sample collection, processing and storing and the choice of biomarker or bioassay, as well as awareness of potential confounding factors, are also essential.

54. **Pernot, E,** Cardis, E and Badie, C.: Usefulness of Saliva Samples for Biomarker Studies in Radiation Research. Cancer Epidemiol Biomarkers Prev; 23(12); 2673–80. (Task 6.6; CREAL and DH-PHE)
Cancer
Non-cancer
Individual sensitivity
Epidemiology

Salivary biomarkers have important potential to facilitate breakthroughs in epidemiologic studies, management of emergency situations, and detection and surveillance of diseases by medical staff. During the last decade, an increasing number of studies on salivary biomarkers have been published as a consequence of the impressive development of new high-throughput technologies. Here, we present a review of salivary biomarkers potentially useful in ionizing radiation (IR) research, particularly in molecular epidemiologic studies. Although several salivary biomarkers of cancer and other IR-associated diseases have been identified, few salivary biomarkers of exposure and no biomarker of susceptibility or effects specific to IR have been reported so far. Further studies are therefore needed to fully assess the potential of saliva as a source of biomarkers in the radiation research field. Although the use of saliva samples is not without drawbacks, it could represent an ideal noninvasive alternative to blood, particularly in children and in the context of large molecular epidemiology studies on the effects of low doses of IR, where, given the expected limited magnitude of effects, an extensive number of samples is required to reach statistical significance. See all the articles in this CEBP Focus section, "Biomarkers, Biospecimens, and New Technologies in Molecular Epidemiology."

55. **Perriaud L,** Marcel V, Sagne C, Favaudon V, Guédin A, De Rache A, Guetta C, Hamon F, Teulade-Fichou MP, Hainaut P, Mergny JL and Hall J.: Impact of G-quadruplex structures and intronic polymorphisms rs17878362 and rs1642785 on basal and ionizing radiation-induced expression of alternative p53 transcripts. Carcinogenesis. 2014 Dec; 35(12):2706-15. (Task 6.4; IC)
Cancer
Individual sensitivity

G-quadruplex (G4) structures in intron 3 of the p53 pre-mRNA modulate intron 2 splicing, altering the balance between the fully spliced p53 transcript (FSp53, encoding full-length p53) and an incompletely spliced transcript retaining intron 2 (p53I2 encoding the N-terminally truncated Δ40p53 isoform). The nucleotides forming G4s overlap the polymorphism rs17878362 (A1 wild-type allele, A2 16-base pair insertion) which is in linkage disequilibrium with rs1642785 in intron 2 (c.74+38 G>C). Biophysical and biochemical analyses show rs17878362 A2 alleles form similar G4 structures as A1 alleles although their position is shifted with respect to the intron 2 splice acceptor site. In addition basal FSp53 and p53I2 levels showed allele specific differences in both p53-null cells transfected with reporter constructs or lymphoblastoid cell lines. The highest FSp53 and p53I2 levels were associated with combined rs1642785-GG/rs17878362-A1A1 alleles, whereas the presence of rs1642785-C with either rs17878362 allele was associated with lower p53 pre-mRNA, total TP53, FSp53 and p53I2 levels, due to the lower stability of transcripts containing rs1642785-C. Treatment of lymphoblastoid cell with the G4 binding ligands 360A or PhenDC3 or with ionizing radiation increased FSp53 levels only in cells with rs17878362 A1 alleles, suggesting that under this G4 configuration full splicing is favoured. These results demonstrate the complex effects of intronic TP53 polymorphisms on G4 formation and identify a new role for rs1642785 on mRNA splicing and stability, and thus on the differential expression of isoform-specific transcripts of the TP53 gene.

56. **Pottier G,** Viau M, Ricoul M, Shim G, Bellamy M, Cuceu C, Hempel WM, Sabatier L.: Lead Exposure Induces Telomere Instability in Human Cells. PLoS One. 2013Jun 26;8(6):e67501. (Task 6.2; CEA)
Cancer

Lead (Pb) is an important environmental contaminant due to its widespread use over many centuries. While it affects primarily every organ system of the body, the most pernicious effects of Pb are on the central nervous system leading to cognitive and behavioral modification. Despite decades of research, the mechanisms responsible for Pb toxicity remain poorly understood. Recent work has suggested that Pb exposure may have consequences on chromosomal integrity as it was shown that Pb exposure leads to the generation of γH2Ax foci, a well-established biomarker for DNA double stranded break (DSB formation). As the chromosomal localization of γH2Ax foci plays an important role in determining the molecular mechanism responsible for their formation, we examined the localization of Pb-induced foci with respect to telomeres. Indeed, short or dysfunctional telomeres (uncapped or damaged telomeres) may be recognized as DSB by the DNA repair machinery, leading to "telomere-Induced Foci" (TIFs). In the current study, we show that while Pb exposure did not increase intra-chromosomal foci, it significantly induced TIFs, leading in some cases, to chromosomal abnormalities including telomere loss. The evidence suggests that these chromosomal abnormalities are likely due to perturbation of telomere replication, in particular on the lagging DNA strand. We propose a mechanism by which Pb exposure leads to the loss of telomere maintenance. As numerous studies have demonstrated a role for telomere maintenance in brain development and tissue homeostasis, our results suggest a possible mechanism for lead-induced neurotoxicity

57. **Pressyanov, D.,** Mitev, K., Georgiev, S. and Dimitrova, I.: Optimization of etching conditions for CD's/DVDs used as detectors for 222Rn. Radiation Measurements, 2015. (Tasks 4.10 and 5.5; SUN)
Radiation quality

To use the combination of the high radon absorption ability of the polycarbonate material of CDs/DVDs with its track-etch properties for retrospective radon measurements in dwellings was first proposed in 2001. Since then the applications of this method have expanded significantly, including measurements of high radon concentrations, e.g. in soil gas, underground mines, radon spas and buildings with exceptionally high radon levels. As the method employs electrochemical etching of alpha-tracks at a certain depth beneath the disk surface, saturation at high track density might occur. In this report we explore how the depth at which the alpha-tracks are etched and the voltage applied for electrochemical etching can be varied, in order to expand the range of the method towards high radon concentrations and to achieve the best accuracy. As a result, optimized regimes for etching CDs/DVDs are proposed and the expanded range of the method estimated.

58. **Raj, K.,** Bouffler S.: DoReMi stem cells and DNA damage workshop. Int J Radiat Biol. 2012, Oct 88(10), 671-676. (Task 5.3; DH-PHE)
Cancer
Non-cancer
Tissue sensitivity

Purpose: The target cells for radiation carcinogenesis are widely held to be stem or stem-like cells. Classically, stem cells are considered to be those capable of renewing tissues while differentiated cells lose the potential to replicate. More recently it has become apparent that greater developmental plasticity exists and that cells can be reprogrammed to form induced pluripotent stem cells. Modelling of radiation cancer risk requires understanding of the characteristics, numbers and responses of target stem cells to radiation. Therefore progress in understanding mechanisms of radiation-induced carcinogenesis is dependent on knowledge of stem cell radiobiology.
Results : In this context, the European Community ' s network of excellence on low dose radiation risk called, ' Low Dose Research towards Multidisciplinary Integration (DoReMi) ' (www. doremi-noe.net) and the United Kingdom ' s Health Protection Agency organised a workshop on *Stem Cells and DNA damage* in Oxfordshire on 7/8 December 2011 to address issues relating to radiation, DNA damage and stem cells. In keeping with the aim of improving understanding of low dose ionising radiation health risk, a panel of experts in stem cells and radiobiology were invited to this workshop. This summary includes all presentations at this workshop and is accompanied by full reports of several speakers.

59. **Roedel, F,** Frey B, Gaipl U, Keilholz L, Fournier C, Manda K, Schöllnberger H, Hildebrandt G, Rödel C.: Modulation of inflammatory immune reactions by low-dose ionizing radiation: molecular mechanisms and clinical application. Curr Med Chem 2012, 19(12), 1741-1750. (Tasks 5.2.1 and 7.6; GUF, UKER and UROS)
Cancer
Non-cancer
Non-targeted effects (bystander)

During the last decade, a multitude of experimental evidence has accumulated showing that low-dose radiation therapy (single dose 0.5-1 Gy) functionally modulates a variety of inflammatory processes and cellular compounds including endothelial (EC), mononuclear (PBMC) and polymorphonuclear (PMN) cells, respectively. These modulations comprise a hampered leukocyte adhesion to EC, induction of apoptosis, a reduced activity of the inducible nitric oxide synthase, and a lowered oxidative burst in macrophages. Moreover, irradiation with a single dose between 0.5-0.7 Gy has been shown to induce the expression of X-chromosome linked inhibitor of apoptosis and transforming growth factor beta 1, to reduce the expression of E-selectin and L-selectin from EC and PBMC, and to hamper secretion of Interleukin-1, or chemokine CCL20 from macrophages and PMN. Notably, a common feature of most of these responses is that they display discontinuous or biphasic dose dependencies, shared with "non-targeted" effects of low-dose irradiation exposure like the bystander response and hyper-radiosensitivity. Thus, the purpose of the present review is to discuss recent developments in the understanding of low-dose irradiation immune modulating properties with special emphasis on discontinuous dose response relationships.

60. **Roedel, F.,** Frey B, Manda K, Hildebrandt G, Hehlgans S, Keilholz L, Seegenschmiedt MH, Gaipl US, Rödel C.: Immunomodulatory properties and molecular effects in inflammatory diseases of low-dose X-irradiation. Front Oncol 2012, 25 Sept, 2 Article 120, p. 1-9. (Tasks 5.2.1 and 7.6; GUF, UKER and UROS)
Cancer
Non-cancer
Non-targeted effects (bystander)

Inflammatory diseases are the result of complex and pathologically unbalanced multicellular interactions. For decades, low-dose X-irradiation therapy (LD-RT) has been clinically documented to exert an anti-inflammatory effect on benign diseases and chronic degenerative disorders. By contrast, experimental studies to confirm the effectiveness and to reveal underlying cellular and molecular mechanisms are still at their early stages. During the last decade, however, the modulation of a multitude of immunological processes by LD-RT has been explored *in vitro* and *in vivo*. These include leukocyte/endothelial cell adhesion, adhesion molecule and cytokine/chemokine expression, apoptosis induction, and mononuclear/polymorphonuclear cell metabolism and activity. Interestingly, these mechanisms display comparable dose dependences and dose-effect relationships with a maximum effect in the range between 0.3 and 0.7 Gy, already empirically identified to be most effective in the clinical routine. This review summarizes data and models exploring the mechanisms underlying the immunomodulatory properties of LD-RT that may serve as a prerequisite for further systematic analyses to optimize low-dose irradiation procedures in future clinical practice.

61. **Roedel F**., Frey B., Multhoff G, Gaipl U.: Contribution of the immune system to bystander and non-targeted effects of ionizing radiation. Cancer Letters 2013, Oct 15, p. 1-9. (Tasks 5.2.1 and 7.6; GUF and UKER)
Cancer
Non-cancer
Non-targeted effects (bystander)

Considerable progress has recently been achieved in the understanding of molecular mechanisms involved in cellular radiation responses and radiation mediated microenvironmental communication. In line with that, it has become more and more obvious that X-irradiation causes distinct immunological effects ranging from anti-inflammatory activities if applied at low (<1 Gy) doses to harmful inflammatory side effects, radiation-induced immune modulation or induction of anti-tumour immune responses at higher doses. Moreover, experimental and clinical evidences indicate that these effects not only originate from direct nuclear damage but also include non-(DNA)
targeted mechanisms including bystander, out of field distant bystander (abscopal) effects and genomic instability. The purpose of the present review is to elucidate immune responses that

are initiated or affected by ionizing radiation, with a special emphasis on anti-inflammatory and abscopal effects and the induction of stress-induced anti-tumour immunity.

62. **Rombouts, C**, Aerts A, Beck M, De Vos WH, Van Oostveldt P, Benotmane MA, Baatout S, Differential response to acute low dose radiation in primary and immortalized endothelial cells. Int J Radiat Biol. 2013, 89(10), 841-850. (Task 7.3; SCK-CEN)
Non-cancer
Tissue sensitivity

Purpose: The low dose radiation response of primary human umbilical vein endothelial cells (HUVEC) and its immortalized derivative, the EA.hy926 cell line, was evaluated and compared. *Material and methods:* DNA damage and repair, cell cycle progression, apoptosis and cellular morphology in HUVEC and EA.hy926 were evaluated after exposure to low (0.05 – 0.5 Gy) and high doses (2 and 5 Gy) of acute X-rays. *Results :* Subtle, but significant increases in DNA double-strand breaks (DSB) were observed in HUVEC and EA.hy926 30 min after low dose irradiation (0.05 Gy). Compared to high dose irradiation (2 Gy), relatively more DSB/Gy were formed after low dose irradiation. Also, we observed a dose-dependent increase in apoptotic cells, down to 0.5 Gy in HUVEC and 0.1 Gy in EA.hy926 cells. Furthermore, radiation induced significantly more apoptosis in EA.hy926 compared to HUVEC. *Conclusions :* We demonstrated for the first time that acute low doses of X-rays induce DNA damage and apoptosis in endothelial cells. Our results point to a non-linear dose-response relationship for DSB formation in endothelial cells. Furthermore, the observed difference in radiation-induced apoptosis points to a higher radiosensitivity of EA.hy926 compared to HUVEC, which should be taken into account when using these cells as models for studying the endothelium radiation response.

63. **Rombouts, C**, Aerts A, Quintens R, Baselet B, El-Saghire H, Harms-Ringdahl M, Haghdoost S, Janssen A, Michaux A, Yentrapalli R, Benotmane MA, Van Oostveldt P, Baatout S.: Transcriptomic profiling suggests a role for IGFBP5 in premature senescence of endothelial cells after chronic low dose rate irradiation. Int J Radiat Biol. 2014, Jul, 90, 7:560-574. (Task 7.3; SCK-CEN, SU and HMGU)
Non-cancer
Tissue sensitivity

Purpose: Ionizing radiation has been recognized to increase the risk of cardiovascular diseases (CVD). However, there is no consensus concerning the dose-risk relationship for low radiation doses and a mechanistic understanding of low dose effects is needed. Material and methods: Previously, human umbilical vein endothelial cells (HUVEC) were exposed to chronic low dose rate radiation (1.4 and 4.1 mGy/h) during one, three and six weeks which resulted in premature senescence in cells exposed to 4.1 mGy/h. To gain more insight into the underlying signaling pathways, we analyzed gene expression changes in these cells using microarray technology. The obtained data were analyzed in a dual approach, combining single gene expression analysis and Gene Set Enrichment Analysis. Results: An early stress response was observed after one week of exposure to 4.1 mGy/h which was replaced by a more inflammation-related expression profile after three weeks and onwards. This early stress response may trigger the radiation-induced premature senescence previously observed in HUVEC irradiated with 4.1mGy/h. A dedicated analysis pointed to the involvement of insulin-like growth factor binding protein 5 (IGFBP5) signaling in radiation-induced premature senescence. Conclusion: Our findings motivate further research on the shape of the dose-response and the dose rate effect for radiation-induced vascular senescence

64. **Rosemann M,** Gonzalez-Vasconcellos I, Domke T, Kuosaite V, Schneider R, Kremer M, Favor J, Nathrath M, Atkinson MJ.: Rb1 promoter variant with reduced activity contributes to osteosarcoma susceptibility in irradiated mice. Mol Cancer. 2014 Aug 4; 13:182. (Task 6.2;

HMGU)
Cancer
Individual sensitivity
Radiation quality

BACKGROUND: Syndromic forms of osteosarcoma (OS) account for less than 10% of all recorded cases of this malignancy. An individual OS predisposition is also possible by the inheritance of low penetrance alleles of tumor susceptibility genes, usually without evidence of a syndromic condition. Genetic variants involved in such a non-syndromic form of tumor predisposition are difficult to identify, given the low incidence of osteosarcoma cases and the genetic heterogeneity of patients. We recently mapped a major OS susceptibility QTL to mouse chromosome 14 by comparing alpha-radiation induced osteosarcoma in mouse strains which differ in their tumor susceptibility. **METHODS:** Tumor-specific allelic losses in murine osteosacoma were mapped along chromosome 14 using microsatellite markers and SNP allelotyping. Candidate gene search in the mapped interval was refined using PosMed data mining and mRNA expression analysis in normal osteoblasts. A strain-specific promoter variant in Rb1 was tested for its influence on mRNA expression using reporter assay. **RESULTS:** A common Rb1 allele derived from the BALB/cHeNhg strain was identified as the major determinant of radiation-induced OS risk at this locus. Increased OS-risk is linked with a hexanucleotide deletion in the promoter region which is predicted to change WT1 and SP1 transcription factor-binding sites. Both in-vitro reporter and in-vivo expression assays confirmed an approx. 1.5 fold reduced gene expression by this promoter variant. Concordantly, the 50% reduction in Rb1 expression in mice bearing a conditional hemizygous Rb1 deletion causes a significant rise of OS incidence following alpha-irradiation. **CONCLUSION:** This is the first experimental demonstration of a functional and genetic link between reduced Rb1 expression from a common promoter variant and increased tumor risk after radiation exposure. We propose that a reduced Rb1 expression by common variants in regulatory regions can modify the risk for a malignant transformation of bone cells after radiation exposure

65. **Rubner, Y,** Wunderlich R, Rühle PF, Kulzer L, Werthmöller N, Frey B, Weiss EM, Keilholz L, Fietkau R, Gaipl US.: How does ionizing radiation contribute to the induction of anti-tumor immunity? Front Oncol 2012, Jul 25, 2: (article 75), 1-11. (Tasks 5.2.1 and 7.6; UKER)
Cancer
Tissue sensitivity
Non-targeted effects (bystander)

Radiotherapy (RT) with ionizing irradiation is commonly used to locally attack tumors. It induces a stop of cancer cell proliferation and finally leads to tumor cell death. During the last years it has become more and more evident that besides a timely and locally restricted radiation-induced immune suppression, a specific immune activation against the tumor and its metastases is achievable by rendering the tumor cells visible for immune attack. The immune system is involved in tumor control and we here outline how RT induces anti-inflammation when applied in low doses and contributes in higher doses to the induction of anti-tumor immunity. We especially focus on how local irradiation induces abscopal effects. The latter are partly mediated by a systemic activation of the immune system against the individual tumor cells. Dendritic cells are the key players in the initiation and regulation of adaptive anti-tumor immune responses. They have to take up tumor antigens and consecutively present tumor peptides in the presence of appropriate co-stimulation. We review how combinations of RT with further immune stimulators such as AnnexinA5 and hyperthermia foster the dendritic cell-mediated induction of anti-tumor immune responses and present reasonable combination schemes of standard tumor therapies with immune therapies. It can be concluded that RT leads to targeted killing of the tumor cells and additionally induces non-targeted systemic immune effects. Multimodal tumor treatments should therefore tend to induce immunogenic tumor cell death forms within a tumor microenvironment that stimulates immune cells.

66. **Rubner, Y,** Muth C, Strnad A, Derer A, Sieber R, Buslei R, Frey B, Fietkau R, Gaipl US.: Fractionated radiotherapy is the main stimulus for the induction of cell death and of Hsp70 release of p53 mutated glioblastoma cell lines. Radiat Oncol 2014, Mar 30, 9(1), 89. (Task 5.2.1; UKER)
Cancer
Non-cancer
Tissue sensitivity

BACKGROUND: Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. Despite a multimodal therapy consisting of resection followed by fractionated radiotherapy (RT) combined with the chemotherapeutic agent (CT) temozolomide (TMZ), its recurrence is almost inevitable. Since the immune system is capable of eliminating small tumor masses, a therapy should also aim to stimulate anti-tumor immune responses by induction of immunogenic cell death forms. The histone deacetylase inhibitor valproic acid (VPA) might foster this. METHODS: Reflecting therapy standards, we applied in our in vitro model fractionated RT with a single dose of 2Gy and clinically relevant concentrations of CT. Not only the impact of RT and/or CT with TMZ and/or VPA on the clonogenic potential and cell cycle of the glioblastoma cell lines T98G, U251MG, and U87MG was analyzed, but also the resulting cell death forms and release of danger signals such as heat-shock protein70 (Hsp70) and high-mobility group protein B1 (HMGB1). RESULTS: The clonogenic assays revealed that T98G and U251MG, having mutated tumor suppressor protein p53, are more resistant to RT and CT than U87MG with wild type (WT) p53. In all glioblastoma cells lines, fractionated RT induced a G2 cell cycle arrest, but only in the case of U87MG, TMZ and/or VPA alone resulted in this cell cycle block. Further, fractionated RT significantly increased the number of apoptotic and necrotic tumor cells in all three cell lines. However, only in U87MG, the treatment with TMZ and/or VPA alone, or in combination with fractionated RT, induced significantly more cell death compared to untreated or irradiated controls. While necrotic glioblastoma cells were present after VPA, TMZ especially led to significantly increased amounts of U87MG cells in the radiosensitive G2 cell cycle phase. While CT did not impact on the release of Hsp70, fractionated RT resulted in significantly increased extracellular concentrations of Hsp70 in p53 mutated and WT glioblastoma cells. CONCLUSIONS: Our results indicate that fractionated RT is the main stimulus for induction of glioblastoma cell death forms with immunogenic potential. The generated tumor cell microenvironment might be beneficial to include immune therapies for GBM in the future.

67. **Sagne C,** Marcel V, Amadou A, Hainaut P, Olivier M, Hall J.: A meta-analysis of cancer risk associated with the TP53 intron 3 duplication polymorphism (rs17878362): geographic and tumor-specific effects. Cell deaths and disease 2013, Feb 14, 4(2): e492. (Task 6.4; IC)
Cancer
Individual sensitivity
Epidemiology
Modeling

We have performed a meta-analysis of cancer risk associated with the rs17878362 polymorphism of the TP53 suppressor gene (PIN3, (polymorphism in intron 3), 16 bp insertion/duplication in intron 3), using a compilation of a total of 25 published studies with 10 786 cases and 11 760 controls. Homozygote carriers of the duplicated allele (A2A2) had a significantly increased cancer risk compared with A1A1 carriers (aggregated odds ratio (OR)¼1.45, 95% confidence interval (CI)¼1.22–1.74). However, there was no significant effect for the A1A2 heterozygotes (A1A2 versus A1A1 aggregated OR¼1.08, 95% CI¼0.99–1.18). No significant heterogeneity or publication bias was detected in the data set analysed. When comparing populations groups, increased cancer risk was associated with A2A2 carriage in Indian, Mediterranean and Northern Europe populations but not in the Caucasian population of the United States. Analysis by cancer site showed an increased risk for A2A2 carriers for breast and colorectal, but not for lung cancers. These results support that the A2A2 genotype of rs17878362 is associated with increased cancer risk, with population and tumor-specific effects.

68. **Sagne C**, Marcel V, Bota M, Martel-Planche G, Nobrega A, Palmero EI, Perriaud L, Boniol M, Vagner S, Cox DG, Chan CS, Mergny JL, Olivier M, Ashton-Prolla P, Hall J, Hainaut P, Achatz MI.: Age at cancer onset in germline TP53 mutation carriers: association with polymorphisms in predicted G-quadruplex structures. Carcinogenesis 2014, Apr, 35(4), 807-815. (Task 6.4; IC)
Cancer
Individual sensitivity
Epidemiology
Modeling

Germline TP53 mutations predispose to multiple cancers defining Li-Fraumeni/Li-Fraumeni-like syndrome (LFS/LFL), a disease with large individual disparities in cancer profiles and age of onset. G-quadruplexes (G4s) are secondary structural motifs occurring in guanine tracks, with regulatory effects on D DNA and RNA. We analyzed 85 polymorphisms within or near five predicted G4s in TP53 in search of modifiers of penetrance of LFS/LFL in Brazilian cancer families with (n = 35) or without (n = 110) TP53 mutations. Statistical analyses stratified on family structure showed that cancer tended to occur ~15 years later in mutation carriers who also carried the variant alleles of two polymorphisms within predicted G4-forming regions, rs17878362 (TP53 PIN3, 16 bp duplication in intron 3; P = 0.082) and rs17880560 (6 bp duplication in 3' flanking region; P = 0.067). Haplotype analysis showed that this inverse association was driven by the polymorphic status of the remaining wild-type (WT) haplotype in mutation carriers: in carriers with a WT haplotype containing at least one variant allele of rs17878362 or rs17880560, cancer occurred ~15 years later than in carriers with other WT haplotypes (P = 0.019). No effect on age of cancer onset was observed in subjects without a TP53 mutation. The G4 in intron 3 has been shown to regulate alternative p53 messenger RNA splicing, whereas the biological roles of predicted G4s in the 3' flanking region remain to be elucidated. In conclusion, this study demonstrates that G4 polymorphisms in haplotypes of the WT TP53 allele have an impact on LFS/LFL penetrance in germline TP53 mutation carriers.

69. **Salomaa S**, Prise KM, Atkinson MJ, Wojcik A, Auvinen A, Grosche B, Sabatier L, Jourdain JR, Salminen E, Baatout S, Kulka U, Rabus H, Blanchardon E, Averbeck D, Weiss W.: State of the art in research into the risk of low dose radiation exposure- findings of the fourth MELODI workshop. J Radiological Protection, 2013, Sep, 33(3): 589-603. (WP1-7; STUK, HMGU, SU, BfS, CEA, IRSN, SCK-CEN and BfS)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Tissue sensitivity
Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

The fourth workshop of the Multidisciplinary European Low Dose Initiative (MELODI) was organized by STUK—Radiation and Nuclear Safety Authority of Finland. It took place from 12 to 14 September 2012 in Helsinki, Finland. The meeting was attended by 179 scientists and professionals engaged in radiation research and radiation protection. We summarize the major scientific findings of the workshop and the recommendations for updating the MELODI Strategic Research Agenda and Road Map for future low dose research activities.

70. **Salomaa S**, Prise KM, Atkinson MJ, Wojcik A, Auvinen A, Grosche B, Sabatier L, Jourdain JR, Salminen E, Baatout S, Kulka U, Rabus H, Blanchardon E, Averbeck D, Weiss W.: Reply to 'State of the art in research into the risk of low dose radiation exposure'. J Radiol Prot. 2014 Mar; 34 (1):259-260. (WP1-7; STUK, HMGU, SU, BfS, CEA, IRSN, SCK-CEN and BfS)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Tissue sensitivity
Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

Comment on: State of the art in research into the risk of low dose radiation exposure--findings of the fourth MELODI workshop. [J Radiol Prot. 2013]; State of the art in research into the risk of low dose radiation exposure. [J Radiol Prot. 2014]

71. **Salomaa, S,** Averbeck, D, Ottolenghi, A, Sabatier, L, Bouffler, S, Atkinson, M and Jourdain, J-R.: European low-dose radiation risk research strategy: Future of research on biological effects at low doses. Radiation Protection Dosimetry (2014), pp. 1–4. (WP1-7; STUK, IRSN, UNIPV, CEA, DH-PHE and HMGU)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Tissue sensitivity
Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

In 2009, the European High Level and Expert Group identified key policy and scientific questions to be addressed through a strategic research agenda for low-dose radiation risk. This initiated the establishment of a European Research Platform, called MELODI (Multidisciplinary European Low Dose Research Initiative). In 2010, the DoReMi Network of Excellence was launched in the Euratom 7th Framework Programme. DoReMi has acted as an operational tool for the sustained development of the MELODI platform during its early years. A long-term Strategic Research Agenda for European low-dose radiation risk research has been developed by MELODI. Strategic planning of DoReMi research activities is carried out in close collaboration with MELODI. The research priorities for DoReMi are designed to focus on objectives that are achievable within the 6-y lifetime of the project and that are in areas where stimulus and support can help progress towards the longer-term strategic objectives.

72. **Samari N.** De Saint-Georges L, Pani G, Baatout S, Leyns L, Benotmane MA.: Non-conventional apoptotic response to ionizing radiation mediated by N-methyl D-aspartate receptors in immature neuronal cells. Int J Mol Med 2013, Mar, 31(3), 516-524. (Task 7.5; SCK-CEN)
Non-cancer
Tissue sensitivity
Non-targeted effects (bystander)

During cortical development, N-methyl D-aspartate (NMDA) receptors are highly involved in neuronal maturation and synapse establishment. Their implication in the phenomenon of excitotoxicity has been extensively described in several neurodegenerative diseases due to the permissive entry of Ca2+ ions and massive accumulation in the intracellular compartment, which is highly toxic to cells. Ionising radiation is also a source of stress to the cells, particularly immature neurons. Their capacity to induce cell death has been described for various cell types either by directly damaging the DNA or indirectly through the generation of reactive oxygen species responsible for the activation of a battery of stress response effectors leading in certain cases, to cell death. In this study, in order to determine whether a link exists between NMDA receptors-mediated excitotoxicity and radiation-induced cell death, we evaluated radiation-induced cell death in vitro and in vivo in maturing neurons during the fetal period. Cell death induction was assessed by TUNEL, caspase-3 activity and DNA ladder assays, with or without the administration of dizocilpine (MK-801), a non-competitive NMDA receptor antagonist which blocks neuronal Ca2+ influx. To further investigate the possible involvement of Ca2+-dependent enzyme activation, known to occur at high Ca2+ concentrations, we examined the protective effect of a calpain inhibitor on cell death induced by radiation. Doses ranging from 0.2 to 0.6 Gy of X-rays elicited a clear apoptotic response that was prevented by the injection of dizocilpine (MK-801) or calpain inhibitor. These data demonstrate the involvement of NMDA receptors in radiation-induced neuronal death by the activation of downstream effectors, including calpain-related pathways. An increased apoptotic process elicited by radiation, occurring independently of the normal developmental scheme, may eliminate post-mitotic but immature neuronal cells and deeply impair the establishment of the neuronal network, which in the case of cortical development is critical for cognitive capacities.

73. **Schanz, S.,** Flockerzi, E., Schuberth, K., Rübe, CE.: Genetically-Defined DNA Repair Capacity Determines the Extent of DNA Damage Accumulation in Healthy Mouse Tissues after Very Low Doses of Ionizing Radiation. J Carcinog Mutagen 2014, 5:6, 2014, pp. 1-8. (Tasks 6.1 and 6.10; USAAR)
Non-cancer effects
Individual sensitivity
Tissue Sensitivity

The biological impact of low doses of ionizing radiation on human health and the genetic factors influencing whole organism radio-sensitivity at low doses are unclear. Using mouse strains that varied in genetic DNA repair capacity (C57BL/6, ATM +/+, ATM +/-, ATM -/-, SCID), we analyzed DNA damage in differentiated cell populations of healthy tissues after repeated low doses of radiation. After 2, 4, 6, 8, and 10 weeks of daily, low-dose radiation (10 mGy), persistent DNA damage foci were counted in the lung (bronchiolar and alveolar cells), heart (cardiomyocytes), and brain (cortical neurons). In all analyzed tissues, the gradual accumulation of DNA damage with increasing doses of fractionated radiation was observed. No verifiable threshold-dose was detected, even in repair-proficient organisms (C57BL/6, ATM +/+). The number of radiation-induced foci varied significantly between the different cell populations, suggesting differing vulnerability to ionizing radiation. Genetic DNA repair capacity also determined the cumulative amount of low-dose radiation damage, with the highest foci levels observed in repair-deficient ATM -/- and SCID mice. The repair capacity of ATM heterozygous mice (ATM +/-), however, was sufficient to cope with the DNA damage burden induced by repetitive low-dose radiation. Collectively, our findings suggest that even very low doses of DNA-damaging radiation increase the health risks of individuals, particularly of those with compromised DNA repair capacity.

74. **Schauer C,** Janko C, Munoz LE, Zhao Y, Kienhöfer D, Frey B, Lell M, Manger B, Rech J, Naschberger E, Holmdahl R, Krenn V, Harrer T, Jeremic I, Bilyy R, Schett G, Hoffmann M, Herrmann M.: Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat Med. 2014 May;20 (5):511-7. (Task 5.2.1; UKER)
Cancer
Non-cancer

Non-targeted effects (bystander)

Gout is characterized by an acute inflammatory reaction and the accumulation of neutrophils in response to monosodium urate (MSU) crystals. Inflammation resolves spontaneously within a few days, although MSU crystals can still be detected in the synovial fluid and affected tissues. Here we report that neutrophils recruited to sites of inflammation undergo oxidative burst and form neutrophil extracellular traps (NETs). Under high neutrophil densities, these NETs aggregate and degrade cytokines and chemokines via serine proteases. Tophi, the pathognomonic structures of chronic gout, share characteristics with aggregated NETs, and MSU crystals can induce NETosis and aggregation of NETs. In individuals with impaired NETosis, MSU crystals induce uncontrolled production of inflammatory mediators from neutrophils and persistent inflammation. Furthermore, in models of neutrophilic inflammation, NETosis-deficient mice develop exacerbated and chronic disease that can be reduced by adoptive transfer of aggregated NETs. These findings suggest that aggregated NETs promote the resolution of neutrophilic inflammation by degrading cytokines and chemokines and ,disrupting neutrophil recruitment and activation.

75. **Schmitt E,** Friedland W, Kundrat P, Dingfelder, M and Ottolenghi, A.: Cross-section scaling for track structure simulations of low-energy ions in liquid water. Radiation Protection Dosimetry Advanced access May 2015. (Task 5.6; HMGU and UNIPV)
Radiation quality
Modelling

Radiation damage by low-energy ions significantly contributes to the high biological efficiency of ion beams in distal Bragg peak regions as well as to the energy-dependent efficiency of neutron irradiation. To enable assessing biological effects of ions at energies <1 MeV u^{-1} with track-structure based models, a Barkas-like scaling procedure is developed that provides ion cross sections in liquid water based on those for hydrogen ions. The resulting stopping power and range for carbon ions agree with the ICRU 73 database and other low-energy stopping power data. The method represents the basis for extending PARTRAC simulations of light ion track structures and biological effects down to the keV u^{-1} range.

76. **Shim G,** Ricoul M, Hempel WM, Azzam EI, Sabatier L.: Crosstalk between telomere maintenance and radiation effects: A key player in the process of radiation-induced carcinogenesis. Mutat Res. Rev Mutat Res 2014, Jan 31, 760, 1-17. (Tasks 5.1 and 6.2; CEA)
Cancer
Individual sensitivity
Radiation quality

It is well established that ionizing radiation induces chromosomal damage, both following direct radiation exposure and via non-targeted (bystander) effects, activating DNA damage repair pathways, of which the proteins are closely linked to telomeric proteins and telomere maintenance. Long-term propagation of this radiation-induced chromosomal damage during cell proliferation results in chromosomal instability. Many studies have shown the link between radiation exposure and radiation-induced changes in oxidative stress and DNA damage repair in both targeted and non-targeted cells. However, the effect of these factors on telomeres, long established as guardians of the genome, still remains to be clarified. In this review, we will focus on what is known about how telomeres are affected by exposure to low- and high-LET ionizing radiation and during proliferation, and will discuss how telomeres may be a key player in the process of radiation-induced carcinogenesis

77. **Tanori M,** Pasquali E, Leonardi S, Casciati A, Giardullo P, De Stefano I, Mancuso M, Saran A, Pazzaglia S.: Developmental and oncogenic radiation effects on neural cells and their differentiating progeny in mouse cerebellum. Stem Cells 2013, Nov, 31(11), 2506-2516. (Task

4.6; ENEA)
Cancer
Tissue sensitivity

Neural stem cells are highly susceptible to radiogenic DNA damage, however, little is known about their mechanisms of DNA damage response (DDR) and the long-term of genotoxic exposure. Patched1 heterozygous mice (Ptc11/2) provide a powerful model of medulloblastoma (MB), a frequent pediatric tumor of the cerebellum. Irradiation of newborn Ptc11/2 mice dramatically increases the frequency and shortens the latency of MB. In this model, we investigated the mechanisms through which multipotent neural progenitors (NSCs) and fate restricted progenitor cells (PCs) of the cerebellum respond to DNA damage induced by radiation, and the long-term developmental and oncogenic consequences. These responses were assessed in mice exposed to low (0.25 Gy) or high (3 Gy) radiation doses at embryonic day 13.5(E13.5), when NSCs giving rise to the cerebellum are specified but the external granule layer (EGL) has not yet formed, or at E16.5, during the expansion of granule PCsto form the EGL. We found crucial differences in DDR and apoptosis between NSCs and fate-restricted PCs, including lack of p21 expression in NSCs. NSCs also appear to be resistant to oncogenesis from low-dose radiation exposure but more vulnerable at higher doses. In addition, the pathway to DNA repair and the pattern of oncogenic alterations were strongly dependent on age at exposure, highlighting a differentiation-stage specificity of DNA repair pathways in NSCs and PCs. These findings shed light on the mechanisms used by NSCs and PCs to maintain genome integrity during neurogenesis and may have important implications for radiation risk assessment and for development of targeted therapies against brain
tumors.

78. **Verbiest T,** Bouffler S, Nutt SL, Badie C.: PU.1 downregulation in murine radiation-induced acute myeloid leukaemia (AML): from molecular mechanism to human AML. Carcinogenesis. 2015 Mar 6. pii: bgv016. [Epub ahead of print] (Task 5.3; DH-PHE)
Cancer

The transcription factor PU.1, encoded by the murine Sfpi1 gene (SPI1 in humans), is a member of the Ets transcription factor family and plays a vital role in commitment and maturation of the myeloid and lymphoid lineages. Murine studies directly link primary acute myeloid leukaemia (AML) and decreased PU.1 expression in specifically modified strains. Similarly, a radiation-induced chromosome 2 deletion and subsequent Sfpi1 point mutation in the remaining allele lead to murine radiation-induced AML. Consistent with murine data, heterozygous deletion of the SPI1 locus and mutation of the -14kb SPI1 upstream regulatory element were described previously in human primary AML, although they are rare events. Other mechanisms linked to PU.1 downregulation in human AML include TP53 deletion, FLT3-ITD mutation and the recurrent AML1-ETO [t(8;21)] and PML-RARA [t(15;17)] translocations. This review provides an up-to-date overview on our current understanding of the involvement of PU.1 in the initiation and development of radiation-induced AML, together with recommendations for future murine and human studies

79. **Wunderlich R**, Ernst A, Rödel F, Fietkau R, Ott O, Lauber K, Frey B, Gaipl US.: Low and moderate dose of ionising radiation up to 2 Gy modulates transmigration and chemotaxis of activated macrophages, provokes an anti-inflammatory cytokine milieu, but does not impact on viability and phagocytic function. Clin Exp Immunol. 2015 Jan;179(1):50-61. (Task 5.2.1; GUF and UKER)
Cancer
Tissue sensitivity
Non-targeted effects (bystander)

Benign painful and inflammatory diseases are treated for decades with low/moderate doses of ionizing radiation (LD-X-irradiation). Tissue macrophages regulate initiation and resolution of inflammation by the secretion of cytokines and by acting as professional phagocytes. Having these pivotal functions, we were interested in how activated macrophages are modulated by LD-X-irradiation, also with regard to radiation protections issues and carcinogenesis. We set up an ex-vivo model in which lipopolysaccharide pre-activated peritoneal macrophages (pMΦ) of radiosensitive BALB/c mice, mimicking activated macrophages under inflammatory conditions, were exposed to X-irradiation from 0.01 Gy up to 2 Gy. Afterwards, the viability of the pMΦ, their transmigration and chemotaxis, the phagocytic behavior, the secretion of inflammatory cytokines and underlying signaling pathways were determined. Exposure of pMΦ up to a single dose of 2 Gy did not influence their viability and phagocytic function, an important fact regarding radiation protection. However, a significantly reduced migration, but an increased chemotaxis of pMΦ after exposure to 0.1 or 0.5 Gy was detected. Both might get along with resolution of inflammation. Cytokine analyses revealed that especially the moderate dose of 0.5 Gy applied in low dose radiotherapy for inflammatory diseases results in an anti-inflammatory cytokine microenvironment of pMΦ, as the secretion of the pro-inflammatory cytokine IL-1ß was reduced and that of the anti-inflammatory cytokine TGF-ß increased. Further, the reduced secretion of IL-1ß correlated with reduced nuclear translocation of NFκB p65, starting at exposure of pMΦ to 0.5 Gy of X-irradiation. We conclude that inflammation is modulated by LD-X-irradiation via changing the inflammatory phenotype of macrophages.

80. **Yentrapalli R,** Azimzadeh O, Barjaktarovic Z, Sarioglu H, Wojcik A, Harms-Ringdahl M, Atkinson MJ, Haghdoost S, Tapio S.: Quantitative proteomic analysis reveals induction of premature senescence in human umbilical vein endothelial cells exposed to chronic low dose rate gamma radiation, Proteomics 2013,Apr, 13(7), 1096-1107. (Task 7.3; HMGU and SU)
Non-cancer
Tissue sensitivity

Chronic low-dose ionizing radiation induces cardiovascular disease in human populations but the mechanism is largely unknown. We suggested that chronic radiation exposure may induce endothelial cell senescence that is associated with vascular damage in vivo. We investigated whether chronic radiation exposure is causing a change in the onset of senescence in endothelial cells in vitro. Indeed, when exposed to continuous low-dose rate gamma radiation (4.1 mGy/h), primary human umbilical vein endothelial cells (HUVECs) initiated senescence much earlier than the non irradiated control cells. We investigated the changes in the protein expression of HUVECs before and during the onset of radiation-induced senescence. Cellular proteins were quantified using isotope-coded protein label technology after 1, 3, and 6 weeks of radiation exposure. Several senescence-related biological pathways were influenced by radiation, including cytoskeletal organization, cell-cell communication and adhesion, and inflammation. Immunoblot analysis showed an activation of the p53/p21 pathway corresponding to the progressing senescence. Our data suggest that chronic radiation-induced DNA damage and oxidative stress result in induction of p53/p21 pathway that inhibits the replicative potential of HUVECs and leads to premature senescence. This study contributes to the understanding of the increased risk of cardiovascular diseases seen in populations exposed to chronic low-dose irradiation.

81. **Yentrapalli R,** Azimzadeh O, Sriharshan A, Malinowsky K, Merl J, Wojcik A, Harms-Ringdahl M, Atkinson MJ, Becker KF, Haghdoost S, Tapio S.: The PI3K/Akt/mTOR pathway is implicated in the premature senescence of primary human endothelial cells exposed to chronic radiation, PLos One 2013,Aug 1; 8(8): e70024. (Task 7.3; HMGU and SU)
Non-cancer
Tissue sensitivity

The etiology of radiation-induced cardiovascular disease (CVD) after chronic exposure to low doses of ionizing radiation is only marginally understood. We have previously shown at a chronic low-dose rate exposure (4.1 mGy/h) causes human umbilical vein endothelial cells (HUVECs) to prematurely senesce. We now show that a dose rate of 2.4 mGy/h is also able to trigger premature senescence in HUVECs, primarily indicated by a loss of growth potential and the appearance of the senescence-associated markers ß-galactosidase (SA-ß-gal) and p21. In contrast, a lower dose rate of 1.4 mGy/h was not sufficient to inhibit cellular growth or increase SA-ß-gal-staining despite an increased expression of p21. We used reverse phase protein arrays and triplex Isotope Coded Protein Labeling with LC-ESI-MS/MS to study the proteomic changes associated with chronic radiation-induced senescence. Both technologies identified inactivation of the PI3K/Akt/mTOR pathway accompanying premature senescence. In addition, expression of proteins involved in cytoskeletal structure and EIF2 signaling was reduced. Age- associated with increased endothelial cell senescence. We postulate that a similar endothelial aging may contribute to the increased rate of CVD seen in populations chronically exposed of low-dose-rate radiation.