# WP5: Shape of the dose-response

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on behalf of Simon Bouffler - DoReMi WP5 lead

# Major aims for WP5

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

| Task | Work | Starting |
|---|---|---|
| 5.1 | Phase – shifts in responses and processes at high/low doses and dose rates | 2010 |
| **5.1.1** | **Low dose Gene Expression signature (LoGiC)** | **2011** |
| 5.2 | Assessing the relative contribution of targeted (DNA), non-targeted and systemic processes to radiation carcinogenesis | 2010 |
| **5.2.1** | **Modulation of Inflammation by low and moderate dose Ionising Radiation (ModInIR)** | **2011** |
| 5.3 | The dynamics of pre-neoplastic change and clonal development | 2010 |
| 5.4 | Mathematical models to link experimental findings and epidemiological data | 2010 |
| 5.5 | Assessing the risk from internal exposures | 2010 |
| **5.5.1** | **Internal Emitters in Uranium Miners (INTEMITUM)** | **2013** |
| **5.5.2** | **Assembly of internal radiation dose for UKAEA and AWE epidemiology cohorts (AIRDoseUK)** | **2013** |

| Task | Work | Starting |
|---|---|---|
| 5.6 | Track structures and initial events: an integrated approach to assess the issue of radiation quality dependence (INITIUM) | 2012 |
| 5.7 | Induction and facilitation of chromothripsis by low dose ionizing radiation (In-FaCT-IR) | 2013 |
| 5.8 | Concerted Action for an Integrated (biology-dosimetry-epidemiology) Research project on Occupational Uranium Exposure (CURE) | 2013 |
| 5.9 | Low dose radiation-induced non-targeter effects in vivo: the role of microvesicles in signal transduction (Rad-Mvivo) | 2014 |
| 5.10 | Effects of Chronic LOw-dose Gamma Irradiation on GAstrointestinal Tumorigenesis (CLOGICAT) | 2014 |

# Task 5.1: Phase – shifts in responses and processes at high / low doses and dose rates *in stem cells* (Lead - SU, BfS, UNIPV)

- Dose, dose-rate, quality dependence of responses for cancer related processes, incl. stress response, immortalization, senescence, DNA/other lesion distributions, cell cycle checkpoints, apoptosis, cytokine production, lesion repair etc...
- Initial work:
  - Low dose and dose-rate stress responses and associated protein expression in human fibroblasts and stem cells
  - Modulation / adaptive response?
  - Mechanistic models

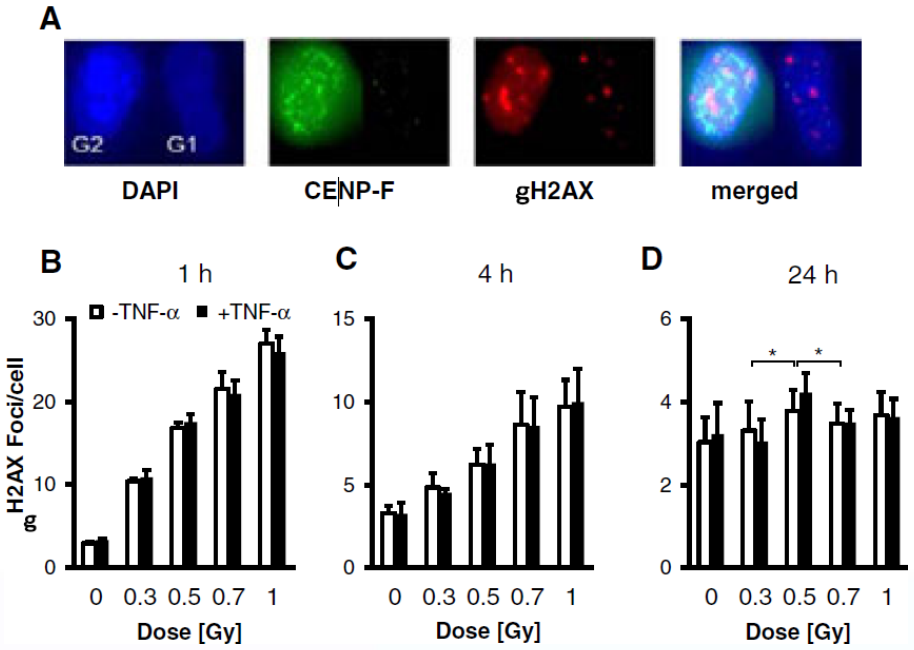
# Task 5.1: Highlighted result

Large *et al*., 2014: Gamma-H2AX foci in HUVEC, 0.3 – 1 Gy:

- Linear response at 1 hr, 4 hr; Non-linear at 24 hr
  - Elevated following ROS inhibition ~ 0.5 Gy
  - SOD decreased  0.5 - 0.7 Gy

-> Non-linear regulation of ROS production and SOD activity < 1 Gy; abolished by ROS inhibition

-> Discontinuous dose-response relationship



# Task 5.1: Cont.

- Shim *et al.*, Mutat Res. Rev 2014:
  - Review of radiation effects on telomeres and the link to carcinogenesis
- Gurtler *et al.*, Arch Physiol Biochem. 2014:
  - Identification of protein biomarkers for inter-individual variation in lymphoblastoid cell lines (10 Gy; 24 hrs)
- Pottier *et al.*, PLoS One 2014:
  - Proposed mechanism of Pb exposure leading to telomeric instability
- Kabacik *et al.*, Radiat. Res. 2015:
  - Characterisation of transcriptional response to IR: gene expression, noncoding microRNAs and long noncoding RNAs in terms of dose, time and ATM status

# Task 5.1.1: Low dose Gene Expression signature, LoGiC (Lead - Erasmus MC)

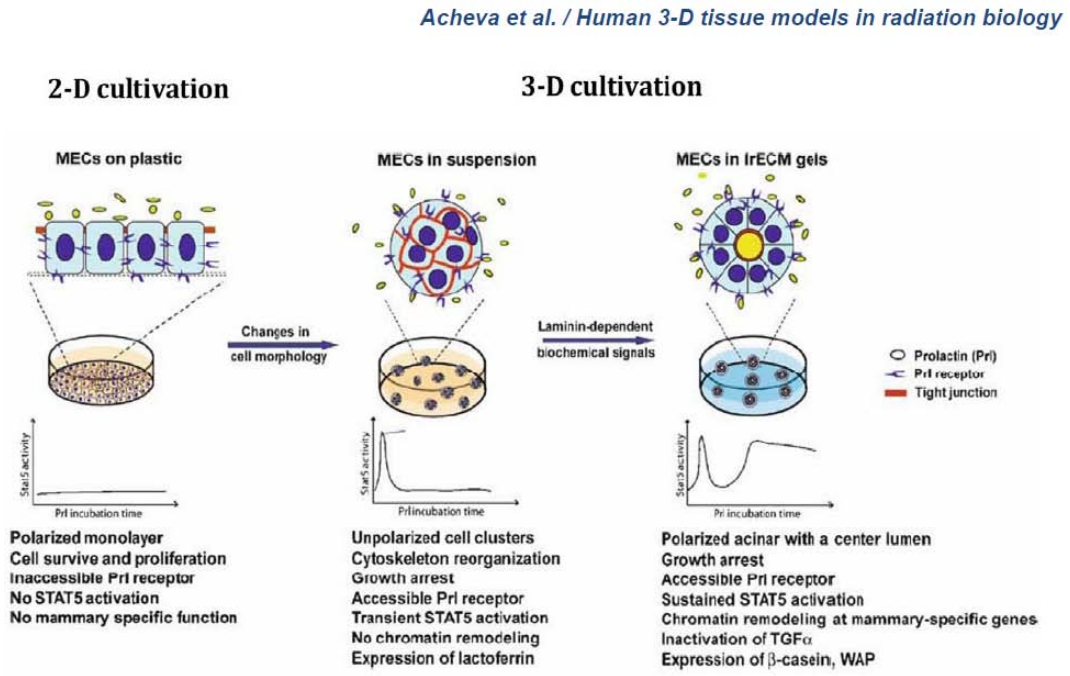
- Investigation of ‘consistent gene expression signature which defines a universal marker profile relevant for the general population’
- Extend mice-human studies to more precise description of these responses in time and in various tissues and cell types
- Prediction of the functional consequences of these changes in expression -> Modelling of radiation response
- Creation of CDKN1A (p21) reporter mouse which will allow further efficient time- and dose-dependent low dose exposure analysis

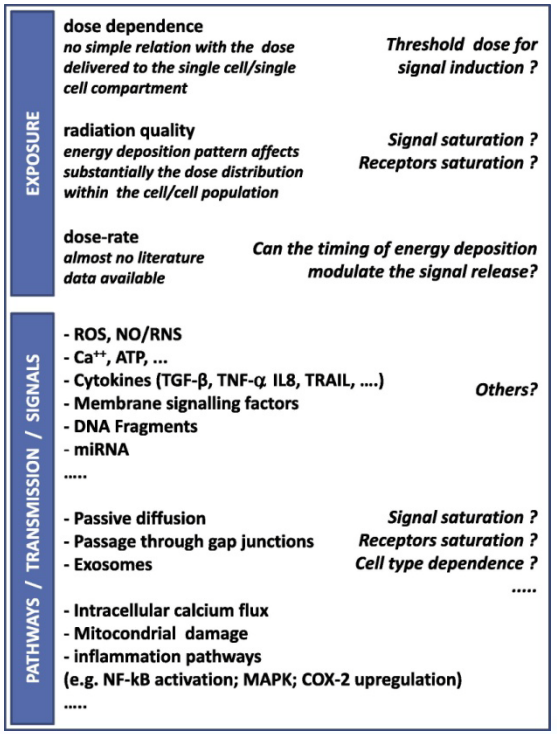
# Task 5.2: Assessing the relative contribution of targeted (DNA), non-targeted and systemic processes to radiation carcinogenesis (Lead – OBU, STUK, SCK-CEN, UNIPV)

- Investigation of contributions that NTE (transmissible genomic instability, bystander phenomena, adaptive responses, effect on systemic processes e.g. inflammatory reactions and immune response, to modulate tumorigenesis) make to carcinogenesis following low dose exposures
- Questions: Relevance in vivo; Effect on tumorigenesis; Interactions between phenomena and net effect
- Initially proposed work:
  - Feasibility study for establishment of a co-culture system for 3-D tissues
  - Mathematical modelling of biophysical processes

# Task 5.2: Results

- Reviews:
  - Role of 3D tissue models (Acheva *et al.*, Int J. Radiat Res. 2014);
  - NTE in general (Campa *et al.*, Cancer Letters 2013)

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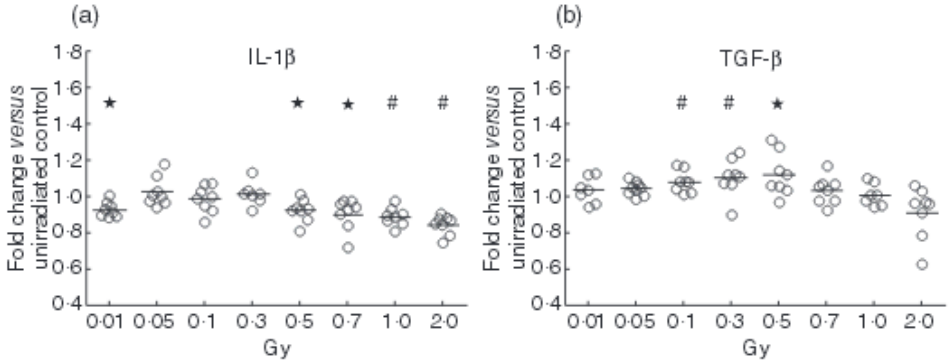
# Task 5.2.1: Modulation of Inflammation by low and moderate dose Ionising Radiation, ModInIR

- Proposed to investigate effects of 0.005 – 1 Gy X-rays on inflammatory reactions and immune modulation:
  - Human cellular assays and *in vivo* models used to examine the impact of irradiation on the function and associated signalling pathways of immune cells, the role of cellular stress and DNA repair in endothelial cells and leukocytes, and the resultant impact on inflammatory conditions
  - Aim to translate cell-based (macrophages, leukocytes, endothelial cells) observations to the whole organism using hTNFα transgenic and air pouch mouse models

# Task 5.2.1: Results

- Immune system and inflammation ~14 publications:
  - *In vitro* studies suggest modest reduction of inflammatory cytokine (IL-1β, TNF-α) production following 0.5-0.7 Gy exposure (Rödel et al 2012)
  - Effects of low (0.1-0.5 Gy) but not high (2 Gy) irradiation on mouse macrophages increased chemotaxis but reduced migration / increased anti-inflammatory cytokine profiles at exposures below 0.5 Gy (Wunderlich et al 2014, 2015)
  - Overall conclusion: Cytokine expression and response may be non-linear in dose-responsiveness




Wunderlich et al., 2014

# Task 5.3: The dynamics of pre-neoplastic change and clonal development (Lead - PHE, CEA, SCK.CEN, HMGU)

- Need for biologically realistic risk projection models to provide mechanistic information for entire carcinogenesis process

- Genomic, transcriptomic and proteomic analyses in AML model to identify time, dose and quality relationships

# Task 5.3: Results

- Progress in fluorescence reported gene systems to monitor critical gene loss events, to monitor disease progression over time (Olme *et al.*, 2013 a; b)
- Development of Integrated transcriptomic and proteomic analysis of mouse acute myeloid leukaemias
- Transcriptome signatures of neutron AMLs differ from those of x-ray AMLs
- Stem cell and DNA damage workshop, December 2011 – influential in development of RISK-IR project (magnitude of cancer risk in humans exposed at doses < 100 mSv; started December 2012…)

# Task 5.4: Modelling of radon-associated lung cancer risk in Eldorado miner cohorts (Lead – HMGU, UNIPV, PHE)

- Mathematical models link experimental models of cancer processes with human population risk estimates
- Proposed models: Inter-cellular apoptosis; Dose-rate threshold for α-particle-induced lung can-cer in uranium miner data; Availability of data from the UK National Registry of Radiation Workers for analyses using cancer models; Carcinogenesis in the thyroid after exposure to radioiodine
- Progress to date:
  - Lung cancer risk modeling comparisons of mechanistic and empirical models (Eidemüller *et al.,* 2012)
  - Model to describe the phenomenon of intercellular induction of apoptosis (Kundrat et al., 2011).

# Task 5.5: Assessing the risk from internal exposures (Lead – IRSN)

- Integrated approach combining epidemiology, biological experiments, dosimetry and modelling in order to improve the assessment of the dose-risk relationship associated with internal exposures on the basis of specific situations
- Workshop: Evaluation of priorities helped build consensus -> CURE (task 5.8; Laurier *et al.*, 2012)
- Uranium miners cohort evaluation (Kreuzer *et al.*,2014)
- Concerted action for an integrated project on occupational uranium exposure (IRSN plus 8 others)

# Task 5.5.1: Internal Emitters in Uranium Miners, INTEMITUM (Lead – SURO)

- Multidisciplinary integration of dosimetry, biology and epidemiology in estimation of leukaemia and non-melanoma skin cancer risk related to low doses from internal and external exposure in uranium mines
- Estimation of parameters needed for calculation doses from long lived radionuclides
- Verification of calculated doses by measurement of uranium in urine among current uranium miners

# Task 5.5.2: Assembly of internal radiation dose for UKAEA and AWE epidemiology cohorts, AIRDoseUK (Lead – NUVIA, AWE, UKAEA)

- Assemble all the data available relating to internal dose in two major UK nuclear industry cohorts, AWE and UKAEA

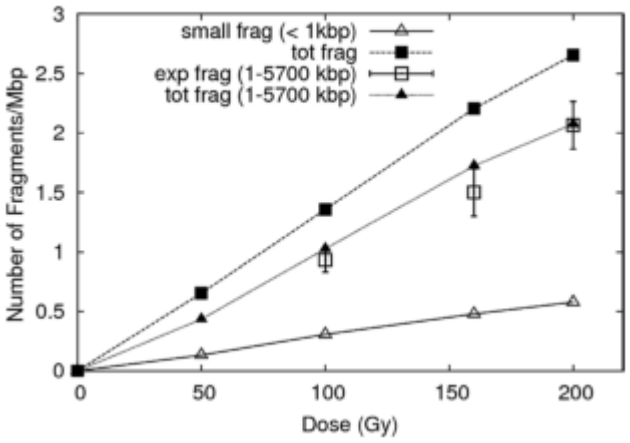
- To develop a methodology to calculate organ doses quickly and efficiently from that data

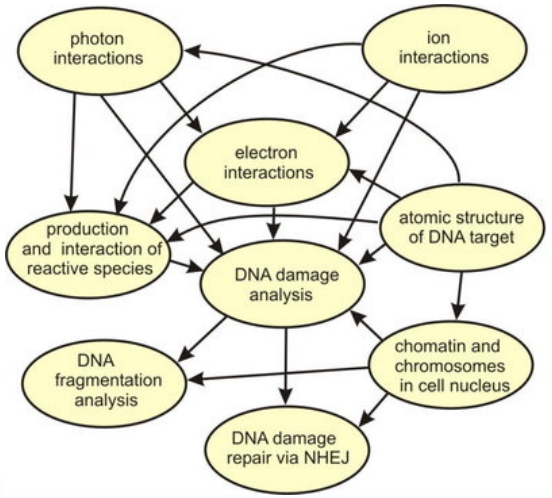
# Task 5.6: Track structures and initial events: an integrated approach to assess the issue of radiation quality dependence, INITIUM (Lead - UNIPV, HMGU)

- PARTRAC models of initial damage, to improve prediction of RBE/quality effects, experimental validation:
  - Modelling of the effects of incorporated low energy β- emitters
  - Modelling of DNA damage induced by low energy light ions/nuclei
  - Kinetic and spacial modelling of foci formation and repair following irradiations of differing quality, dose, dose-rate
  - Comparison of γ- and α- irradiation effects on mitochondrial function, (biological and mathematical models)

# Task 5.6: Results

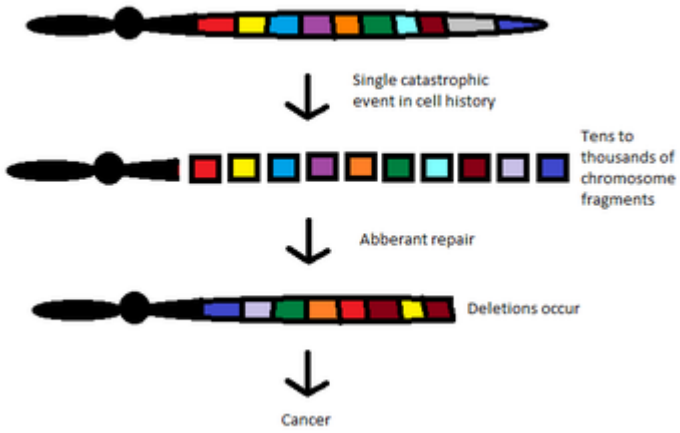
- Review of modelling process for radiation quality and track structure; comparison of experimental data and models (Alloni *et al.*, 2012)
- DNA fragmentation spectra induced by different radiation qualities (Alloni *et al.*, 2011)
- RBE for DNA double-strand break induction by nitrogen ions (Alloni *et al.*, 2013)



# Task 5.7: Induction and facilitation of chromothripsis by low dose ionizing radiation, In-FaCT-IR (Lead – LUMC)

- Development of a model system to determine the impact of radiation exposure frequencies by which micronuclei are taken up and integrated in the genome of recipient cells
- Potential role of TP53 status on uptake and integration of micronuclei
- Investigation of complexity of rearrangements



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# Task 5.8: Concerted Action for an Integrated (biology-dosimetry-epidemiology) Research project on Occupational Uranium Exposure, CURE (Lead - IRSN)

- Verify the feasibility of a molecular epidemiology approach to improve the assessment of health risks associated with uranium exposure

- Three complementary WP:
  - Epidemiology - verify the compatibility of cohorts of uranium workers; reviewing the quality and completeness of data
  - Dosimetry - evaluate the quality and availability of individual monitoring data
  - Biology - identify appropriate biomarkers/samples, develop common SOP

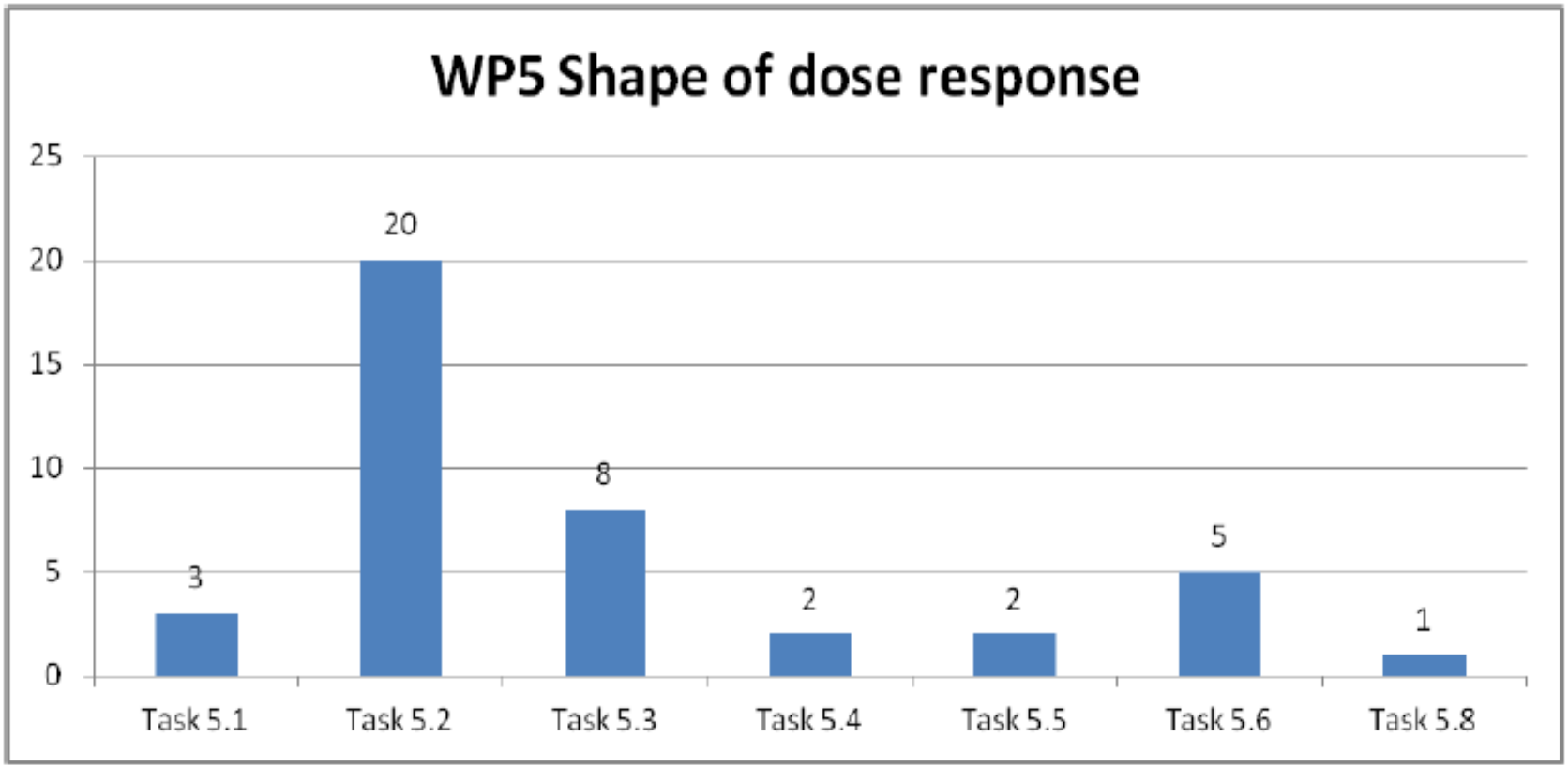
# Task 5.9: Low dose radiation-induced non-targeter effects in vivo: the role of microvesicles in signal transduction, Rad-Mvivo (Lead – OBU, NRIRR, DIT, PHE)

- To test the hypothesis that radiation-induced NTE are likely driven by epigenetic mechanisms
- To investigate:
  - The direct effects of radiation on MV and cellular changes in the spleen and bone marrow *in vivo*
  - The NTE including genomic instability (GI) and bystander effects (BE) of radiation-induced MV on the recipient spleen and bone marrow *in vivo*
  - To validate *in vivo* MV results using an *in vitro* approach

# Task 5.10: Effects of Chronic LOw-dose Gamma Irradiation on GAstrointestinal Tumorigenesis, CLOGICAT (Lead - PHE)

- To increase the understanding of colon cancer development following chronic vs. acute gamma irradiation
- To identify whether the severity of the cancer development is correlated with genotoxic responses observed in blood samples
- To contribute to an improved knowledge of low dose / dose rate radiation cancer
- To contribute to the evidence base for DDREF
- To examine the quantitative effects of exposure to chronic gamma radiation in the well characterised *ApcMin/+* mouse model

# WP5 Publications



# Key research questions:

1) Dependence on energy deposition:

- New epidemiological and/or mechanisitic data on radiation quality in almost all tasks – many experiments and models to elucidate quality effect, e.g. 5.5 and 5.8: Large scale multidisciplinary study of uranium exposure risks

2) Dependence on dose rate:

- Task 5.1 specifically focused on dose rate effects; New mechanistic data in 5.1, 5.4, 5.10; New epidemiological data in 5.8

3) Tissue sensitivities:

- New data in 5.3, 5.4, 5.5, 5.8

4) Genetics and epigenetics:

- New mechanistic data in 5.2, 5.2, 5.3, 5.5

9) Role of non-targeted effects

- New mechanistic data in 5.2, 5.3, 5.7, 5.9

# Have WP5 aims been addressed?

**Aim 1:** To improve knowledge of low dose/dose-rate radiation cancer risk in humans

**Aim 2:** To improve low dose/dose-rate risk projection models based on knowledge of processes that drive carcinogenesis

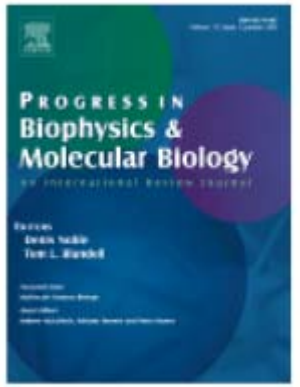
- Substantial progress in exploring dose-responses for early responses to radiation
- Challenge remains relating these early responses to cancer and ensuring adequate experimental models of radiation carcinogenesis are available
- Combination of experimental, epidemiological and modeling approaches continues to be required

# Summary of WP5 progress

- ~ 40 publications to date, more expected…
- Overall, the evidence base for radiation protection has increased, particularly in terms of
  - Dose and dose-rate effects
  - The process of carcinogenesis
  - Impact of the immune function
  - Biophysical interactions
- DoReMi as a whole has contributed to organisational aspects:
  - Highly successful example of a research community commissioning and directing its own research
  - Promotion of collaboration across EU low dose research disciplines

# Proposal for TRA/position papers

- Special issue of ‘Progress in Biophysics and Molecular Biology’
  - Covers ‘ground between physical and biological sciences’
  - Intended audience: biophysicists, biologists, biochemists, cell physiologists, systems biologists,molecular biologists
  - Impact factor: 3.377 (2013)



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- Editorial outlining total progress, followed by
- 2/3/4 review papers from each WP covering areas of scientific advancement within tasks, e.g. for WP5:
  - Immunological and inflammatory responses to radiation, under task 5.2
  - Internal emitters/biophysical responses, under tasks 5.5, 5.6 and 5.8
  - Dose and dose-rate relationships, under tasks 5.1, 5.3, 5.10…
- Editorial and reviews to discuss external advances?

# Acknowledgements

Thanks to all the contributors on WP5

Thank you for your attention!

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