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EPIRADBIO Report Summary

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Final Report Summary - EPIRADBIO (Combining epidemiology and radiobiology to assess cancer risksin the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with total doses in the order of 100 mSv or below)

Executive Summary:
The main aim of EpiRadBio is to combine epidemiology and radiobiology to assess cancer risk in the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with cumulated equivalent doses of the order of 100 mSv or below. In order to supply a new basis for the risk assessment underlying current radiation protection, the key objectives of EpiRadBio are to
• perform measurements of telomere lengths, array-based comparative genomic hybridisation and other 'omics' with cancer tissue and blood samples from members of outstanding radio-epidemiological cohorts in order to characterize key processes of carcinogenesis in humans exposed to low dose radiation
• analyze radiation responses of stem cells and low dose perturbation of intercellular communication in 2D and 3D models using human, normal breast and lung epithelial cells in order to elucidate further key processes of carcinogenesis and supplement the studies of samples from epidemiological cohorts
• integrate the new radiobiological results in models of carcinogenesis in order to include this knowledge in an evaluation of key epidemiological data
• derive cancer risks including individual risk factors after exposures to ionizing radiation with cumulative equivalent doses in the order of 100 mSv or below for supporting radiation protection.
Main achievements include
• A biobank of blood samples from 371 donors belonging to the French Hemangioma Cohort (FHC) comprising cytogenetic slides with metaphase spreads of T- and B-lymphocytes and nucleated blood cells frozen in liquid nitrogen for DNA/RNA- and FACS analysis as well as for future cell culture experiments
• The identification of a biomarker CLIP2 for radiation-induced thyroid cancer diagnosed at age below 20 years
• Isolation of stem cell sub-populations from the human normal breast tissue and determination of their response to low doses of ionizing radiation
• An indication that chronic irradiation is more effective in inducing gene amplification compared to acute irradiation
• A prediction that radiation decreases anti-carcinogenic apoptosis of transformed cells under in vivo conditions in contrast to the experimentally determined increase of this process under in vitro conditions
• An indication that at least for exposure at young ages radiation induces genomic instability and pathways to papillary thyroid cancer and to breast cancer different from spontaneous carcinogenesis
• The hypothesis based on mechanistic models of epidemiological data that a bystander effect may play a central role in the induction of lung cancer by alpha radiation
• A new method allowing a common analysis of cases that occurred before the first screening in a cohort, prevalent cases detected in the first screening, and incident cases detected in subsequent screenings
• A suggestion that life style has a major influence on colon cancer with chromosomal instability but not on colon cancer with microsatellite instability
• Life-time radiation risk estimates based on models developed in the frame of the project.
The project was very effectively managed by a close cooperation of a Project Management Group, the Scientific Advisory Board and the Project Office. Three of the seven persons involved in these leading activities were female. The team was supported by ten Work Package Leaders coordinating the work of specific tasks and implementing the work programm and its changes as decided in common meetings of the leading staff based on new insights having developed during the course of the project.

Project Context and Objectives:
Project context and objectives

The main aim of EpiRadBio is to combine epidemiology and radiobiology to assess cancer risk in the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with cumulated equivalent doses of the order of 100 mSv or below. Such exposures are of central importance for radiation protection, since they correspond to
• the dose limit for occupational exposure (100 mSv in five years)
• exposures currently occurring in the work place (generally below 100 mSv over lifetime)
• exposures currently occurring due to medical diagnostics, e.g., by CT scans causing equivalent doses in the order of

10 mSv per examinations
While most of these exposures are from low-LET radiation, also exposures to high-LET radiation occur. EpiRadBio explored cancer risks from alpha radiation as well.
It is of urgent importance to analyse cancer risks from low doses and from low dose rate, because
• recent epidemiological results challenge the assumption of a dose-dose-rate effectiveness factor (DDREF) made presently for radiation protection
• the non-linear dose response of non-targeted effects and the differences in gene expression in the low-dose and medium/high-dose region question the linear-no-threshold dose-effect model used presently for radiation protection.
Due to lack of statistical power, conventional epidemiology has limitations in studying organ specific health effects from exposures with equivalent doses of the order of 100 mSv and below. Radiobiology can provide insights into basic mechanisms of carcinogenesis after radiation exposures but does not give quantitative results for risks of humans. EpiRadBio explores approaches combining epidemiology and radiobiology in order to address the problem: Incorporation of radiobiological results into the evaluation of epidemiological data on the basis of molecular epidemiology and models of carcinogenesis.
In order to supply a new basis for the risk assessment underlying current radiation protection, the key objectives of EpiRadBio are to
• perform measurements of telomere lengths, array-based comparative genomic hybridisation and other 'omics' with cancer tissue and blood samples from members of outstanding radioepidemiological cohorts in order to characterize key processes of carcinogenesis in humans exposed to low dose radiation
• analyze radiation responses of stem cells and low dose perturbation of intercellular communication in 2D and 3D models using human, normal breast and lung epithelial cells in order to elucidate further key processes of carcinogenesis and supplement the studies of samples from epidemiological cohorts
• integrate the new radiobiological results in models of carcinogenesis in order to include this knowledge in an evaluation of key epidemiological data
• derive cancer risks including individual risk factors after exposures to ionizing radiation with cumulative equivalent doses in the order of 100 mSv or below for supporting radiation protection.

Project Results:
EPIRADBIO: Final Scientific Report
Sub-project 1 Genomic instability and individual susceptibility
WP1.1 Individual susceptibility to genomic instability: Epidemiology and radiation biology studies in French haemangioma patients (CEA, Inserm)
The French Haemangioma Cohort (FHC) presented within the project EpiRadBio represents one of the rare cohorts suitable to study the effect of low doses (<100mGy) of ionizing radiation on the individual cancer risk. More in vivo studies are needed for a better understanding of this effect since all models for low dose risk estimation mostly base on in vitro experiments. Cohort studies might help to identify biomarkers for a better cancer risk assessment for low doses of ionizing radiation.
The special features of the FHC are that it is very homogeneous and consists of healthy persons belonging to a normal population. The only point all person have in common is a skin haemangioma after birth. A part of this cohort was treated locally with radiotherapy for the haemangioma, mostly for esthetical reason, what explains the high amount of women treated from 1946 to 1973. The access to medical documents, describing in detail the treatment, allowed a precise reconstruction of the dose received at every major organ. A long follow up and information about cancer incidence, life style and other confounding factors are helping to estimate the cancer risk only related to the haemangioma treatment that occurred in early infancy (until the age of 3 years) excluding other factors. Epidemiological studies demonstrated that the risk of getting cancer is 3-times higher within the FHC probably related to the radiotherapy.
We are concentrating our analysis on telomeres as potential biomarker, known to be essential in maintaining on one hand the genomic stability by protecting chromosome ends from being recognized as double strand breaks and to act on the other hand as internal clock regulating the lifetime of a cell by telomere shortening during cell division. The ability of telomeres to limit cell proliferation can be thought of as a tumour suppressor mechanism. Telomere shortening is accelerated by many in vivo and ex vivo factors, including radiation. A loss of a single telomere can lead to end to end fusion of chromosomes with further amplification of chromosomal instability through repetition of the break-fusion-bridge (BFB) cycle, which can initiate of new carcinogenic processes. Hence, a loss of telomere maintenance can result in the instability of multiple chromosomes and can generate numerous chromosomal rearrangements that can cause human cancer development (Sabatier et al., 2005; Murnane and Sabatier, 2004; Lo et al., 2002; Desmaze et al., 2003; Raynaud et al., 2008; Ayouaz et al., 2008). There are only few studies analyzing the long-term effect of low doses of ionizing radiation on telomeres. In Ilyenko et al. (2011) described in Chernobyl workers telomere shortening in blood lymphocytes even 20 years after exposure to low-dose ionizing irradiation. However they do not take into account the age of the donors although telomere length is strongly correlated with age.
Within the project EpiRadBio the long-term effect of exposure in early childhood to low-doses of ionizing radiation on the risk of getting cancer was studied. The most important step has been to create a biobank of blood samples from donors belonging to the FHC was established comprising cytogenetic slides with metaphase spreads of T- and B-lymphocytes and nucleated blood cells frozen in liquid nitrogen for DNA/RNA- and FACS analysis as well as for future cell culture experiments. Only donors that declared to have no cancer (evaluated in a first cancer incidence study in 2007, performed by INSERM, Florent deVathaire) have been contacted to perform an exposed/non-exposed study excluding cancer cases to estimate the effect related to the radiotherapy for the haemangioma without side effects like chemo- or radiotherapy applied for cases that developed cancer. The biobank contains nevertheless few cancer cases (19 of 371 donors in total) that have been diagnosed after 2007 (identified thanks to the questionnaire filled by every donor). Cytogenetic analysis on T-lymphocytes from non-exposed donors of the FHC showed comparable values of mean

telomere length and the expected age-related decline in length compared to a normal population and data already published by Vaziri et al. (1993). The differences in telomere length between exposed and non-exposed donors are not substantial. The study is not completed yet since more samples have to be analyzed to increase the statistical significance.
The analysis of intracellular differences of telomere length, taking into consideration the individual length of each telomere, indicates an increased heterogeneity of telomere length within those donors that developed a benign tumour or cancer compared to healthy donors. This result underlines our theory that in fact not a small mean telomere length is inducing cancer but in contrast the presence of few short telomeres within a cell (Pommier and Sabatier, 2002).
Exposure of ionizing radiation seems to affect the genomic instability only to a minor extent within the FHC. The amount of cchromosomal aberrations like dicentric, tricentric, ring or acentric chromosomes and chromosomal fragments was only slightly increased in exposed donors compared to non-exposed while there was a strong correlation for donors that developed a cancer between the cancer treatment and the number of complex chromosomal aberrations that have been significantly increased.
In summary, During EPIRADBIO we create the first biobank of haemangioma patients. With the performed exposed/non-exposed study we demonstrated a strong correlation of telomere length in function of age. In contrast, there are no significant differences in the mean telomere length of exposed donors compared to non-exposed. The study still has to be continued and can be extended now for analysis regarding the heterogeneity of telomere length within individual cells and will be compare to cancer occurrence.
WP 1.2: Genomic instability in tumoral tissues of radiation-related breast and lung cancer
Breast and lung cancer tissues of the MAYAK workers cohort provide a unique source of material to study the possible impact of radiation exposure on cancer development. Dose estimates and excellent medical records are available. However, there was a significant delay in delivery of samples from SUBI due to regulatory affairs. Both lung cancer as well as FFPE breast cancer samples were not received before 2013.
We now have completed a comparative arrayCGH analysis of closely matched breast cancer samples from exposed workers and non-exposed local controls. Bioinformatic analysis has identified significant differences in numerical genomic aberrations between the two groups, as shown below.
As indicated in the figure a significant loss on chromosome 8 was found specifically for the control group, including the region which codes for miRNA miR-3184. Another loss specifically identified for the Mayak group was detected on chromosome 6, which contains the gene TNF (tumor necrosis factor) amongst others. The loss on chromosome 14 involves no annotated gene.
Unfortunately only FFPE autopsy tissue was available from the MAYAK lung cancer cohort, which showed very poor DNA quality, precluding conventional arrayCGH analysis as performed for the breast cancer samples.
In cooperation with WP1.3 the frequently occurring V600E mutation in thyroid cancer was analyzed in a cohort of 121 radioactive radiation exposed and non-exposed tissue samples from Germany (G) and Ukraine (U) using Next-Generation-Sequencing (MiSeq, Illumina) to investigate a possible relation to radiation exposure. Therefore low level frequencies of an A to T exchange in BRAF codon 600, which is the classical V600E mutation, were analyzed in contralateral thyroid tissue of tumor patients as well as in thyroid tissue of healthy individuals. Additionally frequencies of transversions and transitions in codon 600 and neighboring nucleotides were compared.

Figure: Comparison of ArrayCGH results for breast cancer for the exposed group (MAYAK, upper panel) and local control group (lower panel). The chromosomes are depicted from left to right in ascending order. Losses are in blue and gains in red. Boxes highlight statistically significant differences between the two groups ($P<0.05$, $FDR<0.1$).
Table: Read frequencies of adenine to thymine exchange in the codon 600 in exposed and non-exposed tissue samples.

Group	cases	coverage	detected T	% detected T
G thyroid cancer	13	33766	2386	7.07
U thyroid cancer	11	83898	6745	8.04
G thyroid of healthy individuals	19	31487	54	0.17
U thyroid of healthy individuals	11	79264	83	0.10
G thyroid cancer contralateral tissue	13	30331	45	0.15
U thyroid cancer contralateral tissue	30	50189	75	0.15
G normal tissue of diverse organs	24	79877	92	0.11

1. No difference in the frequency of V600E mutations was detected in thyroid cancers of German and Ukrainian individuals (see table). 2. Interestingly no difference in A to T exchange frequencies at position c.1799 was found, while in contrast a higher transversion frequency at c.1798-1800 was found comparing Ukrainian and German healthy tissues. Also the c.1799 transition frequency was significantly increased in Ukrainian in comparison to German tissues. However it is not answerable if this difference is due to radiation exposure or due to material treatment discrepancies between the different countries. 3. The c.1799 transversion frequency is significantly elevated in the whole cohort compared to c.1798 and c.1800, indicating a general susceptibility of this position to mutations. However additional alterations may have to accumulate to develop a malignant tumor.
WP1.3 genomic instability in thyroid cancer
This work package sought to identify radiation-associated genetic and epigenetic alterations in a large cohort of post-Chernobyl papillary thyroid carcinomas. This also included the validation of radiation-associated genomic copy number alterations that were found in the childhood PTC which were investigated in the GENRISK-T project. Moreover, the WP aimed to identify molecular mechanisms on an epigenetic level (micro RNA and methylation) that are linked to radiation exposure in the investigated cases. The work package aimed not only identify alterations on different molecular levels (genomic, expression and epigenetic) but also aimed to gain deeper insights into the interplay of copy number aberrations, mRNA and miRNA expression changes, single-nucleotide polymorphisms and changes on transcriptomic

regulation by using the power of integrative data analysis. As a result from this integrative approach, it was hoped to identify key pathways involved in the radiocarcinogenesis in young onset thyroid cancer. To achieve these aims cases from the Chernobyl Tissue Bank (CTB) where estimates of thyroid dose were available, were investigated to permit correlation analysis of molecular alterations with low or high radiation exposures. The overall strategy was to compare an exposed with a non-exposed cohort of patients that are matched for age at diagnosis, oblast of residence and histopathological subtype of PTC. To investigate the dose-response relationships with respect to expression of the CLIP2 gene, we also used a specific subset of cases from the CTB, those who were enrolled in the NCI sponsored epidemiology cohort – the Ukraine-American (UkrAm) cohort. Direct thyroid measurements and detailed questionnaire data is available for this cohort providing more accurate dosimetry. The work package was also asked to re-examine data from the Genrisk-T project to confirm presence or absence of radiation related changes when the data available from this project on miRNA and DNA methylation from the same patients as used in Genrisk-T were available.
Our initial results were unable to validate the amplification of 7q in an independent cohort. This cohort also included patients born after 1st January 1987 (i.e. after the radioiodine had decayed in the environment) and resident outside the contaminated areas of Ukraine as controls for the radiation exposed cases. A second cohort, again born after 1st January 1987, but with residency in the contaminated oblasts of Ukraine (i.e. the same geographical locations as the exposed cohort, but unexposed to radioiodine by the date of birth) was provided and the analyses repeated. The association of amplification of 7q and radiation exposure was confirmed in this second independent cohort, but this was only valid for those who were young (5 years of age and under) and exposure and under 20 at the time of clinical presentation (see task 1.3.1). A dose-response relationship for CLIP2 was also identified in those aged under 20 at operation or less than 5 at the time of exposure. This may reflect a different molecular mechanism in PTCs induced at lower dose levels from those induced at higher levels (see task 1.3.2).
No evidence of radiation specific changes in miRNA, and a very weak association of changes of DNA methylation was observed, which could not be substantiated in a validation cohort. Associations between morphological subtype of PTC and miRNA and DNA methylation profiles were observed (see tasks 1.3.3 and 1.3.4).
Independent analysis of the transcriptomic data from Genrisk-T confirmed that a transcriptomic profile could be observed that distinguished exposed from non-exposed tumours (Task 1.3.5.3). However, this will require validation in an independent set of PTCs. Integrated analysis suggested that changes in copy number did not correlate with changes in miRNA expression. However, individual mRNAs whose expression levels were positively associated with changes in copy number were identified. In the exposed cases there was tighter correlation between copy number and mRNA expression levels suggesting selection pressure for those regions where copy number was altered. However, the integrated analysis did not confirm an association between CLIP2 expression and radiation exposure, although we were able to confirm the initial finding of correlation of 7q amplification and exposure status in the original Genrisk-T cohort.
Exploratory pathway analyses suggest that there may differing regulatory networks at the pathway level for the unexposed and exposed cases. Our observations on the age at exposure and age at operation restrictions on dose-response suggest that further analysis of larger cohorts with high and low thyroid radiation doses will be required to test this hypothesis as stronger correlations may be identified in PTCs from patients with higher doses. However, it should be noted that there are a limited number of patients with thyroid doses higher than 500 mGy.
Additional tasks to be carried out in the frame of work packages "Thyroid Cancer" (WP 1.3) and "Genomic instability in tumour tissues of radiation- related breast and lung cancer" (WP1.2)
In the course of the project it appeared that also knowledge on normal tissue corresponding to tumours or non-malignant tissue as such from patients who were exposed to radiation should be analysed with regard to known cancer-specific alterations. The overall aim of these works carried out in addition to the planned activities in EpiRadBio was to test the hypothesis that pre-malignant lesions are reflected by the occurrence of cancer specific alterations and thereby allow to identify these.

Deliverables:
• Deliverable D312: CLIP2 mRNA (qRT-PCR) and protein (immunohistochem- istry) expression analysis and RET/PTC analysis of all non-neoplastic thyroid specimens corresponding to the papillary thyroid carcinomas (PTCs) from the UkrAm cohort
• Deliverable D313: CLIP2 mRNA (qRT-PCR) and protein (immunohistochem- istry) expression analysis and RET/PTC analysis of follicular adenomas (FAs) from the UkrAm cohort
• Deliverable D314: CLIP2 mRNA (qRT-PCR) expression analysis and RET/PTC
analysis of the breast cancers from the Mayak cohort
• Deliverable D315: 7q11 copy number analysis from existing array CGH data from the Mayak breast cancer cohort

Sub-project 2 Radiation-induced perturbation and activation of progresses involved in carcinogenesis
WP2.1- Low-dose induction of proliferation, differentiation and genome instability in stem cells
The main hypothesis which was tested in WP 2.1 is that radiation-induced carcinogenesis is related to differences in proliferation, differentiation and genome instability induced by low dose and targeted radiations. This was delivered by two tasks both of which were a close collaboration between the Research Teams at Queen's University Belfast (QUB) and the University of Rostock (UROS) involving transfer of staff for short term visits and shipment of materials between laboratories.

The objectives in order to determine the dose response relationships for proliferation, differentiation and genome stability induced by low dose exposure were:
• To determine the effect of low dose exposure on mammary stem cells
• To determine the effect of low dose exposure on MCF10A normal breast cells

Appropriate ethical approval was obtained to collect breast tissue from patients undergoing elective reduction

mammoplasty surgery at the University of Rostock and Queen's University Belfast. Protocols were developed to isolate epithelial cells from the breast tissue which were allowed to form mammospheres in defined medium. Different sorting strategies including magnetic activated cell sorting have been used and a range of markers including EpCAM, MUC1, CD49f CD44 and CD24 have been characterised. The aldefluor assay has been established as an additional marker of stem cells and experiments have been done in primary cells. Suitable cryopreservation strategies are in place allowing recovery of primary epithelial cells and stromal fibroblasts. Protocols have been developed for low dose irradiations from 0.01 to 5Gy.

Studies of DNA damage response, proliferation, viability, cell surface marker expression, differentiation and cell survival of MCF10A cells have been performed in UROS and QUB in both 2D and mammosphere cultures.
Irradiation studies have been initiated with primary tissue cells from individual patient samples and have included cell growth, colony forming ability, DNA damage response (Gamma-H2AX/53BP1), cell surface marker expression (CD10, CD133/1, CD29, CD49f and CD90) and differentiation markers.

To determine the response of breast cells in 2D and 3D culture to localised irradiation using microbeam approaches 3D acini structures have been generated from the MCF10A cell line in matrigel and dose response relationships obtained for acini size and shape, cytokeratin staining (CK14/18), E-cadherin, GM103 (golgi), Laminin V, cleaved caspase-3, phosphor-ezrin, Ki67, gammaH2AX, MUC1 and DAPI. Quantitative PCR has been used to analyse dose response relationships at doses from 0.01 – 2Gy. Dose dependent changes in growth and circularity are observed up to 12 days after irradiation.
Using primary tissue material we have develop 3D models containing both epithelial and stromal fibroblast compartments. Initial studies have been done with X-rays but there was not sufficient time to develop the models to a suitable level for microbeam irradiation. Instead, co-culture experiments have been performed to determine the impact of bystander signalling between HME1 and MCF10A cultures and this has been extended to stromal interactions. Studies of DNA damage (53BP1) show cell line and time dependent differences in bystander responses.

This project has delivered for the first time defined protocols and procedures for the isolation, purification and analysis of stem cell sub-populations from Human normal breast tissue for radiation carcinogenesis studies in two European Laboratories. More importantly, the low-dose radiation response has been characterised in these subpopulations and in defined 2D and 3D models which replicate the key cellular interactions of the human breast. This work forms the basis for further mechanistic studies which can feed data into biophysical models of radiation-induced carcinogenesis. Future characterisation of these purified subpopulations will require in vivo validation in the cleared mouse/rat mammary fat pad or renal capsule model.
The initial studies have been reported at major scientific meetings including the European Radiation Research Society Annual Meetings. Three papers have been published and two more are in preparation covering the purification of mammary stem cells and a comparison between the radiation response of 2D and 3D cell models.

WP2.2 - Molecular mechanisms of radiation-induced carcinogenesis – role of tissue-environment and stress responses
Task 2.2.1 Role of tissue micro-environment in lung carcinogenesis - Partner 5 (STUK-lead) and SU
The main objective was to study the role of the tissue micro-environment in lung carcinogenesis. The focus areas were: the interactions between epithelial and stromal cells, epithelial to mesenchymal transition (EMT) and the involvement of TGF-β signaling pathway in this process. At first phase of the study the radiation induced EMT was investigated after acute α-particle and γ-radiation exposures using two different human lung epithelial cell lines (BEAS-2B and HBEC-3KT). It was also tested if radiation has a potentiating effect on the EMT induction.
The cells were irradiated with α-particles or γ-rays with or without of TGF-β and cells were harvested 72 hours post treatment. EMT markers (vimentin, fibronectin and E-cadherin) were analysed by immunofluorescence staining and Western blotting. The TGF-β treatment induced EMT in both cell lines in the applied low concentrations. No EMT induction was observed in cells exposed to low or moderate doses of α-particles and γ-rays. Any significant additive effect with radiation and TGF-β was neither observed. We suggest that there might be a different mechanism induced by radiation in bronchial cells after radon and medical exposures that does not involve direct EMT changes. Although, we could not observe any significant direct EMT effect and TGF-β production from the epithelial cells, we obtained data for dose-dependent secretion of active TGF-β from normal lung fibroblast cells (MRC-9) post irradiation with α-particles. We also observed increased levels of α-SMA expression in the fibroblasts indicating radiation-induced myofibroblast activation. These observations were in line with the hypothesis that fibroblasts may have an important role for TGF-β production and secretion after radiation.
The results obtained from the first stage of the study were important step, suggesting that direct irradiation of the epithelial cells might not be a sufficient inducer for EMT and there might be a need to investigate the longer term effects from the irradiated microenvironment. Therefore we explored the role of the radiation-induced signaling from stromal cells in epithelial-stromal co-culture system after protracted exposure to gamma radiation. This part of the work was accomplished together with the consortium partner 7, University of Stockholm (SU). In the course of the study we did three different visits to SU to perform protracted irradiation experiments. The cell lines were irradiated with low dose rate γ-rays with or without addition of TGF-β. In a parallel experiment the epithelial cells were irradiated in co-cultures with MRC-9 lung fibroblasts. EMT markers were analysed. The concentration of 8-oxo-dG, a marker for oxidative stress, was measured in the culture media by SU. TGF-β treatment alone induced EMT in both cell lines in the applied minimum concentrations. Protracted exposure to low LET radiation at total absorbed dose of 1 Gy was able to induce EMT. The results showed that presence of the stromal component in the co-cultures enhances the radiation-induced EMT. In addition, radiation amplified TGF-β-induced EMT with both dose rates and cumulated doses. Protracted irradiation

also induced myofibroblast differentiation of MRC-9 fibroblasts in dose-dependent manner detected as upregulated expression of α-SMA. Oxidative stress was induced in both cell types post irradiation and double treatment with TGF-β.We suggest that radiation-induced phenotypic changes in human bronchial epithelial cells require more than acute exposure and the presence of stromal component enhances the effect. These observations are pointing how the cellular transformation after low dose radiation exposures is dependent on the cell-to-cell interactions. The results could be extrapolated to the processes occurring after protracted radiation exposures in vivo.
BEAS2B cell samples after TGF-β treatment and acute alpha particle irradiation (0.1 and 1.0 gy) were analyzed using two-dimensional difference gel electrophoresis (2DE-DIGE) technique with 2-3 biological replicates for each condition. In total 25 spots were sent for identification with mass spectrometer (MS) German Diabetes Center, Düsseldorf, Germany. Analyses revealed upregulated expression of vimentin in 1 Gy irradiated samples vs control. This result was in line with the results obtained by analyzing changes in EMT marker expression with immunohistochemical stainings.
The current study gives suggestions for the importance of inter-cellular communication for early tumorigenic changes after protracted low dose rate irradiation. It raises attention to the specific 'in vivo'-mimicking conditions that have to be achieved to observe the morphological changes in the epithelial cells. Based on these data, further studies should be conducted on system and organism level to study the early health effects at the low dose rate protracted radiation. This could further have impact on radiation health risk evaluation in contaminated areas where chronic low dose rate exposures might occur (e.g. around Fukushima and Chernobyl accidents, workers in mines).
The results obtained during the first and second periods showed a high level of variability both for telomere transcription and for gene amplification. A detailed analysis of the reproducibility problems suggested that it could be related to the experimental procedure. For the initial experiments the irradiations were carried out in Stockholm, were the facility for chronic irradiation is located. The cells were then frozen and sent to Pavia where, after thawing of the cell samples, all experiments were performed. In addition, we observed that growth conditions in un-irradiated and in chronically irradiated samples were not uniform. To solve these problems a post-doc from Pavia performed the initial part of the telomere transcription experiments and the entire gene amplification experiments in Stockolm, immediately after irradiation. Total TERRA levels were evaluated in human HeLa and MCF cells by slot blot, hybridizing total RNA with a TERRA specific probe. The results of two experiments showed a very limited, statistically not significant, reduction of total TERRA following acute (1 Gy) or chronic (15 mGy/h) irradiation. However, when the transcription of single telomeric ends was evaluated by qPCR, a reproducible variability among different telomeres was observed suggesting that the inhibition of TERRA transcription may contribute to the radiation induced instability of some telomeres. To determine the frequency of DHFR gene amplication in HeLa cells, the number of methotrexate resistant colonies was counted following acute (1 or 2 Gy) or chronic (15 mGy/h) irradiation. The results confirm that low dose rates are particularly effective in inducing gene amplification. Since gene amplification is one of the major mechanisms for oncogene activation and for anticancer drug resistance, these observations suggest low dose rate irradiations may produce significant effects on tumorigenesis and tumour progression.
Evidence from several laboratories, including ours, has demonstrated that the regulation of telomere transcription play a role in the maintenance of telomere stability. Within EpiRadBio, we demonstrated that ionizing radiations inhibit the transcription of some chromosome ends but the total amount of transcripts is not significantly reduced. These results suggest that, a mechanism involving transcription may regulate the radiation induced genome instability in a chromosome-end specific manner. It is well known that gene amplification is one of the major mechanisms for oncogene activation and for anticancer drug resistance. Our results within EpiRadBio indicate that chronic irradiation is more effective in inducing gene amplification compared to acute irradiation.
The objectives to explore low dose induction of endogenous stress response and its role in genotoxic potential and adaptive responses are: (i) to determine the dose rate effects on the shape of the dose response curve for induction of an endogenous stress response and the subsequent formation of reactive oxygen species and (ii) to determine the dose rate factor for induction of adaptive response.
SU has previously shown that exposure of cells in culture to low dose rates of gamma radiation induces and endogenous stress response causing an increased oxidative stress through the formation of free radicals. SU has also shown that the nucleotide pool (dNTP) is a significant target for free radicals. We hypothesized that chronic low dose rates of radiation gives rise to long term elevated levels of endogenously formed free radicals and as well as elevated levels of oxidized dNTP and that this could cause higher yields of mutations in cells exposed to low dose rates of gamma radiation than expected from dose responses seen for unit doses given at high dose rates. The levels of the nucleotide pool sanitizing enzyme MTH1 and extracellular 8-oxo-dG were determined as biomarkers for oxidative stress in chronically exposed cells as well as clonogenic cell survival and mutation frequencies. The second hypothesis tested was that and adaptive dose given at a low dose rate would be more potent compared to the same dose given at a high dose rate. The endpoints investigated were clonogenic survival and mutation frequencies.
Normal human breast epithelial cells (MCF10A) and human lung fibroblasts cells (MRC5) were exposed to doses between 0.1 and 100 mGy, at dose-rates of 1.4, 4.1 and 15 mGy/h and the following stress markers analysed: hMTH1 using Western blot (WB) and extra cellular 8-oxo-dG using ELISA. The results indicate that oxidative stress induced by the doses applied (0.1-100 mGy, different dose rates). The effects of an adapting dose given at different dose rates were studied at the levels of clonogenic cell survival and mutation frequency. The adapting dose (50 mGy) was given at three dose rates: 1.4, 4.1 or 24000 mGy/h followed by a challenging dose of 100, 1000, 3000 or 5000 mGy. The results show that acute or chronic pre-exposure (adaptation) to 50 mGy gamma radiation does not increase the level of clonogenic cell survival in MCF-10A cells subsequently exposed to challenging doses, implying an absence of adaptive response at the level of survival. However, a significant reduction of the mutation frequency was observed when MCF-10A cells were exposed to one adapting dose (50 mGy) given at 1.4 mGy/h and challenged by 1 Gy. The absence of adaptive response at survival level and its presence at the level of mutation is in agreement with the data found in other studies. In any case it is obvious that the adaptive response is not a universal phenomenon that could challenge the validity of the LNT hypothesis in radiation protection. The dose rate effect at mutation levels were studied when MCF10A cells were exposed to 0.1 and 1 Gy but different dose rates. The interesting outcome in this experiment is the

absence of a dose rate effect at the level of mutations (for most of the case). This confirms our previous finding that implies a clear lack of dose rate effect on mutation level and stable type chromosomal aberrations in TK6 cells.
In conclusion, our results suggest that exposure to low dose gamma radiation, 50 mGy, at a dose rate as low as 1.4 mGy/h can induce an adaptive response in MCF-10A cells manifested as reduced level of mutation, however only when the challenging dose is 1000 and not 100 mGy. Such response is absent at the survival level in MCF-10A cell. Moreover in general, there is no sign of a dose rate effect at the level of survival and mutation.
For the purpose of radiation protection, a dose and dose-rate effectiveness factor (DDREF) is defined as the factor by which the risk of causing stochastic effects induced by high acute doses of low-LET IR should be reduced when the same dose is delivered at low dose rates. At present, ICRP publication 103 recommends a DDREF of 2, meaning that the slope of the dose-response at low doses or low dose-rates is half the slope compared to that for high doses and high dose-rates. However, the BEIR (biological effects of ionizing radiation) VII Committee of the National Academy of Sciences (USA) has come up with an even more conservative policy and concludes that the best estimate of the DDREF is 1.5. In support for the BEIR VII policy, recent studies of human populations provide evidence that cancer risks at low dose rates (occupational exposures) are not lower than at high dose rates (atomic bomb survivors). More support comes from the studies for the risk of developing leukemia in humans, as it is not reduced by exposure to protracted radiation. On the other hand, a report by Preston suggests that the health effects of radiation may possibly be significantly reduced after very low dose rate exposures compared to what is currently estimated. Therefore an accurate estimation of DDREF for humans remains an open question.However, considering that mutations play an essential role in carcinogenesis, and DDREF is based on cancer studies, our working hypothesis was that the level of mutations induced by different dose rates could be studied in cells in culture to investigate if DDREF of 2 was valid for mutation induction. We found that the dose rather than the dose rate is an important factor regarding radiation-induced mutagenesis. However, the results are based on a simple cell model system and might be difficult to be applied at the level of organisms as the role of immune system, interaction and communication between different organs etc, cannot be studied in cell culture. Nevertheless, our data from the present project, based on a simple cell model system, suggest that DDREF has the value of 1 in term of radiation mutagenesis.

WP 2.3 Interactions of transformed and healthy cells – pro- and anti-carcinogenic effects
Ionizing radiation can not only affect the traversed cell but can also influence neighbouring cells as a result of intercellular signalling leading to a wide range of biological responses. These non-targeted effects, depending on their relevance to tumourigenesis have the potential to lead to deviations from the “linear no-threshold” dose response used to extrapolate risk determined at high dose and dose rates from epidemiological studies down to low doses associated with typical human exposures. In order to understand this phenomena and its potential impact it is important to understand the underlying mechanisms of how ionising radiation can perturb the existing intercellular signalling network as a function of dose and radiation quality. This was investigated using the previously well-characterised model of intercellular induction of apoptosis (IIA) in non-irradiated oncogenic transformed cells as a reporter of perturbations in intercellular signalling from surrounding normal cells. Previous data from the Oxford group (UOXF.H3) has shown that both high and low-LET radiation perturb intercellular signalling even at very low doses associated with typical human exposures (2 mGy for □-rays and 0.4 mGy for □-particles) with the response plateauing above ~50 mGy. The model system is itself of interest as the transformed cells studied potentially represent a stepping stone for full oncogenic transformation and any modifications of the rate of removal of these transformed cells by radiation could ultimately lead to a modification in cancer risk.

Low dose perturbation of intercellular communication and apoptosis
The main objectives of this task were to identify the pathways involved in low dose perturbation of intercellular communication and apoptosis in non-irradiated oncogenic transformed cells (as a marker of oxidative stress), mediated by surrounding normal human cells and ascertain the generality of stimulation of normal intercellular signalling pathways in a number of normal human cell lines.
The generality of the perturbation of the naturally occurring intercellular signalling by low doses of radiation and associated IIA response between species and in different cell types was demonstrated by the Oxford group (UOXF.H3) for normal human lung fibroblasts (MRC5), human bronchial epithelial cells (BEAS-2B) and human mammary epithelial cells (MCF10A) as well as the original normal rat lung fibroblasts (208F). The addition of specific reactive oxygen/nitrogen species (ROS/RNS) scavengers confirmed a role for similar signalling pathways as those induced by fibroblast effector cells; namely a peroxidase/hypochlorous acid (POD/HOCl) pathway resulting in the production of the apoptosis-inducing hydroxyl radical in close proximity of the transformed cell, and a nitric oxide (NO•) pathway which terminates with the apoptosis-inducing peroxynitrite ion (ONOO•). The NADPH oxidase (and subsequently superoxide, O2•-) inhibitor, apocynin confirmed the importance of the O2•-- cloud that surrounds the transformed cells. Additionally, experiments performed in collaboration with Prof. Bauer (Freiburg) using a competition assay have shown that irradiation of a range of both normal and transformed cells to doses down to 0.7 mGy of either □-particles or □-rays led to maximal release of POD 12 hours post exposure (paper in preparation). Furthermore the key role of TGF-□ in the signalling process was confirmed by using either an anti-TGF-β blocking antibody, which blocked the IIA response or pre-treating cells with TGF-□ which produced a similar enhancement of IIA response following irradiation of the effector cells, with no additional enhancement observed if the effector cells are irradiated in addition to TGF-□ treatment. The non-transformed 208F effector cells (in the absence of the transformed cells) show rapid activation of TGF-□ following irradiation as detected by the nuclear localisation of Smad proteins within 1 hour post irradiation. A putative role for p38MAPK in radiation-induced IIA has also been demonstrated. Oxidative stress in 208Fsrc3 cells was enhanced when co-cultured with each of the effector cell types and was further enhanced by irradiation of these effector cells, however the enhancement on IIA was also found to decrease with decreasing level of oxygenation. These data support the hypothesis that low doses of radiation can enhance the production and/or activation of TGF-□, which subsequently can act on both the normal and transformed cells leading to the production of NO• and POD.

In collaboration with UNIPV, experiments have been performed in Oxford (UOXF.H3) to investigate the spatial and temporal development of IIA perturbed by ionizing radiation to provide data for modelling performed in task 2.3.3. This work was facilitated by the development of a high-throughput facility with automated scoring to quantify the amount of apoptotic cells in a non-invasive manner as a function of time. The apoptotic level observed in the 208Fsrc3 transformed cells, which is a marker of oxidative stress levels, reduced with increasing distance to the normal 208F cells population reaching background levels at 16mm, with irradiation of the 208F cells leading to an enhancement of the response out to 10mm. This data was integrated into an empirical model mapping the induction of IIA as a function of distance and time as part of task 2.3.3. The limiting case was also explored where both cell lines were seeded as a mixed population on the same surface. The rate of apoptosis was again observed to increase following co-culture and increase further still if the normal cells were irradiated prior to seeding. The results of the sham and irradiated experiments show a reduction on percentage of cells undergoing apoptosis predominantly due to continued expansion of the normal cell population and dislodging attached apoptotic cells. Stimulation of the response is also dependent on the fraction of the normal cell population irradiated, with maximal stimulation observed down to ~10% below which the response falls to background levels when ~1% of the cell population was irradiated.
As it stands, the experiments suggest that for small pre-neoplastic lesions composed of a few transformed cells surrounded by lots of healthy cells in close contact, the IIA should be very efficient (due to the long-time combined with a small spatial separation) at removing or reducing the number of these transformed cells, unless a lower level of oxygenation is present. In addition very low doses of radiation have the potential of increasing the rate of removal of these transformed cells, depending on the pre-existing level of TGF-□ in the tissue. This has been shown to relate an increase in the TGF-□ and associated production of NO• and POD in normal and transformed cells. However the relative contribution of the various pathways is very likely to be dependent on the spatial distributions and in particular the local concentration of transformed cells. There is some evidence to suggest that the POD/HOCl pathway may dominate with clumps of transformed cells. Modelling studies performed at HMGU (task 2.3.2) have suggested that under certain conditions where both cell lines were irradiated, that radiation may lead to a decrease in IIA (and therefore a potential increase in carcinogenic risk). Following discussions with HMGU, experiments were performed at UOXF.H3 to try and test model predictions, by irradiating the transformed 208Fsrc3 cells in co-culture with normal 208F cells. As before, this led to an increase rather than a decrease in IIA response. However the relative contribution of the various signalling pathways and interaction between pathways is critically dependent on the spatial distribution of cells and HMGU are currently identifying potential experimental conditions where the radiation may lead to a reduction in IIA in order to test these modelling predictions (see task 2.3.2).

Radiation effects on intercellular induction of apoptosis in transformed cells
The main objective was to develop a mechanism-based mathematical model for intercellular induction of apoptosis (IIA) in oncogenic transformed cells through signalling by surrounding normal cells and radiation-induced perturbations to this phenomenon, based on experimental findings in SP2 and in the literature, to inform the development of carcinogenesis models in SP3.
Selective removal of oncogenic transformed cells via apoptosis upon signalling from surrounding normal cells (intercellular induction of apoptosis, IIA) has been suggested to serve as a natural anti-carcinogenic mechanism (Bauer 2007 Int J Radiat Biol 83, 873-88). Mechanistic modelling supports this concept and predicts that IIA may limit the growth of populations of transformed cells, corresponding to dormant pre-neoplastic lesions, or even completely eradicate the transformed clone (Kundrát et al 2012 Carcinogenesis 33, 253-9; 2015 Radiat Prot Dosimetry, in press, doi: 10.1093/rpd/ncv169).
Low-dose irradiation has been shown to modulate the underlying cytokine and reactive oxygen species signalling and to enhance IIA in vitro (Portess et al 2007 Cancer Res 67, 1246-53; Abdelrazzak et al 2011 Radiat Res 176, 346-55; results obtained within Task 2.3.1). On the other hand, epidemiological data show that radiation induces additional cancers, statistically significantly at least at doses above about 0.5 Gy. To solve this discrepancy, mechanistic modelling of IIA has been performed by HMGU. The two major signalling pathways have been represented, namely the peroxidase/hypochlorous acid and nitric oxide/peroxynitrite pathways. The model has been benchmarked against IIA data from the literature and generated within the project, and then used to predict IIA outcome under conditions that correspond to the situation in vivo more closely than the experiments do. Namely, in typical IIA experiments comparable numbers of transformed and normal cells are used, while in early stages of carcinogenesis only a relatively small clone of transformed cells is surrounded by an overwhelming population of normal cells. The cells are typically seeded relatively sparsely, while most tissues are rather compact. The lifetimes of all species involved in IIA signalling are significantly shorter in vivo than in vitro.
Accounting for all these effects, the modelling predicts that under in vivo-like conditions the two pathways do not contribute equally as in typical IIA experiments but that the peroxynitrite pathway with nitric oxide derived from normal cells largely dominates, and decreases with increasing superoxide release from transformed cells. Low-dose radiation has been shown to transiently enhance the release of superoxide from transformed cells and of peroxidase from all cells, while its effect on the release of nitric oxide is limited (Temme & Bauer 2013 Radiat Res 179, 422-32; Bauer 2011 J Phys Conf Ser 261, 012001). These radiation-induced modifications enhance IIA in vitro, but are predicted to reduce this anti-carcinogenic process under in vivo-like conditions. The performed mechanistic modelling thus solves the discrepancy between radiobiological experiments on IIA and epidemiological data: Radiation is predicted to act on IIA in an anti-carcinogenic way in vitro, but in a pro-carcinogenic way in vivo. Future research should aim at finding conditions (cell densities, scavenger concentrations, inter-culture distances etc.) that could be realized in vitro and where this effect would already be predicted, and at verifying these predictions experimentally.

Modelling regulatory mechanisms of cellular communication following low dose
The objective was to model based on experimental data the effects on intercellular signalling through perturbation of the levels of cytokines induced by different radiation types.

The general objective of UNIPV within task 2.3.3 (and associated contribution to task 2.3.1) was to explore and identify the mechanisms underpinning the intercellular induction of apoptosis (IIA) in non-irradiated transformed cells in co-culture with normal cells, either irradiated or not. The rationale behind the choice of this experimental setup was to develop an in vitro model to represent a simplified in vivo situation of crosstalk between healthy and transformed cells and better understand whether low doses of radiations delivered to healthy tissue/organs have a protective or detrimental role.
For this, two different levels of investigations were considered, i.e. 1) modelling the spatial and temporal features of the IIA comparing both the irradiated and control conditions and with or without inhibitory molecules (in collaboration with UOXF.H3) and 2) evaluating the signalling proteins dynamics and pathways (in collaboration with HMGU). The results of these studies are therefore related to the macroscopic effects of the bystander signalling on the transformed cells, i.e. the apoptosis induction, and to the underpinning signalling pathways that can explain the observed IIA after irradiation.
UNIPV collaborated with UOXF.H3 to perform experiments to systematically explore the variation in the IIA response as a function of time and distance in addition to using a range of scavengers/inhibitors to explore the relative contributions of the various pathways (see also task 2.3.1). This data was integrated into an empirical model mapping the induction of IIA as a function of distance. The response is not only dependent on the diffusion properties of the various signalling molecules but includes potential synergistic effects of different oxidative stress-related molecules.
Experiments were carried out also to evaluate and quantify the radiation-altered signalling protein spectra exchanged between the two cell populations. For this, different complementary studies on both gene expression and protein levels were performed to obtain information on the dynamics and pathways altered in the different co-culture conditions.
Studies on the release dynamics of selected signalling proteins do not show a clear dose dependence in the latent TGF-□1 above 25cGy, whereas differences between control and irradiated samples can be observed, but only at late time points . Gene expression microarray and subsequent pathway analysis showed evidence that radiation exposure alters cell cycle-related pathways of directly irradiated cells and also perturbs signalling pathways involving interleukins related to the immune response. Further, in depth analysis of the gene expression data provided information on the correlation among cytokines and cytokine receptors levels, highlighting the role of the TGF-□ family signalling in the whole process.
In conclusion, the complex effect under investigation is modulated by each of the different conditions we have taken into account (radiation dose and quality, time and space, oxidative stress and TGF-□1 pathways). This complex scenario was particularly evident at the signalling protein level, where different and redundant signalling pathways have been found and recognized to have an important role in such process, although still not having a completely clear understanding of the relative importance of each pathway in the overall IIA. Differently from many other bystander effect studies, this in vitro setup helped in shedding light on the mechanisms underpinning a possible protective effect of low doses of radiation, with consequences in the prediction and in the modelling of radiation risk.
The project results have clearly shown that both high and low-LET irradiation of normal cells can perturb intercellular signalling even at very low doses associated with typical human exposures such as diagnostic exposures or occupational exposures for some mine workers. In particular responses have been observed at doses as low as 2 mGy for □-rays and 0.4 mGy for □-particles. Even at these low doses, radiation enhances the production and/or activation of TGF-□, which subsequently can act on normal (and transformed) cells leading to the production of NO• and POD. More generally, the perturbed intercellular signalling following irradiation of normal cells has previously been shown to lead to a range of biological endpoints including transformation and induction of genomic instability. These may be more important at very low doses associated with typical human exposures, where only a small fraction of cells have a DNA double-strand break, while at higher doses associated with accident or therapy dose (in the region where epidemiology shows statistically significant effects and all cells will have multiple DNA double-strand breaks) it is expected that the classical direct effects of radiation are likely to dominate. Interestingly the work of Eidemüller et al (2012), PLoS ONE 7(8):e41431 for lung cancer mortality after exposure to radon decay products, analysed using a two-staged clonal expansion model showed an increase in the clonal expansion during exposure, with the increase being much stronger for the lower dose rates with the ‘bystander effect’ being proposed as a possible explanation. The initial increase and ‘plateauing’ of the clonal expansion rate occurs over an exposure range (as quantified by the percentage of cells traversed by an □-particle) similar to that producing an increase and subsequent plateauing of the intercellular induction of apoptosis response as a result of enhanced levels of oxidative stress, pointing out the generality of the studied radiation-induced modifications to intercellular signalling.

The model system of intercellular induction of apoptosis (IIA) in transformed cells is itself of interest as the transformed cells studied potentially represent a stepping stone for full oncogenic transformation. Therefore any modifications of the rate of removal of these transformed cells by radiation could ultimately lead to a modification in cancer risk. The experimental data and the mechanistic modelling suggest that for a three-dimensional tissue, with a small pre-neoplastic lesion composed by few transformed cells surrounded by lots of healthy cells in close contact, the IIA should be very efficient, unless a lower level of oxygenation is present. IIA may completely remove the transformed population, or at least stop its growth. In addition very low doses of radiation have the potential of modifying the rate of removal of these transformed cells. In radiobiological experiments, low dose radiation (>2 mGy) of normal cells stimulates various pathways in the removal of transformed cells by apoptosis reaching a maximum enhancement above 50 mGy. While likely being overweighed by other processes at higher doses, this effect may dominate at very low doses, and modify the LNT hypothesis assumed in low-dose risk. First results of the mechanistic modelling studies performed within the project however predict radiation to enhance IIA in vitro but to reduce this anti-carcinogenic process under in vivo conditions. Critical experimental to test these various model predictions are currently being designed.
Sub-project 3 Epidemiology, carcinogenesis and risk
WP 3.1 Breast cancer risk
Breast cancer risk after exposure to low dose of ionising radiation has been analysed in several epidemiological

cohorts. Improved models of carcinogenesis incorporating pathways with genomic instability have been developed. Individual variability in radiation sensitivity has been studied taking familial risk factors into account, such as the number of children, age at first birth and breast cancer occurrence in relatives of exposed individuals. Risk estimates from mechanistic and descriptive models have been derived and compared to the LSS cohort. The results suggest an additive transfer of risk between different populations. Dependence of risk on familial factors, breast dose, exposure history, and age attained has been studied. The mechanistic model for the haemangioma cohort indicates, at least for exposure at young ages, that radiation-induced cellular changes might activate different molecular pathways than spontaneous mutations.

The Swedish Hemangioma Cohort (UGOT, HMGU, KI)

The Swedish Hemangioma Cohort (SHC) consists of about 27000 individuals from two cohorts of patients treated with ionizing radiation because of skin hemangioma in Stockholm and Gothenburg. All individuals were younger than 18 months at time of first treatment. The treatments were performed between 1920 and 1965. All women alive and living in Sweden January,1 1958 were included in the breast cancer analysis.

This part of the SHC consists of 17200 women with 26300 treatments.

The Swedish hemangioma cohort for breast cancer with familial factors

At the start of the EpiRadBio project the follow-up of SHC was extended to the end of 2009. Data on breast cancer incidence was collected not only for the patients, but also for their mothers, sisters and daughters by record linkage of SHC to the Swedish Multi-Generation Register and Swedish Cancer Register. The women in the SHC had a median follow-up of 61 years and 877 of them had a breast cancer. The expected number of women with breast cancer was 705, which gives a SIR of 1.24 (95% confidence interval: 1.18-1.35). Among mothers there were 1,157 that had got a breast cancer and among sisters and children there was 552 and 57 breast cancers respectively. The analyses of SHC in the frame of EpiRadBio have been based on these data. At the end of the project the SHC have been updated to the end of 2013. The updated cohort now includes 1,079 women with breast cancer. Among mother, sisters and daughters there are respectively 1,226, 698 and 100 women with breast cancer.

Updated dosimetry in SHC

Retrospective risk assessments of ionizing radiation demand, among other information, high-quality dosimetry data. Previous dose estimations for the Stockholm hemangioma cohort were based on measurements with thermoluminescent dosimeters (TLD) in an anthropomorphic phantom using the original radium sources, and by a simple dose planning system (DPS). The DPS was used for dose estimations close to the source. The insight of shortcomings of the DPS motivated a reanalysis of the dosimetry of the radium sources. Especially the radiation doses to the female breasts had to be recalculated before this new analysis of radiation-induced breast cancer risk was initiated. The new dosimetry was performed with Monte Carlo (MC) simulation where the models were created with MCNP5 and GEANT4. Since the DPS was merely used for treatments close to the breast nipples only these treatments were affected by the new dosimetry. The result for these treatments showed that the highest doses decreased in average by 15% and the mean value by 75%. As only a small part of all treatments was influenced by the reanalyses, the total breast doses for the women, summarizing all treatments, were less affected. The mean total breast dose for all women in the Stockholm cohort decreased by 49%. The breast doses in the Gothenburg cohort were from the beginning also estimated by a simple DPS. However, some years ago they were recalculated using data from the TLD measurements in Stockholm. This implied that the breast doses in this cohort were only slightly affected by the new MC calculations. After the reanalysis only 3% of the women in the whole cohort had breast doses exceeding 1 Gy (max 32.8 Gy). The mean dose decreased to 0.18 Gy but the median dose remained 0.04 Gy.

Breast cancer risk and possible mechanism of radiation-induced genomic instability in SHC after reanalysed dosimetry

The SHC was analysed for breast cancer incidence with descriptive excess relative risk models and mechanistic models of carcinogenesis. All models agree on the risk estimates. The excess relative and excess absolute risk at the age of 50 years are 0.48 Gy−1 (95% CI 0.28; 0.69) and 10.4 (104 PYR Gy)−1 (95% CI 6.1; 14.4), respectively. These risk estimates are about a factor of 2 higher than previous analyses of this cohort as a consequence of the re-evaluation of the dosimetry system. Explicit models incorporating effects of genomic instability were developed and applied to the SHC. It was found that a radiation-induced transition towards genomic instability was highly significant. The models indicate that the main effect of radiation-induced genomic instability is to increase the rate of transition of non-initiated cells to initiated cells with a proliferative advantage. The magnitude of such acceleration cannot be inferred from epidemiological data alone, but must be complemented by radiobiological measurements. The results indicate, at least for exposure at young ages, that radiation-induced cellular changes might activate different molecular pathways than spontaneous mutations, leading to longer-term processes.

Breast cancer risk in the SHC taking into account breast cancers among mothers, sisters and daugthers

For the family dependent baseline risk there was a 23% increase in risk of the hemangioma patient if a mother had breast cancer ($p=0.10$), a 145% increase in risk if a sister had breast cancer ($p<0.001$), and a 110% increase if a child had breast cancer ($p=0.058$). Both the changes in excess relative risk and excess absolute risk are investigated. The results suggest that the absolute radiation risk for breast cancer is increased 2-4 fold if one of the family members has breast cancer. This should be taken into consideration for medical diagnostic and therapeutic approaches. Statistical power is crucial for this analysis, and an update with the newest available follow-up is planned for publication of the results.

Breast cancer among French, Swedish and Italian thyroid cancer patients (INSERM, UGOT)

In the frame of the EpiRadBio project, the aim was to evaluate the risk of secondary breast cancer in an European cohort of Swedish, Italian, and French patients with papillary or follicular thyroid cancer, and to distinguish any pattern of risk related to exposure to internal radiation therapy given either alone or in association with external beam radiation therapy.

Update of the French, Swedish and Italian thyroid cancer cohorts

The French part of the cohort has been updated up to 2013 for new treatments (therapeutic 131I and external radiotherapy) and new events, by access to the medical records of all the centers.

The update of the Italian part of the cohort has not been performed, due to frequent changes at the head of the department of nuclear medicine of the Hospital of Busto Arsizio. It has to be noted all this part of the cohort had yet been updated, both for new treatments (relapses, metastases, second cancers) and for the new events (second cancers) in 2009.
For the Swedish cohort there was not possible to obtain new information from hospital report. The follow-up data have been improved to included data from the Swedish Cancer Register to the end of 2009 for all the patients.
Update and improvement of the dosimetry
The two main sources of ionizing radiation exposure of the patients of the cohort are 131I and external radiotherapy. For 131I, more realistic exposure estimates were performed (Collaboration with NCI). The dose to the breasts was estimated with the Bolch formulae, using S values from source organ to target organ, computed with the ICRP reference female voxel phantoms and time-integreted activity coefficients of patients treated for a thyroid cancer [Remy, 2008] According to the method applied at this time, for a given activity delivered, a unique breast dose was estimated. Using the ICRP voxel phantoms, the breast doses estimated per 131I administrations were 1.5 times greater than the values derived from the stylized phantoms.
In total, about one tenth of the patients were treated by external radiotherapy. For this source of ionizing radiation, the dosimetry was initially performed for half of the patients with Dos_EG, a computer program developed in our team, in order to estimate the dose received in different parts of the body. In the frame of the EpiRadBio project, we have done additional dose estimations. As technical reports was not available for a significant part of the patients, we have although performed dose-estimations according to standard treatments and used nearest neighbour hot-deck imputation
Breast cancer risk in the French, Swedish and Italian thyroid cancer cohorts
In total, 8475 women were included in the analysis. The median age at diagnosis was 44 years. The median follow-up period after thyroid cancer diagnosis was 13 years (range: 2– 67 years). The median interval of time between thyroid cancer diagnosis and breast cancer occurrence was 14 years (range: 2– 55 years). In all, 11% were treated with external radiotherapy and 62% received therapeutic 131I. The median radiation dose to the breast from 131I was 247 mGy.
Compared to the general population, the women treated for a thyroid cancer had 47% higher risk of breast cancer occurrence (CI 95%: 32-63) than the general population of each country. The same magnitude of increased risk was observed in the three countries. Totally there were 289 thyroid cancer patients with a secondary breast cancer.
The risk of breast cancer increased non-significantly with the dose of ionizing radiation received to the breasts: It increased by 0.12 per Gray received to the breasts (95%CI: -16.26 to +0.697). The ERR was higher for the cumulative doses received 20 years or more before. In conclusion, the overall ERR per Gy is lower but still compatible with the one observed after external ‘instantaneous’ irradiation for HN survivors of same age.
The Mayak workers cohort (HMGU, SUBI)
The Mayak workers cohort (MWC) includes workers, started their employment in 1948-1982 in one of the five plants of Mayak PA: reactor, radiochemical, plutonium production, mechanical repair and water treatment. Cancer incidence analyses are restricted to workers of three main plants: reactor, radiochemical, plutonium. For the purpose of compatibility, analyses of mortality were restricted to the same subcohort. Workers of these plants were subject to exposure to external radiation whilst workers at radiochemical and plutonium production plants were also subject to exposure to plutonium-containing aerosols.
Breast cancer among Mayak workers
During the period 1948-2008 there were 123 incident cases of breast cancer registered among Mayak female workers (and former workers) who remained Ozersk residents. During the same period 56 deaths from breast cancer were registered among those members of MWC who remained Ozersk residents and 37 among those who migrated anywhere else.
Mortality dose response analysis in MWC
The effect of the external gamma dose was modelled as linear dose-response, the ERR/Gy coefficient was 0.17 (95% CI -0.11 – 0.68). Of 93 breast cancer deaths occurred among female members of MWC 85.6 are, according to the model chosen, attributed to background and 7.4 (about 8%) are attributed to ionizing radiation.
Incidence dose-response analysis in MWC
Among female members of MWC, who remained Ozersk residents, there where 123 incident cases of breast cancer. The excess relative risk per Gy was 0.16 (95% CI -0.1 – 0.58), which is almost exactly the risk per unit dose for mortality. Of 123 incident cases 114.9 could be attributed to background and 8.1 (6.6%) to external exposure. Further non-significant trends suggest a slight decrease of ERR with attained age, and an upward curvature of the dose response. Since the lack of power precludes an accurate analysis of the breast-cancer dynamics (~10 radiation-induced cases), no mechanistic models have been constructed.
WP 3.2 Lung cancer risk
Mechanistic models for lung cancer after alpha-particle exposure for the Mayak workers and Wismut miners have been developed and consequences for plutonium and radon risk has been evaluated. Excess relative risk (ERR) models for the radon cohort of the Wismut miners show a linear dose response with a strong dependence of the ERR on attained age, time since exposure and exposure rate. The ERR model for Mayak workers after plutonium exposure has a linear dose response with only an indication for a dependence on attained age.
All cohorts were analyzed with mechanistic models of carcinogenesis. The radiation effect on different stages of carcinogesis was tested. Both in a two-stage and a three-stage model with clonal expansion, it was consistently found for all cohorts that the main effect of radiation leads to an increase of the clonal expansion rate of initiated cells. This indicates a disturbance of cell cycle control or change of cell inactivation (e.g., apoptosis/differentiation rates). The clonal expansion rate depends non-linearly on annual lung dose: after a relatively strong increase for lower exposure rates a levelling is found for larger exposure rates. A radiation-induced bystander effect could be a possible explanation for such an exposure response. This is consistent with the findings from SP2 on intercellular signalling. Since these results

are observed both for radon and plutonium, they might indicate similar underlying mechanisms. The different dependence of the ERR for radon and plutonium in the descriptive models on the radiation modifiers age, time since exposure and exposure rate might be explained by the long-term exposure after plutonium intake when compared to radon.

Mayak worker cohort for lung cancer

The Mayak-workers cohort comprises nuclear workers at the Mayak Plutonium-production facility at Ozyorsk, Russia. The current follow-up includes all years 1948–2008 and comprises 25,757 members. Many of the workers have been exposed to Plutonium-239, predominantly through inhalation. These internal doses have been assessed via urine measurements combined with biokinetic modeling for about 40% of workers in the plants at risk. Furthermore, for most workers, information exists on smoking status and alcohol consumption, as well as on external gamma doses (not significant in this study). Owing to pronounced smoking and Plutonium-inhalation patterns, the dominant cancer-mortality endpoint is lung cancer, with a total of 895 mortality cases. To obtain a sufficiently homogeneous data set amenable to mechanistic modeling, we restrict the cohort to males with full information on smoking/alcohol status and annual internal doses – i.e., 239 Pu doses must be measured or assumed to vanish. This reduced (sub)cohort includes 8,604 persons and 388 lung-cancer deaths.

Lung cancer risk after plutonium exposure with mechanistic models among Mayak workers

To analyze lung-cancer mortality after Plutonium exposure, different mechanistic multi-stage models have been applied: Besides the established two-stage model with clonal expansion, we employed models with three mutation stages as well as a model with two distinct pathways to cancer. The results indicate that three-stage models offer an improved description of the data compared to the two-stage one. No evidence is found for a model with two pathways. All best-fitting models point to a mechanism where radiation increases the clonal-expansion rate of premalignant cells. This may be interpreted in terms of changes in cell-cycle control mediated by bystander signaling or repopulation following cell killing. That mechanism is in line with that found in previous studies on alpha-radiation risk based on two-stage models, both for Plutonium as well as Radon. The present results suggest that this basic mechanism may be of general nature and not limited to the framework of two stages.

Due to a shared basic structure of mechanistic models for Radon and Plutonium risk, many features of the predicted radiation risk agree qualitatively. Among them are: (i) a nonlinear dose-response relationship; (ii) a nearly exponential initial increase of excess relative risk (ERR) with age during exposure, followed by a drop-off for later ages; (iii) an inverse dose-rate effect (i.e., a suppressed risk at high dose rates/short exposure period); and (iv) a similar, if smaller, ERR for smokers compared to non-smokers, i.e., a (sub-)multiplicative interaction between radiation and smoking. It must be stressed that these are model predictions: In fact, most of the effects above cannot be resolved at a significant level within a descriptive analysis of the Mayak data, mainly due to the very long duration of Plutonium exposures compared to Radon and statistical power.

Despite their structural similarity, the different multi-stage models do make qualitatively different predictions for the risk following certain exposure scenarios. Most strikingly, in contrast to the two-stage case, all three-stage models exhibit a critical dose above which the excess risk increases sharply: for low doses, the excess relative risk may be much less than the average value of 5 per Gy, as found in a descriptive analysis, but much higher above the critical dose of about 0.25 Gy. The dose response levels off for doses much higher than about 1 Gy.

Cohort of Wismut miners

The Wismut cohort is the worldwide largest study for lung cancer after radon exposure. The cohort analysed here includes 58695 workers employed to extract the uranium ore in the mountains of Saxony and Thuringia from 1946 to 1990. These workers were exposed to radon and its progeny chiefly working underground or in uranium ore processing facilities. The exposure was relatively high compared to other uranium mines, with an average of 280 WLM (more than 1Sv). Apart from radon, Wismut workers were exposed to external gamma radiation, long-lived radionuclides, arsenic, fine dust and silica, which are all considered to be carcinogenic agents. Previous studies of this cohort have found that among all these agents only exposure to silica dust results in a significant lung cancer risk. Thus silica dust exposure has to be taken into account in our analysis to make an accurate prediction of the effect of radon in lung cancer. In our analysis we considered 2996 lung cancer deaths, which in combination with the large number of workers and long duration of the follow-up results in a strong statistical power.

Lung cancer risk after radon exposure with mechanistic models among Wismut miners

The two-stage clonal expansion model (TSCE) used here has been widely used as a simple mechanistic model for carcinogenesis. It provides us with indications of possible biological processes during the development of cancer in addition to independent estimates of the risk that can be compared with other more traditional epidemiological models. In our analysis a model with an increased rate of clonal expansion of initiated cells caused by radon and silica dust exposure has emerged as the model that best describes the data. Radon exposure affects the clonal expansion rate in a non-linear fashion, having a linear response at low exposure rates which eventually saturates at a certain level as the exposure rates increase. Silica dust has no effect on proliferation until the dust concentrations in air during a working year exceed about 1mg/mm3. There is a residual calendar year adjustment in the clonal expansion rate even after taking into account the silica dust response, which may reflect the improvement of the working safety conditions and other lifestyle factors throughout the post WWII years to the closure of the mines in 1990.

WP 3.3: Thyroid cancer risk

The aim of WP 3.3 is to analyze the risk of thyroid cancer from ionizing radiation with both descriptive and mechanistic models of carcinogenesis. These models have been applied to data sets of Japanese a-bomb survivors and to the UkrAm cohort. For the a-bomb survivors a descriptive model has been developed to assess the effectiveness of the ultrasound screening campaign after the Fukushima accident [1]. However, the major effort of WP 3.3 has been devoted to the radio-epidemiological analysis of papillary thyroid cancer (PTCs) in the UkrAm cohort. For this cohort and for the GENRISK-T cohort, which included Ukrainian and Russian PTCs from the Chernobyl tissue bank, samples of tumor tissue have been used for molecular analysis within EpiRadBio [2, 3]. This choice was made because the integration of results from molecular biology and radiation epidemiology to assess low dose radiation risk is among the core objectives of

EpiRadBio.
Two versions of the UkrAm cohort have been made available in April 2013 and in April 2014 to HMGU by IEM which contain data sets that were used in the two studies of Tronko et al. [4] and Brenner at al. [5]. For the radio-epidemiological analysis within EpiRadBio the UkrAm cohort data set of 13,152 Ukrainian subjects from Brenner et al. [5] has been used. It contained 115 PTCs which were detected in a pre-screening phase and four screening examinations (1998-2000, 2001-2003, 2003-2005 and 2005-2007). The histopathological analysis of thyroid carcinomas was performed by IEM in co-operation with NCI. Most PTC cases were females and their proportion did not significantly vary over time. More than 60% of all PTC cases were children < 10 years old at the time of the Chernobyl accident. While the youngest age at exposure (AaE) was observed in prescreening cases, there was no significant trend in AaE of cases over time. However, a significant increase in age at operation (AaO) over time was observed. A significant decreasing trend with 131I thyroid dose was detected, although this trend did not persist after adjustment for age at operation.
Molecular analysis of PTCs in WP 1.3 confirmed earlier results that overexpression of the CLIP2 gene can be used as a radiation biomarker [2, 3]. For young patients (AaO< 20 yr, AaE < 5 yr) a positive dose response has been detected for thyroid doses under 1 Gy and a flattening of the response for higher doses [6]. Data from older patients (AaO ≥ 20 yr, AaE ≥ 5 yr) revealed no significant dose response of CLIP2 overexpression. In the study of Selmansberger et al. [6] 76 of 141 PTCs belonged to the UkrAm cohort and 65 PTCs belonged to the Genrisk-T cohort. In co-operation with WP 1.3 IEM has analyzed histopathological features of 76 UkrAm PTCs with CLIP2 typing. 72.4% of UkrAm PTCs had a positive CLIP2 biomarker. The frequency of CLIP2 positive cases linearly increased over time from 1st to 4th cycles of screening examination. The average thyroid dose in UkrAm cohort members with CLIP2 positive PTCs by contrast is reduced from 1st to 4th screenings. CLIP2 positive PTCs show a decreasing tumor size with increasing latency. The histopathological analysis showed that mixed subtypes prevailed among CLIP2 positive PTCs with increasing frequency over time. IEM conclude that studies of CLIP2 gene as a biomarker of radiation should certainly continue, in particular, to clarify the question: why is the risk of radiogenic thyroid cancer in exposed subjects decreasing with increasing time since exposure, whereas the frequency of CLIP2 positive PTCs increases in subsequent screening cycles.
The radio-epidemiological analysis comprised all 115 papillary thyroid cancer cases (PTCs) of the data set with individual likelihood regression. Compared to Poisson regression this method uses individual data rather than data in person year tables for calculation of risk estimates. The method allows to combine incidence data for the first (prevalence) screening period and the second to fourth (incidence) screening periods. In the analysis only cases of papillary thyroid cancer have been used because measurements of molecular data are performed only for this cancer type. Both descriptive and mechanistic risk models have been applied to the cohort data. The baseline version of the descriptive model depends on attained age and gender and is corrected for oblast of residence in 1986 similar to the model of Brenner et al. [5]. The excess relative risk (ERR) of the descriptive model depends linearly on dose but is modified with an exponential term which accounts for cell killing at high doses. Attained age and gender were additional dose effect modifiers of the descriptive model. It contained six adjustable parameters and yielded the lowest AIC = deviance + 2 x no. of parameters of all tested models. The age dependence of the baseline hazard and the ERR was modelled by a power law using the same exponent but with opposite sign. Descriptive models of the excess absolute risk (EAR) have not been considered for risk assessment since the described the data not as well as ERR models. Their deviances came out some twenty points higher.
An important result of WP 3.3 is the development of a mechanistic model of thyroid carcinogenesis. The model assumes two pathways to thyroid cancer. The sporadic pathway pertains mainly to older patients with AaO ≥ 20 yr, AaE ≥ 5yr and lower thyroid doses. The radiation-induced pathway pertains mainly to younger patients with AaO < 20 yr, AaE < 5 yr and higher thyroid doses. The dose response of the radiation-induced pathway is linear up to dose of about 1 Gy and exponentially attenuated for higher doses. Compared to the mechanistic model the descriptive model provides a slightly better fit with an AIC difference of about five points. Results of the mechanistic model can be linked to molecular measurements of overexpression of the CLIP2 gene, which has been identified as a radiation biomarker [2, 3]. For younger patients the probability of a positive CLIP2 biomarker shows a striking similarity to the probability of being a radiation induced PTC which can be calculated by the mechanistic model. The comparison supports the hypothesis of separate molecular pathways for spontaneous and radiation-induced PTCs.
A main task of WP 3.3 was to produce estimates of the thyroid cancer risk at low doses. The central estimates of the ERR for attained age 24 yr and the EAR per 10,000 PY from the mechanistic and descriptive models are shown in Tables 1 and 2 for thyroid doses between 0.01-10 Gy. For an exposure of 1 Gy risk estimates for the ERR are compared with pertinent estimates of previous studies on the UkrAm cohort and of a pooled analysis for externally irradiated children [4, 5, 7]. In view of their uncertainties a good agreement was found. However, estimates for the ERR from a study with aggregated data of Chernobyl children and from a-bomb survivors were significantly higher [1, 8]. Estimates of the EAR do not depend on attained age and agree well with the estimate of 4.4 (95% CI 1.9; 10.1) which was recommended in the BEIRVII report [7].
Table: Excess Relative Risk at attained age 24 yr with 95% CI in brackets from the mechanistic model and the descriptive model for thyroid doses 0.01Gy, 0.1Gy, 1Gy and 10Gy.

Mechanistic Model		Descriptive Model	
Thyroid Dose (Gy)	Men	Women	Men	Women
0.01	0.020 (0.008; 0.046)	0.040 (0.018; 0.077)	0.015 (0.004; 0.030)	0.029 (0.009; 0.051)
0.1	0.20 (0.07; 0.46)	0.39 (0.17; 0.77)	0.14 (0.04; 0.30)	0.29 (0.09; 0.50)
1	1.8 (0.7; 4.1)	3.7 (1.7; 7.0)	1.4 (0.3; 2.7)	2.7 (0.9; 4.6)
10	8.5 (2.9; 23)	17 (7; 39)	7.1 (1.9; 18)	14 (5; 32)

Table: Excess Absolute Risk per 10,000 Person Years (PY) with 95% CI in brackets from the mechanistic model and the descriptive model for thyroid doses 0.01Gy, 0.1Gy, 1Gy and 10Gy.
Mechanistic Model Descriptive Model

Thyroid Dose (Gy) Men Women Men Women
0.01 0.040 (0.021; 0.075) 0.081 (0.054; 0.122) 0.031 (0.011; 0.051) 0.063 (0.030; 0.085)
0.1 0.40 (0.21; 0.75) 0.80 (0.53; 1.2) 0.31 (0.11; 0.51) 0.62 (0.30; 0.85)
1 3.7 (2.0; 6.8) 7.4 (5.0; 11) 2.9 (1.1; 4.8) 5.8 (3.0; 7.8)
10 17 (7; 41) 35 (17; 76) 15 (5; 34) 31 (12; 60)

WP3.4 Risk evaluation for radiation protection
We have analysed digestive tract cancer incidence and mortality among the UK NRRW cohort. The NRRW cohort studied here comprehended about 173,000 radiation workers who were monitored for external radiation from 1955 to 2002. The large size of the cohort gives the opportunity to study the effect of protracted low-dose exposure. The dose response is linear, and it gives a value for the excess relative risk of 0.53 and 0.70 per Sievert for incidence and mortality, respectively. A subsidiary analysis excluding workers monitored for internal emitters suggests that the risk may be a factor of 2 larger than for the analysis including all workers. All the above estimates are considerably larger than the ones obtained in the atomic bomb survivors studies, although the uncertainty of the estimates is relatively large with broad confidence intervals.
Stomach cancer mortality has been investigated in the Mayak workers cohort, with follow-up until 2008. A benchmark descriptive analysis has been performed, which indicates that – besides age – birth year, alcohol, and external radiation are risk factors. Smoking is not significantly associated, Plutonium dose is only marginal. The external radiation risk displays a nonlinear dose-response relationship, with levelling at high doses (~1 Gy). The excess relative risk (ERR) falls off at later ages and is elevated at higher alcohol status. To construct a mechanistic model, the two-stage model with clonal expansion has been applied to the data. The results point to a mechanism where external radiation dose rate increases the clonal growth rate of premalignant cells, which tends to saturate at larger dose rates. This leads to a nonlinear dose response and a drop-off in ERR for later attained ages. A manuscript is in preparation.
The work of integrating the new mechanistic and descriptive models into the risk calculation software has been completed. Additional work to enable the software to generate estimates of uncertainty on the risks as a result of the uncertainty associated with the model parameters has additionally been completed. This means that a more informative comparison can be made between the risks generated by the EpiRadBio models and the standard empirical models.
Comparison of the new models and available standard models from ICRP, UNSCEAR and BEIR will be made using appropriate realistic exposure scenarios for the datasets used to create the models. Both excess relative and absolute risk estimates will be generated at a range of ages as will the REICI (Risk of Exposure Induced Cancer Incidence) measure of lifetime risk with uncertainty intervals for the new models. A report detailing the results will be compiled as deliverable D43.2.
A simulation program has been developed that tracks the clone development for mechanistic models developed in EpiRadBio. Therefore it is possible to obtain statistics of the clone size distribution, i.e. the size of preneoplastic lesions, as function of age. This program has been applied to mechanistic models for breast cancer including genomic instability for the Swedish hemangioma cohort, and to the mechanistic models with two pathways for colon cancer in the LSS. In particular, for the colon model the clone size for the microsatellite instability path remains small, whereas the chromosomal instability path leads to much larger clones, in agreement with pathology. The results are described in Deliverable D32.2 (Manuscript on simulation studies of radiobiological data with mechanistic models).

Sub-project 4 Consortium management tasks and achievements
WP4.1 Scientific Management
The key elements of the framework for management has been established and further developed, building upon the approaches adopted for the previous projects. It is anticipated that there will be only minor changes of the framework for management during the course of the project. First periodic report has been reviewed to verify consistency with the project tasks before transmitting them to the Commission. The compliance by beneficiaries with their obligations under this grant agreement has been monitored. During the PMG meetings the progress and developments has been critically reviewed. The list of deliverables and milestones, and the criteria of prioritisation of research activities in the strategic plan are an important metric in the evaluation process. The contingency plans have also been reviewed during the meetings.
WP 4.2 Administrative Management
The financial contribution of Euratom have been administered regarding its allocation between beneficiaries and activities, in accordance with this grant agreement and the decisions taken by the consortium. All the appropriate payments are made to the other beneficiaries without unjustified delay; the records and financial accounts are keeping making it possible to determine at any time what portion of the financial contribution of Euratom has been paid to each beneficiary for the purposes of the project; the Commission was informed of the distribution of the financial contribution. Consortium agreement has been developed and signed by all beneficiaries. The EpiRadBio Web site has been developed and administered. These tools will be accessible using any internet browser.
The Project Office implements the day-to-day operational management of the project:
• transmits of all project information between the consortium and the EC;
• First activity and progress reports has been prepared;
• supports PMG activities;
• supports SAB activities;
• review of PCA
• Review of gender action plan
The project office supports implementation of changes in the activities and the consortium, including new tasks and participants if and when needed.
For administrative and financial management following activity has been performed:

• all administrative and financial information has been transmitted between the consortium and the EC;
• assistance to subproject leaders and contractors, preparation of financial reports and obtaining audit certificates from each participant;
• follow-up to avoid administrative delay;
• reception of the payments made by EC, administration and transfer to the different participants according to the consortium's decisions;
• keeping account of the EC funding, to be able to determine at any time allocation of EC funding;
• one amendment and one information letter to the EC contract have been prepared and accepted during the reporting period;
• DoW modification has been distributed between the partners;
• all the documents related to the project are centralised collected.

Project Communication Action activities
• plan, control and management of PCA activities;
• these activities, which are likely to be conducted in conjunction with the DoReMi consortium, includes support for workshops or wider scientific symposium at which the findings from EpiRadBio will be presented;
• PCA has been reviewed at Month 12
• PCA has been summarised
Training and education
A student exchange programme will be established that provides support for students and junior scientists to work at participant institutes and to attend workshops at month 18. The activities will be coordinated with training activities under the European Network of Excellence Low Dose Research towards Multidisciplinary Integration (DoReMi). One of the participants in the EpiRadBio consortium leads the DoReMi Work Package 3 Education and Training. The Modelling training course is organised and will take place in Ammersee, Germany, April, 2013.
Meetings
Kick-off meeting, Munich, Germany, 5-7 April 2011; Meetings of WP1.3, WP3.1, WP3.3 and WP3.4
Second contractors meeting, Seville, Spain, 9-11 January 2012; Meetings of SP1, SP2, WP1.1, WP1.3, WP3.1, WP3.4
Third contractors meeting, Eze, France, 9 -11 October 2012, Tissue-sample quality-control task group, Meetings of SP1, SP2, WP3.1, WP3.4
The 5th Contractors Meeting; Lisbon, Portugal, 28 - 29 April, 2014, WP and SP meetings, Workshop
Final Contractors Meeting; Regensburg, Germany, 24 - 25 March, 2015, Workshop
Communication between beneficiaries, exchange of researchers and materials, co-operation with other projects/programmes
During the reporting period, a high level communication between the partner institutions has been established. The researchers have visited partner institutions for consultancy, joint working, and exchange of knowledge.
The work in EpiRadBio project is being performed in close contact with the large EU projects DoReMi and Melodi. Shortly after the project start, the memorandum of understanding with DoReMi has been signed.

Potential Impact:

EpiRadBio Potential Impact
Present epidemiological evidence indicates increased cancer risks after low-dose-rate exposures with cumulative effective doses in the order of 100 mSv (Jacob et al. Occup Environ Med 2009). These studies do not support the assumption that cancer risk per unit dose after low-dose-rate exposures with moderate dose is lower than among the atomic bomb survivors. This assumption is, however, fundamental to the present risk assessment used for radiological protection. It is therefore of utmost importance to quantify cancer risks of low- dose-rate exposures, not only at low dose (< 100 mSv) but also at doses of a few hundred millisievert. The current dose limit of occupational exposures to ionising radiation is 100 mSv in five years and thus does not exclude exposures with cumulative doses of a few hundred millisievert.
Analyses of cancer risk using data from epidemiological studies of radiation-exposed populations have generally used an empirical approach. In other words, the shape of the dose-response and the way in which the dose-response might vary by factors such as age and time have been estimated using simple empirical models that aim to describe the patterns seen in the data. These approaches are quite limited in extrapolating epidemiological results on cancer risk at moderate dose to lower dose. Further, there is a wealth of radiobiological information at moderate and low dose. However, implications for health risk after low-dose exposure have remained unclear.
The main potential impact of EpiRadBio is in the invention and exploration of approaches to combine radiobiology and epidemiology in order to derive estimates of health risks at low doses. Modeling of carcinogenesis is one important approach to combine radiobiological and epidemiological data with promising prospective. Compared to models presently used in radiation protection, first results in EpiRadBio indicate a stronger dependence of colon cancer risk on age, and a higher excess absolute risk of thyroid cancer cancer after exposure to I-131 during childhood and adolescence

EpiRadBio contributions to expected impacts
The project contributes towards practically all expected impacts (in bold) listed in the work programme:
• EpiRadBio contributes to low-dose risk research for an optimisation of radiation protection by improving the understanding of carcinogenic processes after exposure to low-dose radiation and delivering quantitative cancer risk estimates for exposure scenarios of relevance for radiation protection
• The shape of the dose response has been elucidated for exposures with equivalent doses in the order of 100 mSv or below, i.e. in the region where the shape of dose response is currently under debate and which is relevant to the

risks from environmental, occupational and medical exposures. This objective has been achieved by analysing molecular characteristics of samples from members of key epidemiological cohorts in two approaches (cancer samples from exposed and non-exposed, cancer samples vs. normal tissue samples from exposed), exploring processes of carcinogenesis by in vitro studies, and analysing a variety of key epidemiological data sets with models of carcinogenesis and with empirical models
• Tissue sensitivity has been analysed by performing the same kind of 'omics', especially array comparative genomic hybridisation, to breast, lung and thyroid cancer tissues. Tissue sensitivity has been quantified by studying cancer risks in the breast, lung, thyroid and digestive tract in a number of epidemiological studies. Thus, EpiRadBio covers risk of the most frequent and the most radiosensitive cancer types
• Individual variability in radiation sensitivity has been addressed by analysing genomic instability in an exposed vs. non-exposed study nested in the French Haemangioma Cohort. Individual variability has also been analysed in epidemiological studies by taking account of familial factors (e.g. breast cancer among close relatives or influence of the number of children and age at birth of first child on breast cancer risk) and behavioural factors (e.g. smoking and alcohol consumption)
• Cancer risks of different radiation quality types have been analysed by comparing lung cancer risks from plutonium and external radiation of Mayak workers, thyroid cancer risks from incorporated iodine and external radiation, and influences of gamma and alpha radiation in in vitro studies with 2D cell cultures including stem cells and 3D tissue models
• Cancer risks from internal exposure to radiation has been quantified by analysing lung cancer risks (and to some degree also cancers risks for the digestive tract) in the Mayak Worker Cohort after incorporation of plutonium, breast cancer risks in the French-Swedish- Italian Thyroid Cancer Cohort after incorporation of 131I, and thyroid cancer risks after incorporation of different radioiodines, especially of 131I, in the aftermath of the Chernobyl accident
• All these areas/directions have been identified and prioritised by the HLEG as most promising in terms of addressing/resolving key policy questions
• EpiRadBio applies a multi-disciplinary approach involving clinicians, pathologists, radiobiologists, radiation chemists, radiation physicists, epidemiologists, statisticians, mathematical modellers and dosimetrists
• The innovative approach of EpiRadBio assesses health effects through integration of radiobiological research and epidemiological studies of groups exposed to low-dose-rates by computational modelling
• The consortium EpiRadBio includes non-radiation research biological communities, e.g., the Microarray Core Facility at the University of Tübingen
• EpiRadBio does not duplicate past or on-going research. Its genuine approach is based on success and insights gained in earlier projects.

One impact listed in call not addressed
One expected impact listed in the Topic: Fission-2010-3.1.1: Contribution to low-dose risk research in Europe of the Euratom Work Programme 2010, non-cancer effects, is not addressed by EpiRadBio. The main reason is the current epidemiological evidence that low- dose-rate exposures with cumulative equivalent doses of the order of 100 mSv do cause excess cancer risks and that the related excess cancer risks per unit dose do not appear to be lower than those among the atomic bomb survivors (Jacob et al Occup Environ Med 2009). Thus a main assumption (DDREF of 2) for deriving present dose limits in radiation protection is severely challenged. The consortium EpiRadBio considers the solution of the question of cancer risks from exposures to ionizing radiation with equivalent doses of 100 mSv or below as being of utmost urgency. The fundamentals laid by earlier projects and the innovative approach proposed by EpiRadBio do now allow this problem to be addressed. However, the efforts necessary to achieve the aim are too large to allow the inclusion of further research, i.e. on non-cancer effects. Non-cancer effects of ionising radiation are or have been studied in Euratom projects like CARDIORISK, SOLO, NOTE and DARKRISK.

Main steps to bring about impacts
The four main steps proposed by EpiRadBio to bring about the impacts listed above were to:
• perform array-based comparative genomic hybridisation and other 'omics' with cancer tissue and blood samples from members of outstanding radio-epidemiological cohorts in order to characterize key processes of carcinogenesis and contribute to the understanding of individual sensitivity
• analyze radiation responses of stem cells and radiation perturbation of intercellular communication and the tissue microenvironment in normal human breast and lung cells in order to elucidate further key processes of carcinogenesis and supplement the studies of samples from epidemiological cohorts
• integrate the new radiobiological results in models of carcinogenesis in order to include this knowledge in an evaluation of key epidemiological data
• derive cancer risks after exposures with cumulative equivalent doses in the order of 100 mSv or below for supporting radiation protection.
The European collaboration settled in the EpiRadBio project could play an important role in the development of further researches.

Dissemination and exploitation of results
All results obtained in the framework of the project are intended to contribute to an improvement of our knowledge on risks due to radiation exposures at low dose rates and have been made available to the scientific community, decision makers, stakeholders and the public. Furthermore, the involvement in this project of scientists from national radiological protection institutes and of members of national and international radiation protection commissions means that new findings can be brought to the attention of national and international bodies (ICRP, UNSCEAR) and policy makers in this area in a timely fashion. The consortium has defined a coherent and consistent Project Communication Action Plan that

was implemented by the consortium during the life of the project. Among the issues to be considered by the consortium in the definition of the Project's Communication Action Plan the following has been considered:
• publications in scientific popular press and peer-reviewed journals
• issuing of press releases to local, national or international press at suitable occasions
• participation at international conferences and meetings.
The dissemination of results has been achieved through:
• a public website
• common workshops with other groups involved in radiation risk research and the organization of international symposia
• the use of specific products of interest to each specific stakeholder group (e.g., policy-makers, technical community, students, the public etc) using the latest web-based technology, such as webinar, listserv and social networking (Facebook).

Contractor meetings
Six contractors meetings were held for the whole project. These consisted of both parallel work package and subproject meetings, and plenary meetings where researchers presented the state of progress of their work. Discussion of work plans and approaches led several times lead to an adaptation to new insights. These meetings also provided valuable opportunities for interactions between researchers working on the same aspects of the project in widely separated institutions. The deliverables of the project, mainly in report format were also made available to all researchers involved.
An important aspect of internal dissemination within the project was the extremely helpful interaction with the Scientific Advisory Board (SAB). The three members of the SAB supported the project from its inception by providing critical comment on study design and interpretation of results, generously providing detailed analyses and commentary to help ensure the best scientific outcomes

Peer-reviewed journals and presentations at international meetings
This is the prime method by which the findings from EpiRadBio has been disseminated to the scientific and medical communities. More than 30 jointly-authored publications based on this work have been published or prepared for submission to international peer-reviewed high-impact journals. Results from EpiRadBio have been presented and discussed at national and international conferences both for the radiation research communities as well as the cancer research and pathology communities.

Dedicated workshops and symposia
EpiRadBio has supported the organisation of the conference on "Late Health Effects of Radiation Exposure: New Statistical, Epidemiological, and Biological Approaches", June 10-13, 2012, The Colony Hotel, Kennebunkport, Maine, USA. The participants of EpiRadBio took part at this conference as speakers.
The Interdisciplinary Workshop, Landshut, Germany, 16-17 May 2013 was organized to identify/understand the needs of carcinogenesis modelling for improving cancer risk assessment at low doses or low dose rates, and to address possibilities of radiation biology to fulfil these needs.
In order to contribute to the integration of EURATOM research, EpiRadBio contributes to meetings of MELODI, DOREMI and other international meetings in order to achieve integration of different European projects and of expertise outside of Europe and from other disciplines than radiation research.

Training and education
Education and training are important components of the EpiRadBio project.
In frame of a student exchange program a training course "Modelling in the Field of Radiation Biology and Radiation Epidemiology" has been organised and carried out in Herrsching, Ammersee, Germany, 8-19 April 2013. Basic principles and recent research results on modelling in radiation epidemiology and radiation biology have been presented and explained by expert scientists. 25 young people from different countries have studied during two weeks the latest results of research in the field of Retrospective dosimetry, Radiation epidemiology, Initial events and damage response, Mechanistic modelling and Systems biology.
The activities were coordinated with training activities under the European Network of Excellence Low Dose Research towards Multidisciplinary Integration (DoReMi). One of the participants in the EpiRadBio consortium leads the DoReMi Work Package 3 Education and Training.

EpiRadBio public website
The public website targets two groups of users, on the one hand the general public and on the other hand the scientific community. The website infrastructure was set-up and maintained by the Project Office.
The public part of the web site includes descriptions of project objectives, perspectives and progress. Links to peer reviewed publications and other dissemination activities are also available on the website. Whilst it was anticipated that the major users of the website would be scientists with interests in radiation and/or health studies, the site was designed to be accessible to a lay audience, by explaining clearly the background to the research and the aims and expected outcomes of EpiRadBio.
It is anticipated that the website will remain accessible and will be updated with information on subsequent publications for at least one year after the end of the project.

List of Websites:
www.epiradbio.eu

Related information

Documents and Publications

- final1-logo.pdf
- final1-figures.pdf

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