January 2017, version 5

DoReMi Barometer of publications

Aim of the barometer

The DoReMi project started in January 2010 and ended 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: WP4 Infrastructures, WP5 Shape of dose response, WP6 Individual sensitivities and WP7 Non-cancer effects (for description of scientific content of each WP, see here).

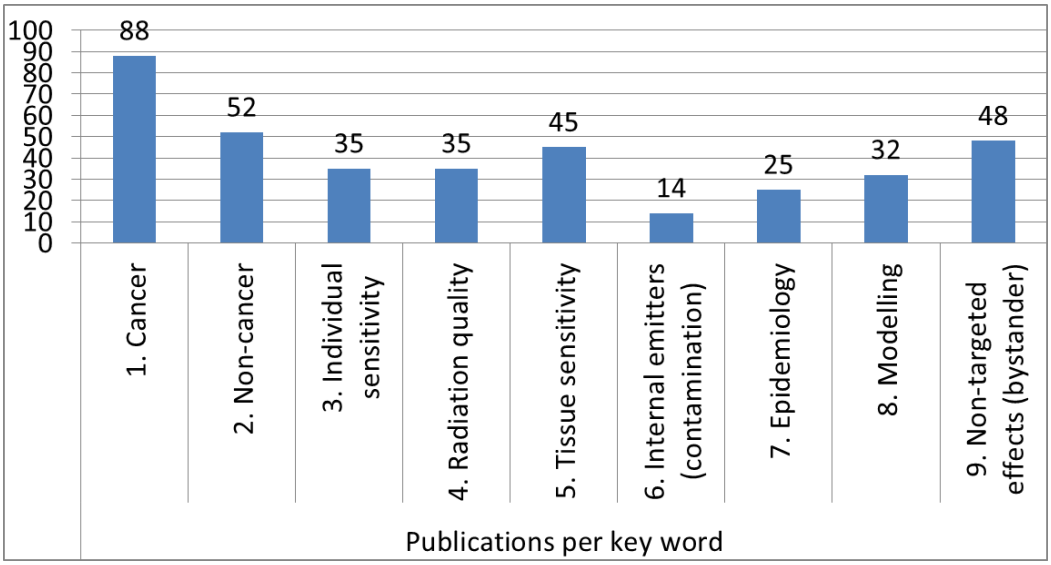
The results are presented in form of a DoReMi Barometer of the publications that is subject to regular updating due to still forthcoming DoReMi publications.

This document is the version 5 of the Barometer, published in January 2017, representing the status of the publications after 12 months from the end of the project.

Results

Progress by key word

By December 2016, there are altogether 129 DoReMi publications (see list of publications as Appendix 1). Looking by the key words, most of the DoReMi publications are related to key word "Cancer" (88 publications out of 129 are related to this key word). In the second place, there is the key word "Non-cancer", with 52 publications, closely followed by Non-targeted effects (bystander) (48 publications) and "Tissue sensitivity" (45 publications). For the rest of the key words, the number of publications varies from 14 to 35.



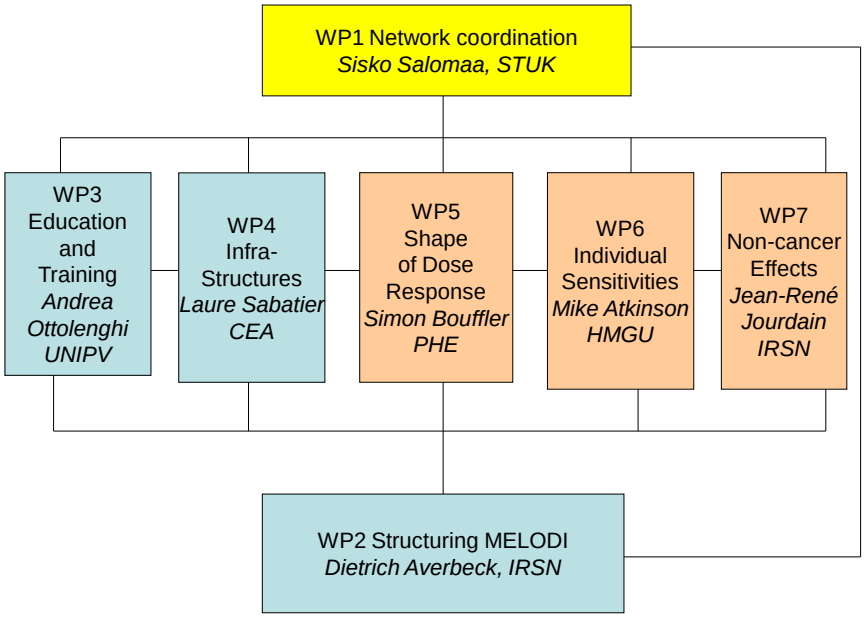
Picture 1: Publications per key word

Some analysis on the basis of key wording has also been performed. Most publications are on the impact of immunological and non-targeted processes regarding low dose radiation induced cancers (influence of microenvironment on cancer development or on treatments by radiotherapy). Less publications concern low dose and low dose rate and radiation quality on cancer or non-cancer effects. The number of publications concerning the development of biomarkers has increased favorably. Furthermore, there is clear progress in the mechanisms of low dose radiation induced cancer and non-cancers. Also, there is new information on the use of biomarkers in molecular epidemiology and the use of biomarkers for the detection of individual sensitivity among populations.

Progress by WP and Task

The distribution of publications differs according to the different tasks of the work packages concerning RTD research on low dose health risks. It should be noted that the DoReMi work plan was amended several times, mainly via three competitive calls for new partners, as well as via three internal calls, providing opportunities to existing partners. It should also be taken into account that many tasks that started in the beginning of the project were extended via internal ad hoc mechanism that allowed the DoReMi programme to develop further and to respond to current and topical needs.

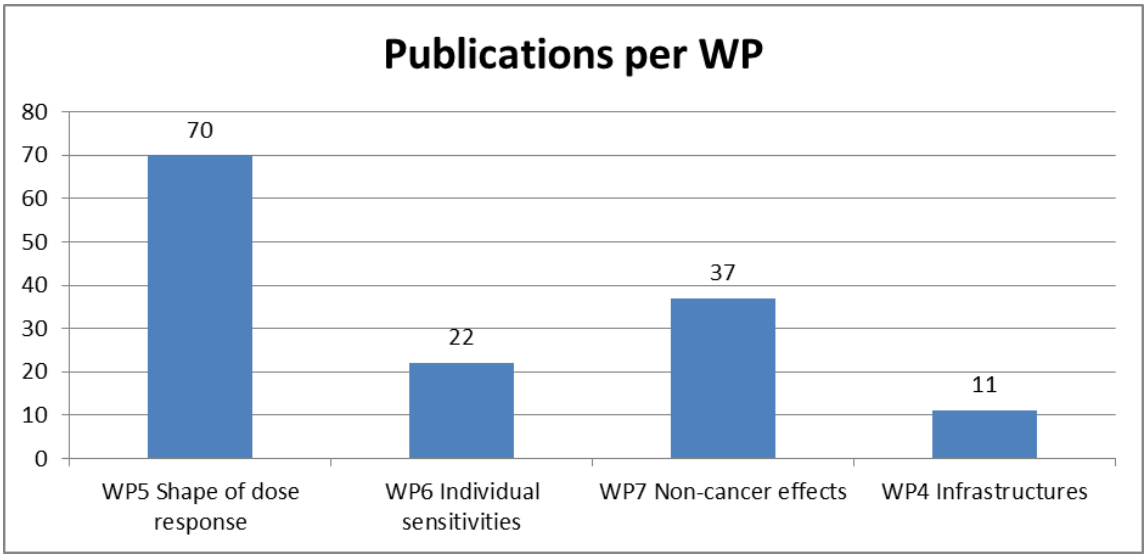
The work package structure of DoReMi was as follows:



Picture 2: DoReMi WP structure

Conclusion and outlook

The DoReMi barometer of publications and the updated publication list give a good account of the scientific progress of RTD studies achieved after the end of DoReMi. A decent number of publications are still expected to become available in the forthcoming months.

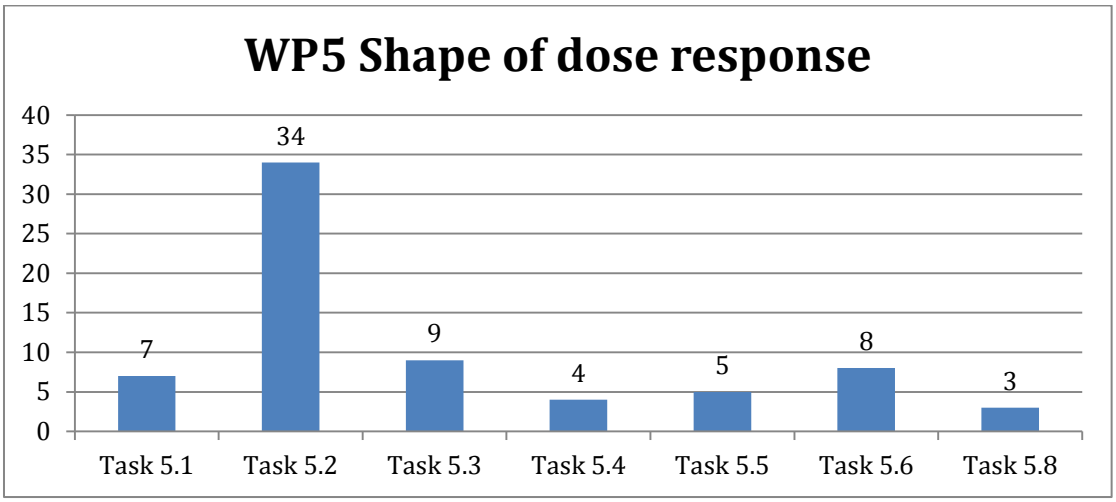


Picture 3: Publications per WP: The numbers indicate that apart from the effects low dose exposures on cancer, within DoReMi, the research issues low dose induced non-cancer effects and individual sensitivity have gained increased importance in the research for the improvement of radiation protection.

Out of 129 DoReMi publications:

- Nine publications could not be assigned solely to any individual work package as they are more general publications related to DoReMi activities as a whole.
- Ten publications are related to both WP5 and WP7, two publications to both WP5 and WP6 and two publications to both WP4 and WP5.
- In WP6, three publications are related to two different Tasks, and in WP5 and WP7, two publications are related to two different Tasks

Comparison within work packages has also been performed. In WP5 Shape of dose response, the publications are divided as follows:



Picture 4: WP5 publications per Task: The numbers reveal that inflammatory processes together with radiation targeted, non-targeted systemic processes have been recognized as important determinants for radiation-induced cancers.

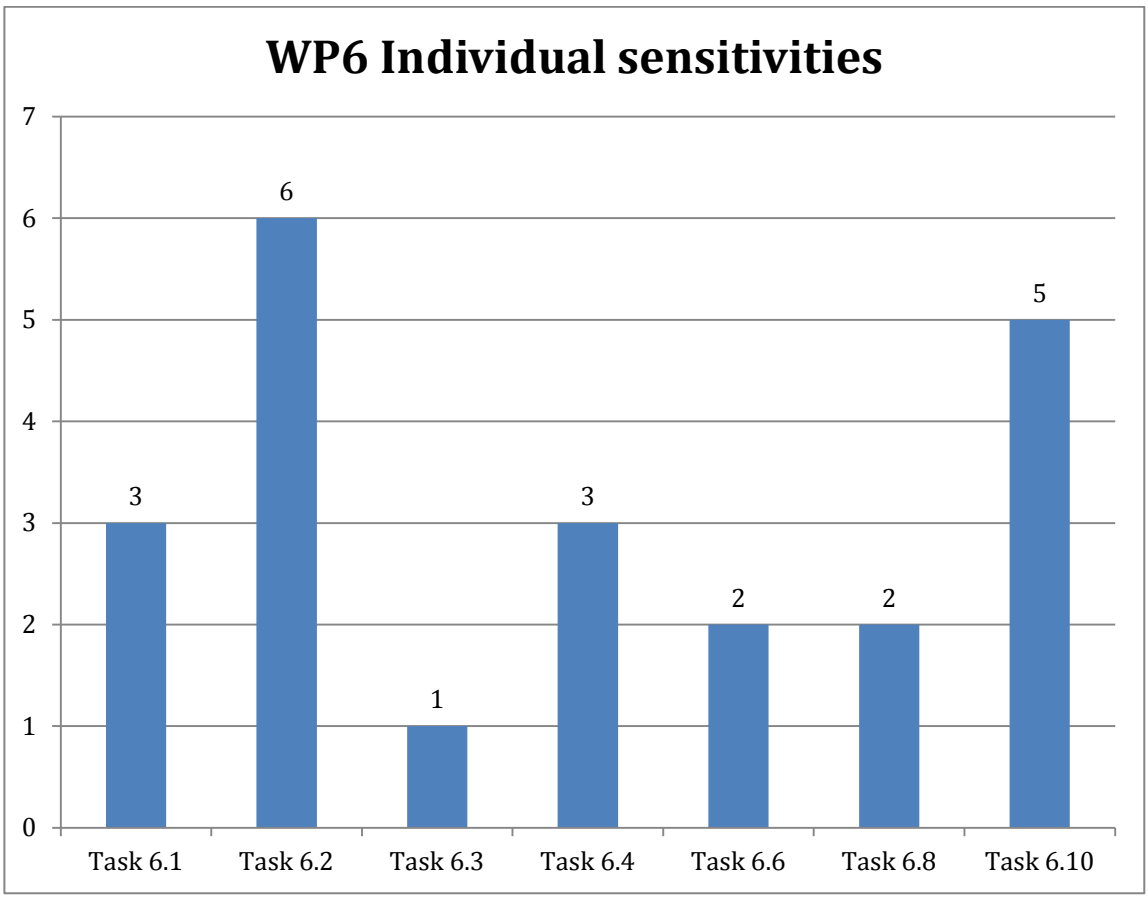
Table 1: WP5 Shape of dose response program enlargement

This table below provides further information on WP5 Tasks, their extensions (due to calls) and starting times.

Task	Work	Starting
5.1	Phase – shifts in responses and processes at high/low doses and dose rates	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	2010
5.2.1	**Modulation of Inflammation by low and moderate dose Ionising Radiation (ModInIR)**	**2011**
5.3	The dynamics of pre-neoplastic change and clonal development	2010
5.4	Mathematical models to link experimental findings and epidemiological data	2010

5.5	Assessing the risk from internal exposures	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)**	**2012**
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)**	**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**

In WP6 Individual sensitivities, the publications are divided as follows:



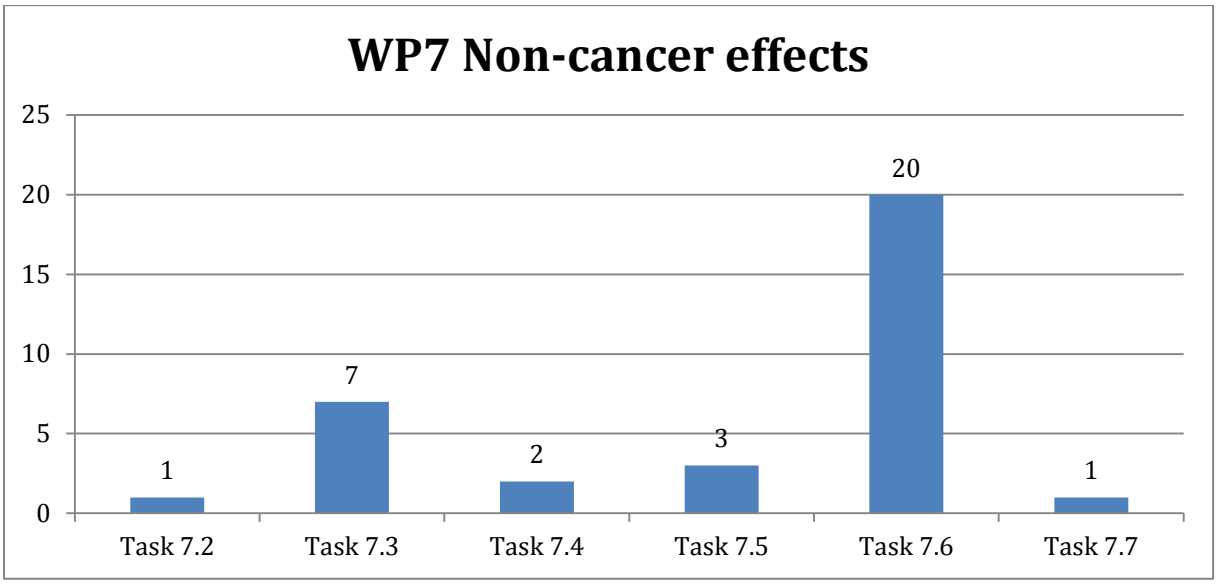
Picture 5: WP6 publications per Task. The numbers reflect the importance of genetic modifiers, the repair capacity for radiation induced DNA damage and the development of suitable biomarkers for the detection of individual radiation sensitivity among human populations.

Table 2: WP6 Individual sensitivities program enlargement

The table below provides further information on WP6 Tasks, their extensions (due to calls) and starting times.

Task	Work	Starting
6.1	Molecular epidemiological studies to address the role of individual genetic variation in determining susceptibility to low doses	2010
6.2	Identification of genetic modifiers of individual cancer susceptibility and their mechanisms of action	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 rate exposure	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	2010
6.7	Planning expansion of research portfolio	2010
6.8	**Predicting individual radiation sensitivity with Raman microspectroscopy (PRISM)**	**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)**	**2013**
6.11	**Mechanism of low dose response to ionizing radiation and its significance in radiation protection (RADSENS)**	**2013**

In WP7 Non-cancer effects, the publications are divided as follows:



Picture 7: WP7 publications per Task. The numbers indicate the growing importance of low dose induced non-cancer effects, the involvement of pro-and anti-inflammatory effects, and the focus within DoReMi on cardiovascular (endothelial cells), eye lens opacities and neurological effects.

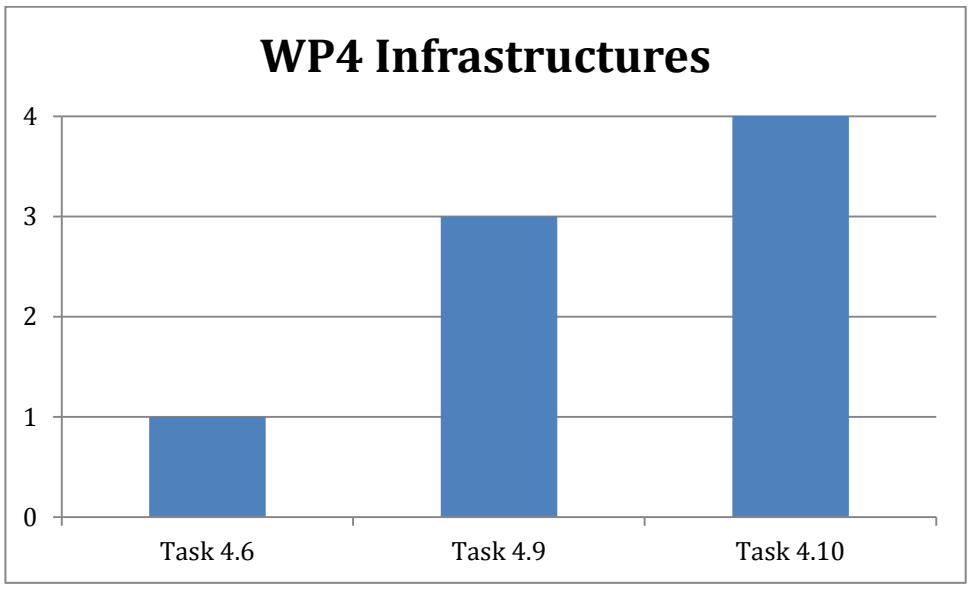
Table 3: WP7 Non-cancer effects program enlargement

The table below provides further information on WP7 Tasks, their extensions (due to calls) and their starting times.

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	2010
7.3	Feasibility study towards a systems biology approach of radiation response of the endothelium	2010
7.4	Pilot epidemiological study of lens opacities among a cohort of interventional 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	2010
7.6	**Study on contribution of low dose X-radiation in induction of anti-inflammation**	**2011**

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: invivo and invitro studies (RadCat)**	**2014**

In WP4 Infrastructures, the publications are divided as follows:



Picture 8: WP4 publications per Task: The numbers reflect the growing importance of suitable infrastructures for low dose and low dose rate research.

Table 4: WP4 Infrastructures program enlargement

The table below provides further information on WP4 Tasks, their extensions (due to calls) and their starting times.

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)**	**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)**	**2013**
4.10	**Laboratory infrastructure for retrospective radon and thoron dosimetry (RETRODOS)**	**2014**

Appendix I : List of DoReMi publications – by December 2016

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 November 2016) 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.

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, 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.

6. **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.

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. Alloni D, Baiocco G, Babini G, Friedland W, Kundrat P, Mariotti L, Ottolenghi A. Energy dependence of the complexity of DNA damage induced by carbon ions. Radiat Prot Dosimetry 9 May 2015, 166 (1-4) 86-90. (Task 5.6, UNIPV)
Cancer
Radiation quality
Modeling

To assess the complexity of DNA damage induced by carbon ions as a function of their energy and LET, 2-Gy irradiations by 100 keV u(-1)-400 MeV u(-1) carbon ions were investigated using the PARTRAC code. The total number of fragments and the yield of fragments of <30 bp were calculated. The authors found a particularly important contribution of DNA fragmentation in the range of <1 kbp for specific energies of <6 MeV u(-1). They also considered the effect of different specific energies with the same LET, i.e. before and after the Bragg peak. As a first step towards a full characterisation of secondary particle production from carbon ions interacting with tissue, a comparison between DNA-damage induction by primary carbon ions and alpha particles resulting from carbon break-up is presented, for specific energies of >1 MeV u(-1).

9. 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 non-linear 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.

10. Babini G, Morini J, Baiocco G, Mariotti L, Ottolenghi A. In vitro γ-ray-induced inflammatory response is dominated by culturing conditions rather than radiation exposures. Scientific Reports 2015, 5, 9343 (Task 5.2, UNIPV)
Cancer
Non-targeted effects (bystander)

The inflammatory pathway has a pivotal role in regulating the fate and functions of cells after a wide range of stimuli, including ionizing radiation. However, the molecular mechanisms governing such responses have not been completely elucidated yet. In particular, the complex activation dynamics of the Nuclear transcription Factor kB (NF-kB), the key molecule governing

the inflammatory pathway, still lacks a complete characterization. In this work we focused on the activation dynamics of the NF-kB (subunit p65) pathway following different stimuli. Quantitative measurements of NF-kB were performed and results interpreted within a systems theory approach, based on the negative feedback loop feature of this pathway. Time-series data of nuclear NF-kB concentration showed no evidence of γ-ray induced activation of the pathway for doses up to 5 Gy but highlighted important transient effects of common environmental stress (e.g. CO2, temperature) and laboratory procedures, e.g. replacing the culture medium, which dominate the in vitro inflammatory response

11. Badie, C., Agnieszka Blachowicz, Zarko Barjaktarovic, Rosemary Finnon, Arlette Michaux, Hakan Sarioglu, Natalie Brown, Grainne Manning, M.Abderrafi Benotmane, Soile Tapio, Joanna Polanska, Simon D. Bouffler: Transcriptomic and proteomic analysis of mouse radiation–induced acute myeloid leukaemia (AML). Oncotarget. 2016 Jun 28;7(26):40461-40480. (Task 5.3; DH-PHE, HMGU, SCK-CEN)
Cancer

A combined transcriptome and proteome analysis of mouse radiation-induced AMLs using two primary AMLs, cell lines from these primaries, another cell line and its in vivo passage is reported. Compared to haematopoietic progenitor and stem cells (HPSC), over 5000 transcriptome alterations were identified, 2600 present in all materials. 55 and 3 alterations were detected in the proteomes of the cell lines and primary/in vivo passage material respectively, with one common to all materials. In cell lines, approximately 50% of the transcriptome changes are related to adaptation to cell culture, and in the proteome this proportion was higher. An AML 'signature' of 17 genes/proteins commonly deregulated in primary AMLs and cell lines compared to HPSCs was identified and validated using human AML transcriptome data. This also distinguishes primary AMLs from cell lines and includes proteins such as Coronin 1, pontin/RUVBL1 and Myeloperoxidase commonly implicated in human AML. C-Myc was identified as having a key role in radiation leukaemogenesis. These data identify novel candidates relevant to mouse radiation AML pathogenesis, and confirm that pathways of leukaemogenesis in the mouse and human share substantial commonality.

12. Baiocco, G., S. Barbieri, G. Babini, J. Morini, D. Alloni, W. Friedland, P. Kundrát, E. Schmitt, M. Puchalska, L. Sihver & A. Ottolenghi: The origin of neutron biological effectiveness as a function of energy. Sci Rep. 2016; 6: 34033. (Task 5.6 ; UNIPV and HMGU)
Radiation quality
Modeling

The understanding of the impact of radiation quality in early and late responses of biological targets to ionizing radiation exposure necessarily grounds on the results of mechanistic studies starting from physical interactions. This is particularly true when, already at the physical stage, the radiation field is mixed, as it is the case for neutron exposure. Neutron Relative Biological Effectiveness (RBE) is energy dependent, maximal for energies ~1 MeV, varying significantly among different experiments. The aim of this work is to shed light on neutron biological effectiveness as a function of field characteristics, with a comprehensive modeling approach: this brings together transport calculations of neutrons through matter (with the code PHITS) and the predictive power of the biophysical track structure code PARTRAC in terms of DNA damage evaluation. Two different energy dependent neutron RBE models are proposed: the first is phenomenological and based only on the characterization of linear energy transfer on a microscopic scale; the second is purely ab-initio and based on the induction of complex DNA damage. Results for the two models are compared and found in good qualitative agreement with current standards for radiation protection factors, which are agreed upon on the basis of RBE data.

13. Baselet, B., Rombouts, C., Benotmane, A.M., Baatout, S. and Aerts, An.: Cardiovascular

diseases related to ionizing radiation: The risk of low-dose exposure (Review). Int J Mol Med. 2016 Oct 17. (Tasks 7.1 and 7.3, SCK-CEN).
Non-cancer

Traditionally, non-cancer diseases are not considered as health risks following exposure to low doses of ionizing radiation. Indeed, non-cancer diseases are classified as deterministic tissue reactions, which are characterized by a threshold dose. It is judged that below an absorbed dose of 100 mGy, no clinically relevant tissue damage occurs, forming the basis for the current radiation protection system concerning non-cancer effects. Recent epidemiological findings point, however, to an excess risk of non-cancer diseases following exposure to lower doses of ionizing radiation than was previously thought. The evidence is the most sound for cardiovascular disease (CVD) and cataract. Due to limited statistical power, the dose-risk relationship is undetermined below 0.5 Gy; however, if this relationship proves to be without a threshold, it may have considerable impact on current low‐dose health risk estimates. In this review, we describe the CVD risk related to low doses of ionizing radiation, the clinical manifestation and the pathology of radiation-induced CVD, as well as the importance of the endothelium models in CVD research as a way forward to complement the epidemiological data with the underlying biological and molecular mechanisms.

14. **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.

15. **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.

16. **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.

17. Campa, A, Balduzzi M, Dini V, Esposito G, Tabocchini MA.: The complex interactions between radiation induced non-targeted effects and cancer. Volume 356, Issue 1, 1 January 2015, pages 126–136 (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.

18. Candeias SM, Gaipl US. The immune system in cancer prevention, development and therapy. Anti-Cancer Agents Med CHem 2016 16: 101-107. (Task 5.2.1; CEA and UKER)
Cancer
Non-targeted effects (bystander)

The immune system plays a pivotal role in the maintenance of the integrity of an organism. Besides the protection against pathogens, it is strongly involved in cancer prevention, development and defense. This review focuses on how the immune system protects against infections and trauma and on its role in cancer development and disease. Focus is set on the interactions of the innate and adaptive immune system and tumors. The role of IFN-γ as a pleiotropic cytokine that plays a very important role at the interface of innate and adaptive immune systems in tumor development and induction of anti-tumor immune responses is outlined. Further, immune cells as prognostic and predictive markers of cancer will be discussed. Data are provided that even the brain as immune privileged organ is subjected to immune surveillance and consequently also brain tumors. Immune therapeutic approaches for glioblastoma multiforme, the most frequent and malignant brain tumor, based on vaccination with dendritic cells are outlined and application of hyperthermia in form of magnetic nanoparticles is discussed. We conclude that the immune system and developing tumors are intimately intertwined. Anti-tumor immune responses can be prominently boosted by multimodal therapies aiming on the one hand to induce immunogenic tumor cell death forms and on the other hand to actively counteract the immune suppressive microenvironment based on the tumor itself.

19. Derer A, Deloch L, Rubner Y, Fietjkau Y, Frey B, Gaipl US. Radio-immunotherapy-induced immunogenic cancer cells as basis for induction of systemic anti-tumor immune responses-pre-clinical evidence and ongoing clinical applications. Frontiers in Immunology (review) 2015 Oct, 6: art 505, pp. 1-19. (Tasks 5.2.1 and 7.6; UKER and GUF)
Cancer
Non-targeted effects

Radiotherapy (RT) primarily aims to locally destroy the tumor via the induction of DNA damage in the tumor cells. However, the so-called abscopal, namely systemic and immune-mediated, effects of RT move over more and more in the focus of scientists and clinicians since combinations of local irradiation with immune therapy have been demonstrated to induce anti-tumor immunity. We here summarize changes of the phenotype and microenvironment of tumor cells after exposure to irradiation, chemotherapeutic agents, and immune modulating agents rendering the tumor more immunogenic. The impact of therapy-modified tumor cells and damage-associated molecular patterns on local and systemic control of the primary tumor, recurrent tumors, and metastases will be outlined. Finally, clinical studies affirming the bench-side findings of interactions and synergies of radiation therapy and immunotherapy will be discussed. Focus is set on combination of radio(chemo)therapy (RCT) with immune checkpoint inhibitors, growth factor inhibitors, and chimeric antigen receptor T-cell therapy. Well-deliberated combination of RCT with selected immune therapies and growth factor inhibitors bear the great potential to further improve anti-cancer therapies.

20. Derer A, Frey B, Fietkau R, Gaipl US **.** Immune-modulating properties of ionizing-radiation: ratyionale for the treatment of cancer by combination radiotherapy and immune checkpoint inhibitors. Cancer Immunol Immunother online 21 November 2015 pp1-7 (Task 5.2.1, UKER)
Cancer
Non-targeted effects (bystander)

Radiotherapy (RT) utilizes the DNA-damaging properties of ionizing radiation to control tumor growth and ultimately kill tumor cells. By modifying the tumor cell phenotype and the tumor microenvironment, it may also modulate the immune system. However, out-of-field reactions of RT mostly assume further immune activation. Here, the sequence of the applications of RT and immunotherapy is crucial, just as the dose and fractionation may be. Lower single doses may impact on tumor vascularization and immune cell infiltration in particular, while higher doses may impact on intratumoral induction and production of type I interferons. The induction of immunogenic cancer cell death seems in turn to be a common mechanism for most RT schemes. Dendritic cells (DCs) are activated by the released danger signals and by taking up tumor peptides derived from irradiated cells. DCs subsequently activate T cells, a process that has to be tightly controlled to ensure tolerance. Inhibitory pathways known as immune checkpoints exist for this purpose and are exploited by tumors to inhibit immune responses. Cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) on T cells are two major checkpoints. The biological concepts behind the findings that RT in combination with anti-CTLA-4 and/or anti-PD-L1 blockade stimulates CD8+ T cell-mediated anti-tumor immunity are reviewed in detail. On this basis, we suggest clinically significant combinations and sequences of RT and immune checkpoint inhibition. We conclude that RT and immune therapies complement one another

21. 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.

22. Dimitrova I, Georgiev S, Pressyanov D, Sabot B, Michielsen N, Bondiquel S, Mitev K. Influence of the type of CD case on the track density distribution in CDs exposed to thoron. Appl Radiat Isot 2015 Dec 290015: doi: 10.1016. (Tasks 4.10; SUN)
Radiation quality

A novel approach for retrospective thoron (Rn-220) measurements in buildings was recently proposed. It employs CDs/DVDs as detectors, in which the alpha-tracks formed by thoron's progeny Po-212 are analyzed. Compact disks and DVDs that had been stored in their cases are suitable, because the case provides a fixed and reproducible geometry of the Po-212 source. Since the measurement and calibration procedures involve analysis of different pieces of the disk, it is important to test the homogeneity of the track density over the disk area. This report presents results of such a study, in which disks in different containers were exposed to thoron. In almost all disks, the track density was inhomogeneous, increasing significantly near the holes in the container through which thoron could enter. However, as demonstrated, in disks regularly used and randomly rotated in their containers, the track density is expected to homogenize. It is concluded that the homogeneity of the tracks should be tested in disks exposed to thoron in homes and should be estimated or compensated in calibration exposures.

23. Domienik, J., Farah, J. and Struelens, L.: Validation of ELDO approaches for retrospective assessment of cumulative eye lens doses of interventional cardiologists—results from DoReMi project. J. Radiol. Prot. 36 (2016) 736–745. (Task 7.11; NIOM, IRSN and SCK-CEN).
Non-cancer
Epidemiology

The first validation results of the two approaches developed in the ELDO project for retrospective assessment of eye lens doses for interventional cardiologists (ICs) are presented in this paper. The first approach (a) is based on both the readings from the routine whole body dosimeter worn above the lead apron and procedure-dependent conversion coefficients, while the second approach (b) is based on detailed information related to the occupational exposure history of the ICs declared in a questionnaire and eye lens dose records obtained from the relevant literature. The latter approach makes use of various published eye lens doses per procedure as well as the appropriate correction factors which account for the use of radiation protective tools designed to protect the eye lens. To validate both methodologies, comprehensive measurements were performed in several Polish clinics among recruited physicians. Two dosimeters measuring whole body and eye lens doses were worn by every physician for at least two months. The estimated cumulative eye lens doses, calculated from both approaches, were then compared against the measured eye lens dose value for every physician separately. Both approaches results in comparable estimates of eye lens doses and tend to overestimate rather than underestimate the eye lens doses. The measured and estimated doses do not differ, on average, by a factor higher than 2.0 in 85% and 62% of the cases used to validate approach (a) and (b), respectively. In specific cases, however, the estimated doses differ from the measured ones by as much as a factor of 2.7 and 5.1 for method (a) and (b), respectively. As such, the two approaches can be considered accurate when retrospectively estimating the eye lens doses for ICs and will be of great benefit for ongoing epidemiological studies.

24. Domienik, J., Gryglak, S. and Jurewicz, J.: Characteristics of interventional cardiologists and their work practices for the study on radiation-induced lens opacities based on the methodology developed by ELDO—preliminary results. Journal of Radiation Research, Vol. 57, No. 4, 2016, pp. 431–437. (Task 7.11; NIOM).
Non-cancer
Epidemiology

Preliminary results of the Polish epidemiology study on eye lens opacities among interventional cardiologists (ICs), based on the methodology proposed by ELDO (epidemiological studies of radio-induced cataracts in interventional cardiologists and radiologists: methodology implementation), are presented. The aim of the study is to test the hypothesis concerning the excess risk of cataract in the group of ICs. The first results concern the study population characteristics, including the most important confounding factors for cataract, as well as a detailed description of the work practices in interventional cardiology needed in order to reconstruct the cumulative eye lens dose. The data from 69 ICs and 23 controls collected based on the general medical questionnaire and the occupational questionnaire (for ICs only) were analyzed. The mean age of ICs and of the control group was 41 and 44, respectively, while the mean duration of work for exposed physicians was 9 years. The analysis of the data from the occupational questionnaire concerning the procedures performed, the use of various access routes, as well as radiation protection tools (eye lens glasses, ceiling suspended transparent shield, etc.) are also presented. On the basis of this information and additional assumptions about the doses per procedure (as well as reduction factors for various types of radiation measures), the cumulative doses to the eye lens of ICs were evaluated. They ranged up to 1.55 Sv and 0.4 Sv for left and right eye, respectively; however, the dose to only 3% of ICs exceeded the new threshold for development of eye lens opacities (0.5 Gy) proposed by the ICRP.

25. 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.

26. 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.

27. 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

28. 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.

29. Esposito, G., Anello, P., Pecchia, I., Tabocchini, M.A., Campa, A.: Facility for gammairradiations of cultured cells at low dos erates: design, physical characteristics and functioning. Applied Radiation and Isotopes 115 (2016) 227–234. (Task 4.7; ISS).
Cancer
Non-cancer
Individual sensitivity
Tissue sensitivity
Modelling
Non-targeted effects (bystander)

We describe a low dose/dose rate gamma irradiation facility (called LIBIS) for in vitro biological systems, for the exposure, inside a CO2 cell culture incubator, of cells at a dose rate ranging from few μGy/h to some tens of mGy/h. Three different 137Cs sources are used, depending on the desired dose rate. The sample is irradiated with a gamma ray beam with a dose rate uniformity of at least 92% and a percentage of primary 662 keV photons greater than 80%. LIBIS complies with high safety standards.

30. 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.

31. Farah J, Struelens L, Auvinen A, Jacob S, Koukorava C, Schnelzer M, Vanhavere F, Clairand I. Application of the ELDO approach to assess cumulative eye lens doses for

interventional cardiologists. Radiat Prot. Dosimetry 2015, 164(1-2) 84-88. (Task 7.4; IRSN, SCK-CEN and STUK)
Non-cancer

In preparation of a large European epidemiological study on the relation between eye lens dose and the occurrence of lens opacities, the European ELDO project focused on the development of practical methods to estimate retrospectively cumulative eye lens dose for interventional medical professionals exposed to radiation. The present paper applies one of the ELDO approaches, correlating eye lens dose to whole-body doses, to assess cumulative eye lens dose for 14 different Finnish interventional cardiologists for whom annual whole-body dose records were available for their entire working period. The estimated cumulative left and right eye lens dose ranged from 8 to 264 mSv and 6 to 225 mSv, respectively. In addition, calculations showed annual eye lens doses sometimes exceeding the new ICRP annual limit of 20 mSv. The work also highlights the large uncertainties associated with the application of such an approach proving the need for dedicated dosimetry systems in the routine monitoring of the eye lens dose.

32. Finkel, P., Frey, B., Mayer, F., Bösl, K., Werthmöller, N., Mackensen, A., Gaipl, U.S. and Ullrich, E.: The dual role of NK cells in antitumor reactions triggered by ionizing radiation in combination with hyperthermia. OncoImmunology, 5:6, e1101206. (Task 5.2.1, UKER, GUF).
Cancer
Non-targeted effects (bystander)

Classical tumor therapy consists of surgery, radio(RT)- and/or chemotherapy. Additive immunotherapy has gained in impact and antitumor in situ immunization strategies are promising to strengthen innate and adaptive immune responses. Immunological effects of RT and especially in combination with immune stimulation are mostly described for melanoma. Since hyperthermia (HT) in multimodal settings is capable of rendering tumor cells immunogenic, we analyzed the in vivo immunogenic potential of RT plus HT-treated B16 melanoma cells with an immunization and therapeutic assay. We focused on the role of natural killer (NK) cells in the triggered antitumor reactions. In vitro experiments showed that RT plus HT-treated B16 melanoma cells died via apoptosis and necrosis and released especially the danger signal HMGB1. The in vivo analyses revealed that melanoma cells are rendered immunogenic by RT plus HT. Especially, the repetitive immunization with treated melanoma cells led to an increase in NK cell number in draining lymph nodes, particularly of the immune regulatory CD27 CD11b NK cell subpopulation. While permanent NK cell depletion after immunization led to a significant acceleration of tumor outgrowth, a single NK cell depletion two days before immunization resulted in significant tumor growth retardation. The therapeutic model, a local in situ immunization closely resembling the clinical situation when solid tumors are exposed locally to RT plus HT, confirmed these effects. We conclude that a dual and time-dependent impact of NK cells on the efficacy of antitumor immune reactions induced by immunogenic tumor cells generated with RT plus HT exists.

33. 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.

34. **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.

35. **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.

36. **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.

37. **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.

38. **Frey B**, Hehlgans S, Rödel F, Gaipl US. Modulation of inflammation by low and high doses of ionizing radiation: Implications for benign and malign diseases. Cancer Letters 2015, 368, 230-237. (Tasks 5.2.1 and 7.6; UKER and GUF)
Cancer
Non-cancer
Non-targeted effects (bystander)

Inflammation is a homeostatic mechanism aiming to maintain tissue integrity. The underlying immunological mechanisms and the interrelationship between ionizing radiation and inflammation are complex and multifactorial on cellular and chemical levels. On the one hand, radiation with single doses exceeding 1 Gy might initiate inflammatory reactions and thereby impact on tumor development. On the other hand, radiation is capable of attenuating an established inflammatory process, which is clinically used for the treatment of inflammatory and degenerative diseases with low-dose radiotherapy (single dose <1 Gy). At higher doses, ionizing radiation, especially in combination with additional immune stimulation, fosters the induction of immunogenic forms of tumor cell death and shifts the tumor microenvironment as well as the infiltration of immune cells from an anti- to a pro-inflammatory state. Distinct tumor infiltrating immune cells predict the response to radiochemotherapy in a multitude of tumor entities. While a high tumor infiltration of these adaptive immune cells mostly predicts a favorable disease outcome, a high infiltration of tumor-associated macrophages predicts an unfavorable response. Pro-inflammatory events should dominate over anti-inflammatory ones in this scenario. This review focuses on how ionizing radiation modulates inflammatory events in benign inflammatory and in malign diseases. A special focus is set on the role of tumor infiltrating lymphocytes and macrophages as biomarkers to predict treatment response and anti-tumor immunity and on mechanisms implicated in the anti-inflammatory effects of low-dose radiation therapy

39. **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.

40. 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.

41. 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.

42. Georgiev S, Dimitrova I, Pressyanov D, Mitev K Retrospective Rn-220 measurements by compact discs. IEEE Transactions on Nuclear Science 2016, 63: 333-340. (Task 4.10 and 5.5; SUN)

Radiation quality
Epidemiology

Recently, a method for retrospective ^{220}Rn measurements by etching alpha-tracks in Compact Discs (CDs) or Digital Video Discs (DVDs) has been proposed. In that method the tracks at the front surface (with respect to the reading laser beam) of the discs are analyzed. These measurements are influenced by ^{222}Rn absorbed in the disc material and the signal must be corrected for ^{222}Rn presence. In this work we study whether the influence of ^{222}Rn can be eliminated if the back (labeled) side of the CDs is analyzed. Because of the coating on the back side of the disc, ^{222}Rn absorption is prevented and its influence on ^{220}Rn signal is significantly reduced. Moreover, it has been found that in most discs the signal at the back side is an order of magnitude greater than that at the front side, which significantly improves the sensitivity of the method. Thus, this work proposes a new practical approach for retrospective ^{220}Rn measurements of improved sensitivity and reduced ^{222}Rn influence.

43. Graupner A, Instanes C, Andersen JM, Brede DA, Dertinger SD, Eide DM, Lind OC, Brandt-Kjelsen A, Bjerke H, Salbu B, Oughton D, Brunborg G, Olsen AK. The genotoxic effects of continuous low dose rate gamma irradiation and selenium deficiency in mice blood cells. Mutagenesis, 2015, 30, 217–225. (Task 7.10; NIPH)
Cancer
Non-cancer
Tissue sensitivity

Many studies have investigated genotoxic effects of high Se diets but very few have addressed the genotoxicity of Se deprivation and its consequences in germ cells and none in somatic cells. To address these data gaps, C57BL/6 male mice were subjected to Se deprivation starting in the parental generation, i.e. before conception. Mice were given a diet of either low (0.01 mg Se/kg diet) or normal (0.23 mg Se/kg diet) Se content. Ogg1-deficient (*Ogg1−/−*) mice were used as a sensitive model towards oxidative stress due to their reduced capacity to repair oxidised purines. *Ogg1−/−* mice also mimic the repair characteristics of human post-meiotic male germ cells which have a reduced ability to repair such lesions. The genotoxicity of Se deficiency was addressed by measuring DNA lesions with the alkaline single cell gel electrophoresis (+ Fpg to detect oxidized DNA lesions) in somatic cells (nucleated blood cells and lung cells) and male germ cells (testicular cells). Total Se concentration in liver and GPx activity in plasma and testicular cells were measured. Gene mutation was evaluated by an erythrocyte-based *Pig-a* assay. We found that Se deprivation of F1 from their conception and until early adulthood led to the induction of DNA lesions in testicular and lung cells expressed as significantly increased levels of DNA lesions, irrespective of the mouse genotype. In blood cells, Se levels did not appear to affect DNA lesions or mutant cell frequencies. The results suggest that the testis was the most sensitive tissue. Thus, genotoxicity induced by the low Se diet in the spermatozoal genome has potential implications for the offspring.

44. Graupner, A., Dag M. Eide, Christine Instanes, Jill M. Andersen, Dag A. Brede,Stephen D. Dertinger, Ole C. Lind,3, Anicke Brandt-Kjelsen, Hans Bjerke, Brit Salbu, Deborah Oughton, Gunnar Brunborg & Ann K. Olsen: Gamma radiation at a human relevant low dose rate is genotoxic in mice. Sci Rep 6:32977. (Task 4.5; NIPH and NMBU)
Cancer

Even today, 70 years after Hiroshima and accidents like in Chernobyl and Fukushima, we still have limited knowledge about the health effects of low dose rate (LDR) radiation. Despite their human relevance after occupational and accidental exposure, only few animal studies on the genotoxic effects of chronic LDR radiation have been performed. Selenium (Se) is involved in oxidative stress defence, protecting DNA and other biomolecules from reactive oxygen species (ROS). It is hypothesised that Se deficiency, as it occurs in several parts of the world, may

aggravate harmful effects of ROS-inducing stressors such as ionising radiation. We performed a study in the newly established LDR-facility *Figaro* on the combined effects of Se deprivation and LDR γ exposure in DNA repair knockout mice (*Ogg1*−/−) and control animals (*Ogg1*+/−). Genotoxic effects were seen after continuous radiation (1.4 mGy/h) for 45 days. Chromosomal damage (micronucleus), phenotypic mutations (*Pig-a* gene mutation of RBCCD24−) and DNA lesions (single strand breaks/alkali labile sites) were significantly increased in blood cells of irradiated animals, covering three types of genotoxic activity. This study demonstrates that chronic LDR γ radiation is genotoxic in an exposure scenario realistic for humans, supporting the hypothesis that even LDR γ radiation may induce cancer.

45. 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.

46. 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.

47. Hall, Janet; Penny A. Jeggo, Catharine West, Maria Gomolka, Roel Quintens, Christophe Badie, Olivier Laurent, An Aerts, Nataša Anastasov, Omid Azimzadeh, Tamara Azizova, Sarah Baatout, Bjorn Baselet, Rafi Benotmane, Eric Blanchardon, Yann Guéguen, Siamak Haghdoost, Mats Harms-Ringhdahl, Julia Hess, Michaela Kreuzer, Dominique Laurier, Ellina Macaeva,, Grainne Manning, Eileen Pernot, Jean-Luc Ravanat, Laure Sabatier, Karine Tack, Soile Tapio, Horst Zitzelsberger, Elisabeth Cardis: Ionizing radiation biomarkers in epidemiological studies – An update. Mutation Research reviews. (WP1-7; IC/Inserm, BfS, SCK-CEN, DH-PHE, IRSN, HMGU, SU and CEA)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Tissue sensitivity
Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

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

48. Hartmann, J., Wölfelschneider, J., Stache, C., Buslei, R., Derer, A., Schwarz, M., Bäuerle, T., Fietkau, R., Gaipl, U.S., Bert, C., Hölsken, A. and Frey, B.: Novel technique for high-precision stereotactic irradiation of mouse brains. Strahlenther Onkol. 2016 Nov;192(11):806-814. Epub 2016 Jul 11. Task 5.2.1; UKER.
Cancer
Tissue sensitivity

Background and purpose: Small animal irradiation systems were developed for preclinical evaluation of tumor therapy closely resembling the clinical situation. Mostly only clinical LINACs are available, so protocols for small animal partial body irradiation using a conventional clinical system are essential. This study defines a protocol for conformal brain tumor irradiations in mice.
Materials and methods: CT and MRI images were used to demarcate the target volume and organs at risk. Three 6 MV photon beams were planned for a total dose of 10 fractions of 1.8 Gy. The mouse position in a dedicated applicator was verified by an X-ray patient positioning

system before each irradiation. Dosimetric verifications (using ionization chambers and films) were performed. Irradiation-induced DNA damage was analyzed to verify the treatment effects on the cellular level.
Results: The defined treatment protocol and the applied fractionation scheme were feasible. The in-house developed applicator was suitable for individual positioning at submillimeter accuracy of anesthetized mice during irradiation, altogether performed in less than 10 min. All mice tolerated the treatment well. Measured dose values perfectly matched the nominal values from treatment planning. Cellular response was restricted to the target volume.
Conclusion: Clinical LINAC-based irradiations of mice offer the potential to treat orthotopic tumors conformably. Especially with respect to lateral penumbra, dedicated small animal irradiation systems exceed the clinical LINAC solution.

49. **Jacquet P**, van Buul P, van Duijn-Goedhart A, Reynaud K, Buset J, Neefs M, Michaux 1, Monsieurs P, de Boer P. Radiation sensitivity of the gastrula-stage embryo : Chromosome aberrations and mutation in lacZ transgenic mice: The roles of DNA double-strand break repair systems. Mutat Res/Genet Toxicol Environ Mutagenesis 2015, 792, 26-34. (Task 6.10, SCK-CEN)
Individual sensitivity
Tissue sensitivity

At the gastrula phase of development, just after the onset of implantation, the embryo proper is characterized by extremely rapid cell proliferation. The importance of DNA repair is illustrated by embryonic lethality at this stage after ablation of the genes involved. Insight into mutation induction is called for by the fact that women often do not realize they are pregnant, shortly after implantation, a circumstance which may have important consequences when women are subjected to medical imaging using ionizing radiation. We screened gastrula embryos for DNA synthesis, nuclear morphology, growth, and chromosome aberrations (CA) shortly after irradiation with doses up to 2.5Gy. In order to obtain an insight into the importance of DNA repair for CA induction, we included mutants for the non-homologous end joining (NHEJ) and homologous recombination repair (HRR) pathways, as well as Parp1-/- and p53+/- embryos. With the pUR288 shuttle vector assay, we determined the radiation sensitivity for point mutations and small deletions detected in young adults. We found increased numbers of abnormal nuclei 5h after irradiation; an indication of disturbed development was also observed around this time. Chromosome aberrations 7h after irradiation arose in all genotypes and were mainly of the chromatid type, in agreement with a cell cycle dominated by S-phase. Increased frequencies of CA were found for NHEJ and HR mutants. Gastrula embryos are unusual in that they are low in exchange induction, even after compromised HR. Gastrula embryos were radiation sensitive in the pUR288 shuttle vector assay, giving the highest mutation induction ever reported for this genetic toxicology model. On theoretical grounds, a delayed radiation response must be involved. The compromised developmental profile after doses up to 2.5Gy likely is caused by both apoptosis and later cell death due to large deletions. Our data indicate a distinct radiation-sensitive profile of gastrula embryos, including some stage-specific aspects that are not as yet understood.

50. **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.

51. Kinzel, L., Ernst, A., Orth, M., Albrecht, V., Hennel, R., Brix, N., Frey, B., Gaipl, U.S., Zuchtriegel, G., Reichel, C.A., Blutke, A., Schilling, D., Multhoff, G., Li, M., Niyazi, M., Friedl, A.A., Winssinger, N., Belka, C. and Lauber, K.: A novel HSP90 inhibitor with reduced hepatotoxicity synergizes with radiotherapy to induce apoptosis, abrogate clonogenic survival, and improve tumor control in models of colorectal cancer. Oncotarget. 2016 Jul 12;7(28):43199-43219. (Task 5.2.1; LMU, UKER).

Cancer
Non-targeter effects

The chaperone heat shock protein 90 (HSP90) crucially supports the maturation, folding, and stability of a variety of client proteins which are of pivotal importance for the survival and proliferation of cancer cells. Consequently, targeting of HSP90 has emerged as an attractive strategy of anti-cancer therapy, and it appears to be particularly effective in the context of molecular sensitization towards radiotherapy as has been proven in preclinical models of different cancer entities. However, so far the clinical translation has largely been hampered by suboptimal pharmacological properties and serious hepatotoxicity of first- and second-generation HSP90 inhibitors. Here, we report on NW457, a novel radicicol-derived member of the pochoxime family with reduced hepatotoxicity, how it inhibits the DNA damage response and how it synergizes with ionizing irradiation to induce apoptosis, abrogate clonogenic survival, and improve tumor control in models of colorectal cancer *in vitro* and *in vivo*.

52. Koetter B, Frey B, Winderl M, Rubner Y, Scheithauer H, Sieber R, Fietkau R, Gaipl US. The in vitro immunogenic potential of caspase-3 proficient breast cancer cells with basal low immunogenicity is increased by hypofractionated irradiation. Radiation Oncology 2015, 10, 197, pp.1-14. (Task 5.2.1, UKER)

Cancer
Non-targeted effects (bystander)

BACKGROUND: Radiotherapy is an integral part of breast cancer treatment. Immune activating properties of especially hypofractionated irradiation are in the spotlight of clinicians, besides the well-known effects of radiotherapy on cell cycle and the reduction of the clonogenic potential of tumor cells. Especially combination of radiotherapy with further immune stimulation induces immune-mediated anti-tumor responses. We therefore examined whether hypofractionated irradiation alone or in combination with hyperthermia as immune stimulants is capable of inducing breast cancer cells with immunogenic potential. METHODS: Clonogenic assay, AnnexinA5-FITC/Propidium iodide assay and ELISA analyses of heat shock protein 70 and high mobility group box 1 protein were applied to characterize colony forming capability, cell death induction, cell death forms and release of danger signals by breast cancer cells in response to hypofractionated radiation (4x4Gy, 6x3Gy) alone and in combination with hyperthermia (41.5 °C for 1 h). Caspase-3 deficient, hormone receptor positive, p53 wild type MCF-7 and caspase-3 intact, hormone receptor negative, p53 mutated MDA-MB231 breast cancer cells, the latter in absence or presence of the pan-caspase inhibitor zVAD-fmk, were used. Supernatants of the treated tumor cells were analyzed for their potential to alter the surface expression of activation markers on human-monocyte-derived dendritic cells. RESULTS: Irradiation reduced the clonogenicity of caspase deficient MCF-7 cells more than of MDA-B231 cells. In contrast, higher amounts of apoptotic and necrotic cells were induced in MDA-B231 cells after single irradiation with 4Gy, 10Gy, or 20Gy or after hypofractionated irradiation with 4x4Gy or 6x3Gy. MDA-B231 cells consecutively released higher amounts of Hsp70 and HMGB1 after hypofractionated irradiation. However, only the release of Hsp70 was further increased by hyperthermia. Both, apoptosis induction and release of the danger signals, was dependent on caspase-3. Only supernatants of MDA-B231 cells after hypofractionated irradiation resulted in slight changes of activation markers on dendritic cells; especially that of CD86 was upregulated and HT did not further impact on it. CONCLUSIONS: Hypofractionated irradiation is the main stimulus for cell death induction and consecutive dendritic cell activation in caspase proficient breast cancer cells. For the assessment of radiosensitivity and immunological effects of radio- and immunotherapies the readout system is crucial.

53. **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

54. 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.

55. 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.

56. 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.

57. Kundrát P and Friedland W.: Impact of intercellular induction of apoptosis on low-dose radiation carcinogenesis. Radiation Protection Dosimetry, 2015. (Task 5.4; HMGU)
Cancer
Modelling
Non-targeted effects (bystander)

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.

58. Kundrát P. and Friedland W.: Enhanced release of primary signals may render intercellular signalling ineffective due to spatial aspects. Nature Scientific Reports 6:33214. (Task 5.4 ; HMGU)
Cancer
Modelling
Non-targeted effects (bystander)

Detailed mechanistic modelling has been performed of the intercellular signalling cascade between precancerous cells and their normal neighbours that leads to a selective removal of the

precancerous cells by apoptosis. Two interconnected signalling pathways that were identified experimentally have been modelled, explicitly accounting for temporal and spatial effects. The model predicts highly non-linear behaviour of the signalling. Importantly, under certain conditions, enhanced release of primary signals by precancerous cells renders the signalling ineffective. This counter-intuitive behaviour arises due to spatial aspects of the underlying signalling scheme: Increased primary signalling by precancerous cells does, upon reaction with factors derived from normal cells, produce higher yields of apoptosis-triggering molecules. However, the apoptosis-triggering signals are formed farther from the precancerous cells, so that these are attacked less efficiently. Spatial effects thus may represent a novel analogue of negative feedback mechanisms.

59. **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.

60. **Laurent, O.,** Maria Gomolka, Richard Haylock, Eric Blanchardon, Augusto Giussani, Will Atkinson, Sarah Baatout, Derek Bingham, Elisabeth Cardis, Janet Hall, Ladislav Tomasek, Sophie Ancelet, Christophe Badie, Gary Bethel, Jean-Marc Bertho, Ségolène Bouet, Richard Bull, Cécile Challeton-de Vathaire, Rupert Cockerill, Estelle Davesne, Teni Ebrahimian, Hilde Engels, Michael Gillies, James Grellier, Stephane Grison, Yann Gueguen, Sabine Hornhardt, Chrystelle Ibanez, Sylwia Kabacik, Lukas Kotik, Michaela Kreuzer, Anne Laure Lebacq, James Marsh, Dietmar Nosske, Jackie O'Hagan, Eileen Pernot, Matthew Puncher, Estelle Rage, Tony Riddell, Laurence Roy, Eric Samson, Maamar Souidi, Michelle C Turner, Sergey Zhivin and Dominique Laurier: Concerted Uranium Research in Europe (CURE): toward a collaborative project integrating dosimetry, epidemiology and radiobiology to study the effects of occupational uranium exposure. J. Radiol. Prot. 36 (2016) 319–345. (Task 5.8; IRSN, BfS, DH-PHE, Nuvia, SCK-CEN, AWE, SURO and CREAL.
Cancer
Non-cancer
Individual sensitivity

Radiation quality
Internal emitters (contamination)
Epidemiology
Modelling

The potential health impacts of chronic exposures to uranium, as they occur in occupational settings, are not well characterized. Most epidemiological studies have been limited by small sample sizes, and a lack of harmonization of methods used to quantify radiation doses resulting from uranium exposure. Experimental studies have shown that uranium has biological effects, but their implications for human health are not clear. New studies that would combine the strengths of large, well-designed epidemiological datasets with those of state-of-the-art biological methods would help improve the characterization of the biological and health effects of occupational uranium exposure. The aim of the European Commission concerted action CURE (Concerted Uranium Research in Europe) was to develop protocols for such a future collaborative research project, in which dosimetry, epidemiology and biology would be integrated to better characterize the effects of occupational uranium exposure. These protocols were developed from existing European cohorts of workers exposed to uranium together with expertise in epidemiology, biology and dosimetry of CURE partner institutions. The preparatory work of CURE should allow a large scale collaborative project to be launched, in order to better characterize the effects of uranium exposure and more generally of alpha particles and low doses of ionizing radiation.

61. 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.

62. Legrand M, Elie C, Stefani J, Florès N, Culeux C, Delissen O, Ibanez C, Lestaevel P, Eriksson P, Dinocourt C. Cell proliferation and cell death are disturbed during prenatal and postnatal brain development after uranium exposure. Neurotoxicology 2015, http://dx.doi.org/10.1016/j.neuro.2015.10.007, pp. 1-36. (Task 7.5, IRSN)
Internal emitters (contamination)

The developing brain is more susceptible to neurotoxic compounds than adult brain. It is also well known that disturbances during brain development cause neurological disorders in adulthood. The brain is known to be a target organ of uranium (U) exposure and previous

studies have noted that internal U contamination of adult rats induces behavioral disorders as well as affects neurochemistry and neurophysiological properties. In this study, we investigated whether depleted uranium (DU) exposure affects neurogenesis during prenatal and postnatal brain development. We examined the structural morphology of the brain, cell death and finally cell proliferation in animals exposed to DU during gestation and lactation compared to control animals. Our results showed that DU decreases cell death in the cortical neuroepithelium of gestational day (GD) 13 embryos exposed at 40 mg/L and 120 mg/L and of GD18 fetuses exposed at 120mg/L without modification of the number of apoptotic cells. Cell proliferation analysis showed an increase of BrdU labeling in the dentate neuroepithelium of fetuses from GD18 at 120 mg/L. Postnatally, cell death is increased in the dentate gyrus of postnatal day (PND) 0 and PND5 exposed pups at 120 mg/L and is associated with an increase of apoptotic cell number only at PND5. Finally, a decrease in dividing cells is observed in the dentate gyrus of PND21rats developmentally exposed to 120 mg/L DU, but not at PND0 and PND5. These results show that DU exposure during brain development causes opposite effects on cell proliferation and cell death processes between prenatal and postnatal development mainly at the highest dose. Although these modifications do not have a major impact in brain morphology, they could affect the next steps of neurogenesis and thus might disrupt the fine organization of the neuronal network.

63. **Lorat Y.,** Schanz S., Rübe CE.: Ultrastructural Insights into the Biological Significance of Persisting DNA Damage Foci after Low Doses of Ionizing Radiation. Clin Cancer Res. 2016 Nov 1;22(21):5300-5311. (Task 6.10 ; USAAR).
Cancer
Individual sensitivity
Tissue sensitivity

Purpose: Intensity-modulated radiotherapy (IMRT) enables the delivery of high doses to target volume while sparing surrounding nontargeted tissues. IMRT treatment, however, substantially increases the normal tissue volume receiving low-dose irradiation, but the biologic consequences are unclear.
Experimental design: Using mouse strains that varied in genetic DNA repair capacity, we investigated the DNA damage response of cortical neurons during daily low-dose irradiation (0.1 Gy). Using light and electron microscopic approaches, we enumerated and characterized DNA damage foci as marker for double-strand breaks (DSBs).
Results: During repeated low-dose irradiation, cortical neurons in brain tissues of all mouse strains had a significant increase of persisting foci with cumulative doses, with the most pronounced accumulation of large-sized foci in repair-deficient mice. Electron microscopic analysis revealed that persisting foci in repair-proficient neurons reflect chromatin alterations in heterochromatin, but not persistently unrepaired DSBs. Repair-deficient SCID neurons, by contrast, showed high numbers of unrepaired DSBs in eu- and heterochromatin, emphasizing the fundamental role of DNA-PKcs in DSB rejoining, independent of chromatin status. In repair-deficient ATM-/- neurons, large persisting damage foci reflect multiple unrepaired DSBs concentrated at the boundary of heterochromatin due to disturbed KAP1 phosphorylation.
Conclusion: Repeated low-dose irradiation leads to the accumulation of persisting DNA damage foci in cortical neurons and thus may adversely affect brain tissue and increase the risk of carcinogenesis. Multiple unrepaired DSBs account for large-sized foci in repair-deficient neurons, thus quantifying foci alone may underestimate extent and complexity of persistent DNA damage.

64. **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.

65. 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.

66. 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.

67. 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.

68. 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.

69. 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

70. Mavragani IV, Laskaratou DA, Frey B, Candeias SM, Gaipl US, Lumniczky K, Georgaklilas AG. Key mechanisms involved in ionizing radiation-induced systemic effects. A current review. Toxicology Research 2016, 6 Aug, pp. 1-22 (Tasks 5.2.1 and 7.6; CEA, NRIRR, UKER and GUF)
Cancer
Non- cancer
Nontargeted effects (bystander)

Organisms respond to physical, chemical and biological threats by a potent inflammatory response, aimed at preserving tissue integrity and restoring tissue homeostasis and function. Systemic effects in an organism refer to an effect or phenomenon which originates at a specific point and can spread throughout the body affecting a group of organs or tissues. Ionizing radiation (IR)-induced systemic effects arise usually from a local exposure of an organ or part of the body. This stress induces a variety of responses in the irradiated cells/tissues, initiated by the DNA damage response and DNA repair (DDR/R), apoptosis or immune response, including inflammation. Activation of this IR-response (IRR) system, especially at the organism level, consists of several subsystems and exerts a variety of targeted and non-targeted effects. Based on the above, we believe that in order to understand this complex response system better one should follow a 'holistic' approach including all possible mechanisms and at all organization levels. In this review, we describe the current status of knowledge on the topic, as well as the key molecules and main mechanisms involved in the 'spreading' of the message throughout the body or cells. Last but not least, we discuss the danger-signal mediated systemic immune effects of radiotherapy for the clinical setup.

71. **Mitev K.,** Georgiev S., Dimitrova, I. and Pressyanov, D.: Application of scintillation counting using polycarbonates to radon measurements. Radiation Measurements 92 (2016) 32e38. (Task 4.10; SUN).
Radiation quality
Epidemiology

This work proposes an approach for radon (222Rn) measurement based on scintillation counting of polycarbonate specimens. The proposed technique takes advantage of the high absorption ability of polycarbonates to 222Rn and of the fact that radiation emitted by 222Rn and its progeny causes the polycarbonate material to emit light. The theoretical background behind the proposed technique is presented and its application to two types of 222Rn measurements is demonstrated. The first application of the proposed technique is in the a posteriori calibration of compact discs for retrospective 222Rn measurements, where it is sometimes necessary to measure 222Rn concentrations higher than 2 MBq/m3 for several days. It is demonstrated that the application of the proposed technique increases the range of applicable 222Rn concentrations in the a posteriori calibration of CDs and adheres to its time and cost efficiency. The second application of the proposed technique is to soil-gas 222Rn measurements. The applicability of the technique is demonstrated in more than 110 radon-in-soil-gas measurements, which were performed in different terrains, covering 222Rn concentrations between 3 and 1200 kBq/m3. It is found that the measurements by scintillation counting of polycarbonates are consistent with the reference measurements by diffusion chambers with Kodak Pathe LR II detectors and very good linear correlations between the techniques are observed. The results from this study imply that the scintillation counting of polycarbonates may be suitable when fast, screening radon-in-soil gas measurements are necessary in a large number of points.

72. **M'kacher R**, Maalouf EE, Ricoul M, Heidingsfelder L, Laplagne E, Cuceu C, Hempel WM, Colicchio B, Dieterlen A, Sabatier L. New tool for biological dosimetry: reevaluation and automation of the gold standard method following telomere and centromere staining. Mutat Res. 2014 Dec;770:45-53. (Task 5.1; CEA)
Cancer
Individual sensitivity

Purpose: The dicentric chromosome (dicentric) assay is the international gold-standard method for bio-logical dosimetry and classification of genotoxic agents. The introduction of telomere and centromere(TC) staining offers the potential to render dicentric scoring more efficient and robust. In this study,we improved the detection of dicentrics and all unstable chromosomal aberrations (CA) leading to asignificant reevaluation of the dose–effect curve and developed an automated approach following TCstaining.Material and methods: Blood samples from 16 healthy donors were exposed to137Cs at 8 doses from0.1 to 6 Gy. CA were manually and automatically scored following uniform (Giemsa) or TC staining. Thedetection of centromeric regions and telomeric sequences using PNA probes allowed the detection of allunstable CA: dicentrics, centric and acentric rings, and all acentric fragments (with 2, 4 or no telomeres)leading to the precise quantification of estimated double strand breaks (DSB).Results: Manual scoring following TC staining revealed a significantly higher frequency of dicentrics($p < 10-3$) (up to 30%) and estimated DSB ($p < 10-4$) compared to uniform staining due to improved detec-tion of dicentrics with centromeres juxtaposed with other centromeres or telomeres. This improvementpermitted the development of the software, TCScore, that detected 95% of manually scored dicentricscompared to 50% for the best currently available software (DCScoreTM).Conclusion: The use of TC staining has permitted a reevaluation of the dose–response curve and thehighly efficient automation of the scoring process, marking a new step in the management and follow-upof populations exposed to genotoxic agents including ionizing radiation.

73. **M'kacher R,** El Maalouf E, Terzoudi G, Ricoul M, Heidingsfelder L, Karachristou I, Laplagne E, Hempel WM, Colicchio B, Dieterlen A, Pantelias G, Sabatier L. Detection and automated scoring of dicentric chromosomes in nonstimulated lymphocyte prematurely condensed chromosomes after telomere and centromere staining. Int J Radiat Oncol Biol Phys. 2015 Mar 1;91(3):640-9. (Task 5.1; CEA)
Cancer
Individual sensitivity

Purpose: To combine telomere and centromere (TC) staining of premature chromosome condensation (PCC) fusions to identify dicentrics, centric rings, and acentric chromosomes, making possible the realization of a doseeresponse curve and automation of the process.
Methods and Materials: Blood samples from healthy donors were exposed to 60Co irradiation at varying doses up to 8 Gy, followed by a repair period of 8 hours. Premature chromosome condensation fusions were carried out, and TC staining using peptide nucleic acid probes was performed. Chromosomal aberration (CA) scoring was carried out manually and automatically using PCC-TCScore software, developed in our laboratory.
Results: We successfully optimized the hybridization conditions and image capture parameters, to increase the sensitivity and effectiveness of CA scoring. Dicentrics, centric rings, and acentric chromosomes were rapidly and accurately detected, leading to a linear-quadratic doseeresponse curve by manual scoring at up to 8 Gy. Using PCC-TCScore software for automatic scoring, we were able to detect 95% of dicentrics and centric rings.
Conclusion: The introduction of TC staining to the PCC fusion technique has made possible the rapid scoring of unstable CAs, including dicentrics, with a level of accuracy and ease not previously possible. This new approach can be used for biological dosimetry in radiation emergency medicine, where the rapid and accurate detection of dicentrics is a high priority using automated scoring. Because there is no culture time, this new approach can also be used for the follow-up of patients treated by genotoxic therapy, creating the possibility to perform the estimation of induced chromosomal aberrations immediately after the blood draw.

74. **M'kacher R**, Girinsky T, Colicchio B, Ricoul M, Dieterlen A, Jeandidier E, Heidingsfelder L, Cuceu C, Shim G, Frenzel M, Lenain A, Morat L, Bourhis J, Hempel WM, Koscielny S, Paul JF, Carde P, Sabatier L. Telomere shortening: a new prognostic factor for cardiovascular disease post-radiation exposure. Radiat Prot Dosimetry. 2015 Apr;164 (1-2):134-7. doi: 10.1093 (Tasks 5.1 and 6.2; CEA)
Non cancer
Tissue sensitivity

Telomere length has been proposed as a marker of mitotic cell age and as a general index of human organism aging. Telomere shortening in peripheral blood lymphocytes has been linked to cardiovascular-related morbidity and mortality. The authors investigated the potential correlation of conventional risk factors, radiation dose and telomere shortening with the development of coronary artery disease (CAD) following radiation therapy in a large cohort of Hodgkin lymphoma (HL) patients. Multivariate analysis demonstrated that hypertension and telomere length were the only independent risk factors. This is the first study in a large cohort of patients that demonstrates significant telomere shortening in patients treated by radiation therapy who developed cardiovascular disease. Telomere length appears to be an independent prognostic factor that could help determine patients at high risk of developing CAD after exposure in order to implement early detection and prevention.

75. **Morini J,** Babini G, Mariotti L, Baiocco G, Nacci L, Maccario C, Rössler U, Minelli A, Savio M, Gomolka M, Kulka U, Ottolenghi A, Danesino C. Radiosensitivity in lymphoblastoid cell lines

derived from Schwachman-Diamond Syndrome patients. Radiat Protect Dosimetry 12 April 2015, 166(1-4) 95-100. (Task 5.2 UNIPV and BfS)
Cancer
Individual sensitivity

Shwachman-Diamond syndrome is an autosomal-recessive disorder characterised by bone marrow failure and a cumulative risk of progression to acute myeloid leukaemia. The Shwachman-Bodian-Diamond syndrome (SBDS) gene, the only gene known to be causative of the pathology, is involved in ribosomal biogenesis, stress responses and DNA repair, and the lack of SBDS sensitises cells to many stressors and leads to mitotic spindle destabilisation. The effect of ionising radiation on SBDS-deficient cells was investigated using immortalised lymphocytes from SDS patients in comparison with positive and negative controls in order to test whether, in response to ionising radiation exposure, any impairment in the DNA repair machinery could be observed. After irradiating cells with different doses of X-rays or gamma-rays, DNA repair kinetics and the residual damages using the alkaline COMET assay and the γ-H2AX assay were assessed, respectively. In this work, preliminary data about the comparison between ionising radiation effects in different patients-derived cells and healthy control cells are presented.

76. Muth C, Rubner Y, Semrau S, Rühle P-F, Frey B, Strnad A, Buslei R, FIetkau R and Gaipl US. Primary glioblastoma multiforme tumors and recurrence. Comparative analysis of the danger suignals HMGB1, HSP70 and calreticulin. Strahlenther Onkol. 2015 , 8 Dec 2015, online: pp. 1-10. (Task 5.2.1., UKER)
Cancer
Non-targeted effects (bystander)

PURPOSE: Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor. Despite improved multimodal therapies, the tumor recurs in most cases. Diverging patient survival suggests great tumor heterogeneity and different therapy responses. Danger signals such as high-mobility group box protein 1 (HMGB1), heat shock protein 70 (HSP70), and calreticulin (CRT) are biomarker candidates, due to their association with tumor progression versus induction of antitumor immune responses. Overexpression of these danger signals has been reported for various types of tumors; however, their role in GBM is still elusive. A direct comparison of their expression in the primary tumor versus the corresponding relapse is still lacking for most tumor entities. PATIENTS AND METHODS: We therefore performed an expression analysis by immunohistochemistry of the danger signals HMGB1, HSP70, and CRT in primary tumors and the corresponding relapses of 9 patients with de novo GBM. RESULTS: HMGB1 was highly expressed in primary tumors with a significant reduction in the respective relapse. The extracellular HSP70 expression was significantly increased in the relapse compared to the primary tumor. CRT was generally highly expressed in the primary tumor, with a slight increase in the relapse. CONCLUSION: The combination of a decreased expression of HMGB1, an increased expression of extracellular HSP70, and an increased expression of CRT in the relapse seems to be beneficial for patient survival. HMGB1, extracellular HSP70, and CRT could be taken into concerted consideration as potential biomarkers for the prognosis of patients with GBM.

77. 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.

78. 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.

79. 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.

80. 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.

81. 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.

82. **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.

83. **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.

84. **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.

85. **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.

86. Ott OJ, Jeremias C, Gaipl US, Frey B, Schmidt M, Fietkau R. Radiotherapy for benign achillodynia. Long term results of the Erlangen Dose Optimization trial. Strahlenther Onkol 191 (12):979-984. (Task 7.6 UKER)
Non-cancer
Tissue sensitivity

BACKGROUND: The aim of this study was to evaluate the long-term efficacy of two dose-fractionation schedules for radiotherapy of achillodynia.PATIENTS AND METHODS: Between February 2006 and February 2010, 112 evaluable patients were recruited for this prospective trial. All patients received orthovoltage radiotherapy. One course consisted of 6 fractions/3 weeks. In the 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. The endpoint was pain reduction. Pain was measured before, right after (early response), 6 weeks after (delayed response), and approximately 2 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 24 months (range, 11-56). The overall early, delayed, and long-term response rates for all patients were 84 %, 88 %, and 95 %, respectively. The mean VAS values before treatment for early, delayed, and long-term responses for the 0.5-Gy and 1.0-Gy groups were 55.7 ± 21.0 and 58.2 ± 23.5 (p = 0.53), 38.0 ± 23.2 and 30.4 ± 22.6 (p = 0.08), 35.5 ± 25.9 and 30.9 ± 25.4 (p = 0.52), and 11.2 ± 16.4 and 15.3 ± 18.9 (p = 0.16), respectively. The mean CPS values before treatment for early, delayed, and long-term responses were 8.2 ± 3.0 and 8.9 ± 3.3 (p = 0.24), 5.6 ± 3.1 and 5.4 ± 3.3 (p = 0.76), 4.4 ± 2.6 and 5.3 ± 3.8 (p = 0.58), and 2.2 ± 2.9 and 2.8 ± 3.3 (p = 0.51), respectively. No significant differences in long-term response quality between the two arms was found (p = 0.73). CONCLUSION: Radiotherapy is a very effective treatment for the management of benign achillodynia. For radiation protection, the dose for a radiotherapy series should not exceed 3.0 Gy.

87. 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.

88. Penterling C, Drexler GA, Böhland C, Stamp R, Wilke C, Braselmann H, et al. (2016): Depletion of Histone Demethylase Jarid1A Resulting in Histone Hyperacetylation and Radiation Sensitivity Does Not Affect DNA Double-Strand Break Repair. PLoS ONE 11(6): e0156599. (Task 4.9; LMU, HMGU, UBWM.)
Individual sensitivity

Histone demethylases have recently gained interest as potential targets in cancer treatment and several histone demethylases have been implicated in the DNA damage response. We investigated the effects of siRNA-mediated depletion of histone demethylase Jarid1A (KDM5A, RBP2), which demethylates transcription activating tri- and dimethylated lysine 4 at histone H3 (H3K4me3/me2), on growth characteristics and cellular response to radiation in several cancer cell lines. In unirradiated cells Jarid1A depletion lead to histone hyperacetylation while not affecting cell growth. In irradiated cells, depletion of Jarid1A significantly increased cellular radiosensitivity. Unexpectedly, the hyperacetylation phenotype did not lead to disturbed accumulation of DNA damage response and repair factors 53BP1, BRCA1, or Rad51 at damage sites, nor did it influence resolution of radiation-induced foci or rejoining of reporter constructs. We conclude that the radiation sensitivity observed following depletion of Jarid1A is not caused by a deficiency in repair of DNA double-strand breaks.

89. 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.

90. **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."

91. **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.

92. **Persa E**, Balogh A, Safrany G, Lumniczky K.. The effect of ionizing radiation on regulatory T cells in health and disease. Cancer Letters 2015, 368:252-261. (7.6, NRIRR)
Cancer
Tissue sensitivity
Non-targeted effects (immunology)

Treg cells are key elements of the immune system which are responsible for the immune suppressive phenotype of cancer patients. Interaction of Treg cells with conventional anticancer therapies might fundamentally influence cancer therapy response rates. Radiotherapy, apart from its direct tumor cell killing potential, has a contradictory effect on the antitumor immune response: it augments certain immune parameters, while it depresses others. Treg cells are intrinsically radioresistant due to reduced apoptosis and increased proliferation, which leads to their systemic and/or intratumoral enrichment. While physiologically Treg suppression is not enhanced by irradiation, this is not the case in a tumorous environment, where Tregs acquire a highly suppressive phenotype, which is further increased by radiotherapy.This is the reason why the interest for combined radiotherapy and immunotherapy approaches focusing on the abrogation of Treg suppression has increased in cancer therapy in the last few years. Here we summarize the basic mechanisms of Treg radiation response both in healthy and cancerous environments and discuss Treg-targeted pre-clinical and clinical immunotherapy approaches used in combination with radiotherapy. Finally, the discrepant findings regarding the predictive value of Tregs in therapy response are also reviewed.

93. **Piqueret-Stephan, L.,** Ricoul, M., Hempel, W.M. and Sabatier, L.: Replication Timing of Human Telomeres is Conserved during Immortalization and Influenced by Respective Subtelomeres. Sci Rep 6:32510. (Task 6.2, CEA).
Cancer
Individual sensitivity

Telomeres are specific structures that protect chromosome ends and act as a biological clock, preventing normal cells from replicating indefinitely. Mammalian telomeres are replicated

throughout S-phase in a predetermined order. However, the mechanism of this regulation is still unknown. We wished to investigate this phenomenon under physiological conditions in a changing environment, such as the immortalization process to better understand the mechanism for its control. We thus examined the timing of human telomere replication in normal and SV40 immortalized cells, which are cytogenetically very similar to cancer cells. We found that the timing of telomere replication was globally conserved under different conditions during the immortalization process. The timing of telomere replication was conserved despite changes in telomere length due to endogenous telomerase reactivation, in duplicated homologous chromosomes, and in rearranged chromosomes. Importantly, translocated telomeres, possessing their initial subtelomere, retained the replication timing of their homolog, independently of the proportion of the translocated arm, even when the remaining flanking DNA is restricted to its subtelomere, the closest chromosome-specific sequences (inferior to 500 kb). Our observations support the notion that subtelomere regions strongly influence the replication timing of the associated telomere.

94. 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.

95. 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. 83 : 36-40. (Tasks 4.10 and 5.5; SUN)
Radiation quality
Epidemiology

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.

96. Pressyanov, D., Mitev, K., Georgiev, S., Dimitrova, I., Kolev, J.: Laboratory facility to create reference radon + thoron atmosphere under dynamic exposure conditions. Journal of Environmental Radioactivity 166 (2017) 181-187. (Task 4.10; SUN)
Radiation quality
Modelling

Radon (222Rn) and thoron (220Rn) levels in the environment are typically subject to significant random and systematic variations. Creation in the laboratory of reproducible and controlled exposure conditions close to that in the real environment can be useful for testing 222 Rn and 220 Rn detectors and for research. In this report the design and performance of a novel laboratory facility with such functionality is presented. The facility allows the exposure of detectors under controlled dynamic as well as static activity concentrations of 222Rn and 220Rn (pure and mixed) and temperature. The temperature is measured and regulated within -15 C ÷ + 60 C by a dedicated programmable thermostat. Different reference activity concentrations in the exposure vessel are made by regulating the flow-rate of the air that flushes 222Rn/220Rn activity from the sources towards the exposure vessel. Reference atmospheres that contain 222Rn, 220Rn or a specified ratio of the two can be created. Pilot experiments that demonstrate the feasibility of the approach are presented. They include follow-up of a pre-defined temperature profile (in the range - 5 C ÷ + 35 C), test of the correspondence between planned and measured 222Rn and 220Rn activity concentrations, follow-up of a pre-defined dynamic profile of 220Rn concentrations and test of the possibility to create mixed 220Rn/222Rn atmospheres (experimentally checked for ratio of the activity concentrations from 0.27 to 4.5). The results from the experimental tests are in agreement with the values obtained by the developed theoretical model. The proposed approach can be used to plan and create stationary and dynamic reference exposure conditions that are close to the real exposure regimes in the environment.

97. Quintens R, Verret T., Janssen A, Neefs M, Leuson L, Michaux A, Versiegers M, Samari N, Pani G, Verheyde J, Baatout, Betnomane MA.: Identification of novel radiation-induced p53-dependent transcripts extensively regulated during mouse brain development. Biology Open 2015, 4: 331-344 (Task 7.5, SCK-CEN)
Non-cancer
Tissue sensititivy

Ionizing radiation is a potent activator of the tumor suppressor gene p53, which itself regulates the transcription of genes involved in canonical pathways such as the cell cycle, DNA repair and apoptosis as well as other biological processes like metabolism, autophagy, differentiation and development. In this study, we performed a meta-analysis on gene expression data from different in vivo and in vitro experiments to identify a signature of early radiation-responsive genes which were predicted to be predominantly regulated by p53. Moreover, we found that several genes expressed different transcript isoforms after irradiation in a p53-dependent manner. Among this gene signature, we identified novel p53 targets, some of which have not yet been functionally characterized. Surprisingly, in contrast to genes from the canonical p53-regulated pathways, our gene signature was found to be highly enriched during embryonic and post-natal brain development and during in vitro neuronal differentiation. Furthermore, we could show that for a number of genes, radiation-responsive transcript variants were upregulated during development and differentiation, while radiation non-responsive variants were not. This suggests that radiation exposure of the developing brain and immature cortical neurons results in the p53-mediated activation of a neuronal differentiation program. Overall, our results further increase the knowledge of the radiation-induced p53 network of the

embryonic brain and provide more evidence concerning the importance of p53 and its transcriptional targets during mouse brain development.

98. **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.

99. **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.

100. **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.

101. Roedel F., Frey B., Multhoff G, Gaipl U.: Contribution of the immune system to bystander and non-targeted effects of ionizing radiation. January 1, 2015, Volume 356, Issue 1, Pages 105–113. (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.

102. 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.

103. 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

104. 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

105. 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.

106. 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.

107. Rühle, P.F., Fietkau, R., Gaipl, U.S and Frey, B.:Development of a Modular Assay for Detailed Immunophenotyping of Peripheral Human Whole Blood Samples by Multicolor Flow Cytometry. Int. J. Mol. Sci. 2016, 17(8), 1316. (Tasks 5.2.1 and 7.6, UKER.)
Individual sensitivity

The monitoring of immune cells gained great significance in prognosis and prediction of therapy responses. For analyzing blood samples, the multicolor flow cytometry has become the method of choice as it combines high specificity on single cell level with multiple parameters and high throughput. Here, we present a modular assay for the detailed immunophenotyping of blood (DIoB) that was optimized for an easy and direct application in whole blood samples. The DIoB assay characterizes 34 immune cell subsets that circulate the peripheral blood including all major immune cells such as T cells, B cells, natural killer (NK) cells, monocytes, dendritic cells (DCs), neutrophils, eosinophils, and basophils. In addition, it evaluates their functional state and a few non-leukocytes that also have been associated with the outcome of cancer therapy. This DIoB assay allows a longitudinal and close-meshed monitoring of a detailed immune status in patients requiring only 2.0 mL of peripheral blood and it is not restricted to peripheral blood mononuclear cells. It is currently applied for the immune monitoring of patients with glioblastoma multiforme (IMMO-GLIO-01 trial, NCT02022384), pancreatic cancer (CONKO-007 trial, NCT01827553), and head and neck cancer (DIREKHT trial, NCT02528955) and might pave the way for immune biomarker identification for prediction and prognosis of therapy outcome.

108. **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.

109. **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.

110. **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.

111. **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]

112. **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.

113. Salomaa, S., Jourdain J-R., Kreuzer M., Jung T. and Repussard J. : Multidisciplinary European Low Dose Initiative – An update of the MELODI program. International Journal of Radiation Biology (2017). (WP1-7 ; STUK, IRSN, BfS).
Cancer
Non-cancer

Individual sensitivity
Radiation quality
Tissue sensitivity
Internal emitters (contamination)
Epidemiology
Modeling
Non-targeted effects (bystander)

114. 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.

115. Samson, E., Piot, I., Zhivin, S., Richardson, D.B., Laroche, P., Serond, AP., Laurier, D. and Laurent, O.: Cancer and non-cancer mortality among French uranium cycle workers: the TRACY cohort. BMJ Open 2016;6:e010316. (Task 5.8; IRSN)
Cancer
Non-cancer
Individual sensitivity
Radiation quality
Internal emitters (contamination)
Epidemiology
Modelling

Objectives: The health effects of internal contamination by radionuclides, and notably by

uranium, are poorly characterised. New cohorts of uranium workers are needed to better examine these effects. This paper analyses for the first time the mortality profile of the French cohort of uranium cycle workers. It considers mortality from cancer and non-cancer causes.
Methods: The cohort includes workers employed at least 6 months between 1958 and 2006 in French companies involved in the production of nuclear fuel. Vital status and causes of death were collected from French national registries. Workers were followed-up from 1 January 1968 to 31 December 2008. Standardised mortality ratios (SMRs) were computed based on mortality rates for the French general population.
Results: The cohort includes 12 649 workers (88% men). The average length of follow-up is 27 years and the mean age at the end of the study is 60 years. Large mortality deficits are observed for non-cancer causes of death such as non-cancer respiratory diseases (SMR=0.51 (0.41 to 0.63)) and circulatory diseases (SMR=0.68 (0.62 to 0.74)). A mortality deficit of lower magnitude is also observed for all cancers combined (SMR (95% CI): 0.76 (0.71 to 0.81)). Pleural mesothelioma is elevated (SMR=2.04 (1.19 to 3.27)).
Conclusions: A healthy worker effect is observed in this new cohort of workers involved in the uranium cycle. Collection of individual information on internal uranium exposure as well as other risk factors is underway, to allow for the investigation of uraniumrelated risks.

116. 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.

117. 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.

118. 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.

119. Selmansberger M, Feuchtinger A, Zurnadzhy L, Michna A, Kaiser JC, Abend M, Brenner A, Bogdanova T, Walch A, Unger K, Zitzelberger H, Hess J. CLIP2 as radiation biomarker in papillary thyroid carcinoma. Oncogene 2015,34:3917-3925 (Task 6.3; HMGU)
Cancer
Tissue sensitivity
Epidemiology

A substantial increase in papillary thyroid carcinoma (PTC) among children exposed to the radioiodine fallout has been one of the main consequences of the Chernobyl reactor accident. Recently, the investigation of PTCs from a cohort of young patients exposed to the post-Chernobyl radioiodine fallout at very young age and a matched nonexposed control group revealed a radiation-specific DNA copy number gain on chromosomal band 7q11.23 and the radiation-associated mRNA overexpression of CLIP2. In this study, we investigated the potential role of CLIP2 as a radiation marker to be used for the individual classification of PTCs into CLIP2-positive and -negative cases—a prerequisite for the integration of CLIP2 into epidemiological modelling of the risk of radiation-induced PTC. We were able to validate the radiation-associated CLIP2 overexpression at the protein level by immunohistochemistry (IHC) followed by relative quantification using digital image analysis software (P=0.0149). Furthermore, we developed a standardized workflow for the determination of CLIP2-positive and -negative cases that combines visual CLIP2 IHC scoring and CLIP2 genomic copy number status. In addition to the discovery cohort (n=33), two independent validation cohorts of PTCs (n=115) were investigated. High sensitivity and specificity rates for all three investigated cohorts were obtained, demonstrating robustness of the developed workflow. To analyse the function of CLIP2 in radiation-associated PTC, the CLIP2 gene regulatory network was reconstructed using global mRNA expression data from PTC patient samples. The genes comprising the first neighbourhood of CLIP2 (BAG2, CHST3, KIF3C, NEURL1, PPIL3 and RGS4) suggest the involvement of CLIP2 in the fundamental carcinogenic processes including

apoptosis, mitogen-activated protein kinase signalling and genomic instability. In our study, we successfully developed and independently validated a workflow for the typing of PTC clinical samples into CLIP2-positive and CLIP2-negative and provided first insights into the CLIP2 interactome in the context of radiation-associated PTC.

120. 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

121. Shim, G., Normil, M.D., Testard, I., Hempel, W.M., Ricoul, M. and Sabatier, L.: Comparison of Individual Radiosensitivity to γ-Rays and Carbon Ions. Front. Oncol. 6:137. (Task 5.1, CEA).
Individual sensitivity
Radiation quality

Carbon ions are an up-and-coming ion species, currently being used in charged par-ticle radiotherapy. As it is well established that there are considerable interindividual differences in radiosensitivity in the general population that can significantly influence clinical outcomes of radiotherapy, we evaluate the degree of these differences in the context of carbon ion therapy compared with conventional radiotherapy. In this study, we evaluate individual radiosensitivity following exposure to carbon-13 ions or γ-rays in peripheral blood lymphocytes of healthy individuals based on the frequency of ionizing radiation (IR)-induced DNA double strand breaks (DSBs) that was either misrepaired or left unrepaired to form chromosomal aberrations (CAs) (simply referred to here as DSBs for brevity). Levels of DSBs were estimated from the scoring of CAs visualized with telomere/centromere-fluorescence *in situ* hybridization (TC-FISH). We examine radiosensitivity at the dose of 2 Gy, a routinely administered dose during fractionated radiotherapy, and we determined that a wide range of DSBs were induced by the given dose among healthy individuals, with highly radiosensitive individuals harboring more IR-induced breaks in the genome than radioresistant individuals following exposure to the same dose. Furthermore, we determined the relative effectiveness of carbon irra-diation in comparison to γ-irradiation in the induction of DSBs at each studied dose (isodose effect), a quality we term "relative dose effect" (RDE). This ratio is advantageous, as it allows for simple comparison of dose–response curves. At 2 Gy, carbon irradiation was three times more effective in inducing DSBs compared with γ-irradiation (RDE of 3); these results were confirmed using a second cytogenetic technique, multicolor-FISH. We also analyze radiosensitivity at other doses (0.2–15 Gy), to represent hypo- and hyperfractionation doses and determined that RDE is dose dependent: high ratios at low doses, and approaching 1 at high doses. These results could have clinical implications as IR-induced DNA damage and the ensuing CAs and genomic instability can have significant cellular consequences that could potentially have profound implications for long-term human health after IR exposure, such as the emergence of secondary cancers and

other pathobiological conditions after radiotherapy.

122. Siebenwirth C., Greubel, C., Drexler, S.E., Girst, S., Reindl, J., Walsh, D.W.M., Dollinger, G., Friedl, A.A., Schmid, T.E. and Drexler, G.A.: Determination of the accuracy for targeted irradiations of cellular substructures at SNAKE. Nuclear Instruments and Methods in Physics Research B 348 (2015) 137–142. (Task 4.9 ; UBWM and LMU).
Cancer
Non-cancer
Radiation quality

In the last 10 years the ion microbeam SNAKE, installed at the Munich 14 MV tandem accelerator, has been successfully used for radiobiological experiments by utilizing pattern irradiation without targeting single cells. Now for targeted irradiation of cellular substructures a precise irradiation device was added to the live cell irradiation setup at SNAKE. It combines a sub-micrometer single ion irradiation facility with a high resolution optical fluorescence microscope. Most systematic errors can be reduced or avoided by using the same light path in the microscope for beam spot verification as well as for and target recognition. In addition online observation of the induced cellular responses is possible. The optical microscope and the beam delivering system are controlled by an in-house developed software which integrates the open-source image analysis software, CellProfiler, for semi-automatic target recognition. In this work the targeting accuracy was determined by irradiation of a cross pattern with 55 MeV carbon ions on nucleoli in U2OS and HeLa cells stably expressing a GFP-tagged repair protein MDC1. For target recognition, nuclei were stained with Draq5 and nucleoli were stained with Syto80 or Syto83. The damage response was determined by live-cell imaging of MDC1-GFP accumulation directly after irradiation. No systematic displacement and a random distribution of about 0.7 lm (SD) in x-direction and 0.8 lm (SD) in y-direction were observed. An independent analysis after immunofluorescence staining of the DNA damage marker yH2AX yielded similar results. With this performance a target with a size similar to that of nucleoli (i.e. a diameter of about 3 lm) is hit with a probability of more than 80%, which enables the investigation of the radiation response of cellular subcompartments after targeted ion irradiation in the future.

123. 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.

124. Te Riet L, van Deel ED, van Thiel BS, Moltzer E, van Vliet N, Ridwan Y, van Veghel R, van Heijningen PM, Robertus JL, Garrelds IM, Vermeij M, van der Pluijm I, Danser AH, Essers J.: AT1-receptor blockade, but not renin inhibition, reduces aneurysm growth and cardiac failure in fibulin-4 mice. J Hypertens. 2016 Apr;34(4):654-665. (Task 7.7; Erasmus MC)
Non-cancer

Aims: Increasing evidence supports a role for the angiotensin II-AT1-receptor axis in aneurysm development. Here, we studied whether counteracting this axis via stimulation of AT2 receptors is beneficial. Such stimulation occurs naturally during AT1-receptor blockade with losartan, but not during renin inhibition with aliskiren.
Methods and results: Aneurysmal homozygous fibulin-4R/R mice, displaying a four-fold reduced fibulin-4 expression, were treated with placebo, losartan, aliskiren, or the bblocker propranolol from day 35 to 100. Their phenotype includes cystic media degeneration, aortic regurgitation, left ventricular dilation, reduced ejection fraction, and fractional shortening. Although losartan and aliskiren reduced hemodynamic stress and increased renin similarly, only losartan increased survival. Propranolol had no effect. No drug rescued elastic fiber fragmentation in established aneurysms, although losartan did reduce aneurysm size. Losartan also increased ejection fraction, decreased LV diameter, and reduced cardiac pSmad2 signaling. None of these effects were seen with aliskiren or propranolol. Longitudinal micro-CT measurements, a novel method in which each mouse serves as its own control, revealed that losartan reduced LV growth more than aneurysm growth, presumably because the heart profits both from the local (cardiac) effects of losartan and its effects on aortic root remodeling.
Conclusion: Losartan, but not aliskiren or propranolol, improved survival in fibulin-4R/R mice. This most likely relates to its capacity to improve structure and function of both aorta and heart. The absence of this effect during aliskiren treatment, despite a similar degree of blood pressure reduction and renin–angiotensin system blockade, suggests that it might be because of AT2-receptor stimulation.

125. **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

126. **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.

127. Wunderlich, R., Ruehle, P.F., Deloch, L., Unger, K., Hess, J., Zitzelsberger, H., Lauber, K., Frey, B. and Gaipl, U.S.: Interconnection between DNA damage, senescence, inflammation, and cancer. Front Biosci (Landmark Ed). 2017 Jan 1;22:348-369. (Tasks 5.2.1 and 7.6; HMGU, UKER, FAU.)
Cancer
Non-cancer
Individual sensititivy

In order to deal with endogenous and exogenous factors, including radiation or pathogens, cells evolved different strategies. This includes highly complex processes such as DNA damage response, senescence, cell death, and inflammatory reactions. Recent research indicates an interconnection between the mentioned cellular pathways whilst all of them seem to play a role in induction and progression, but also the prevention of cancerous diseases and therefore qualify for potential prevention and treatment strategies. On the basis of their pivotal functions in cancer biology in general, each of the cellular processes represents promising single therapeutic targets. Further, due to their strong interconnection, targeting all of them in a multimodal approach could be another promising strategy to treat cancer. We, therefore, review the mechanisms of DNA damage induction, detection and repair as well as the induction of cell death. Further, features of senescence and mechanism of inflammation induction and abrogation are outlined. A special focus is set on how senescence and inflammation are related to diseases and how targeting them, could contribute to improvement of cancer therapies.

128. 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.

129. 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.