Non-targeted effects of ionising radiation (NOTE) - European Integrated project, 2006-2010

Sisko Salomaa[1*], Eric G. Wright[2], Guido Hildebrandt[3], Munira Kadhim[4], Mark P. Little[5], Kevin M. Prise[6], and Oleg V. Belyakov[1]

[1] Research and Environmental Surveillance, STUK - Radiation and Nuclear Safety Authority, Helsinki FI-00881, Finland

[2] University of Dundee, Division of Pathology and Neuroscience, Molecular and Cellular Pathology Laboratories, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland, UK

[3] Department of Radiotherapy and Radiooncology, University of Leipzig, Leipzig 04103, Germany

[4] Oxford Brookes University, OX3 0BP, UK

[5] Department of Epidemiology and Public Health, Imperial College Faculty of Medicine, Norfolk Place, London W2 1PG, UK

[6] Queen's University Belfast, BT9 7BL, UK

Abstract. The general objectives of the NOTE project are: (1) to investigate the mechanisms of non-targeted effects, in particular, bystander effects, genomic instability and adaptive response; (2) to investigate if and how non-targeted effects modulate the cancer risk in the low dose region, and whether they relate to protective or harmful functions; (3) to investigate if ionising radiation can cause non-cancer diseases or beneficial effects at low and intermediate doses; (4) to investigate individual susceptibility and other factors modifying non-targeted responses; (5) to assess the relevance of non-targeted effects for radiation protection and to set the scientific basis for a modern, more realistic, radiation safety system; (6) to contribute to the conceptualisation of a new paradigm in radiation biology that would cover both the classical direct (DNA-targeted) and non-targeted (indirect) effects. The NOTE brings together 20 major European and Canadian groups involved in the discovery, characterisation and mechanistic investigation of non-targeted effects of ionising radiation in cellular, tissue and animal models. The NOTE research activities are organised in six work packages. Four work packages (WPs 2-5) are problem-oriented, focussing on major questions relevant for the scientific basis of the system of radiation protection: WP2 Mechanisms of non-targeted effects, WP3 Non-cancer diseases, WP4 Factors modifying non-targeted responses, WP5 Modelling of non-targeted effects. The integration activities provided by WP6 strengthen the collaboration by supporting the access to infrastructures, mobility and training. WP7 provides dissemination and exploitation activities in the form of workshops and a public website. Managerial activities (WP1) ensure the organisation and structures for decision making, monitoring of progress, knowledge management and efficient flow of information and financing. Progress of the first two years of the project is described in this paper. Coordinator of the NOTE project is Prof. Sisko Salomaa, STUK - Radiation and Nuclear Safety Authority, Helsinki, Finland.

KEYWORDS: non-targeted effects, ionising radiation, bystander effect, genomic instability, adaptive response, Euratom, integrated project.

1. Introduction

The NOTE project (Non-targeted effects of ionising radiation) has reached its second year in 2008. We describe here the project objectives, structure, strategy and main achievements by the mid-term of the project.

The universality of the target theory of radiation-induced effects is challenged by observations on non-targeted effects such as bystander effects, genomic instability and adaptive response. Essential features of non-targeted effects are that they do not require direct nuclear exposure by radiation and they are particularly significant at low doses. This new evidence suggests a need for a new paradigm in radiation biology. The new paradigm should cover both the classical (targeted) and the non-targeted effects. New aspects include the role of cellular communication and tissue-level responses. A better understanding of non-targeted effects may have important consequences for health risk assessment and, consequently, on radiation protection.

In general, there is a need to investigate the link between non-targeted effects and various radiation-induced health effects, like cancer, genetic (hereditary) effects, reproductive/developmental effects and non-cancer diseases. Furthermore, it is important to explore the mechanisms involved in the non-targeted effects of ionising radiation, to determine the dose-effect relationships of non-targeted effects in space and time, to address the role of individual susceptibility in response and to determine whether the non-targeted effects relate to protective or harmful responses to radiation. The linkage between the bystander response, adaptive response and genomic instability needs to be studied. A longer term objective is to establish a conceptual framework for the generation of a new radiobiological paradigm that covers both targeted (direct) and non-targeted (indirect) effects of ionising radiation. This, in turn would help in setting the scientific basis for development of a new, more realistic, radiation protection system.

Some questions can be addressed by employing a range of low-dose broad field and microbeam irradiation approaches to investigate both high- and low-LET responses, and by employing well-defined biological systems, such as human cell cultures, 3D artificial tissue systems and ex vivo tissue explants. At the cell and molecular levels, new research should focus particularly on identifying the signals and signal receptors for the non-targeted effects. It will be important to understand whether such signals are produced by all cell types and whether reception and response is general or limited by cell type or organ. Identifying and understanding the action of the signalling process could lead to a means of predicting the outcome of an exposure in an individual.

While research at the cellular, molecular and ex vivo tissue levels will be critical for understanding the mechanisms of these processes, their influence on risk must also be determined more directly. To properly assess the net impact of targeted and non-targeted radiation effects, new research should specifically employ whole animal models, using both strains that are genetically normal and strains that are suspected to be radiation sensitive or cancer prone. Overall measures of risk need to be used together with tissue specific measures, and these tissues need to be assessed for cellular and molecular changes. These results will also be important in understanding the relationship between dose and tissue weighting factors as dose decreases. The animal models could additionally provide clarification on interactions of non-targeted effects with exogenous (e.g. dietary) and endogenous (heritable) variables as a possible part of an inflammatory-type response to radiation-induced stress under in vivo conditions. Long-term clonal variability of non-targeted responses and cell type differences needs to be studied. More information is required on the influence of LET, and on simultaneous exposures to radiations of different LET. More information is also required on the relationship of dose rate and total dose for induction of these responses. Mathematical and statistical modelling is likely to improve the understanding of the potential role of non-targeted effects in the development of different pathologies.

Teratogenic and carcinogenic endpoints in foetal and young animals should receive particular attention. Measurement of non-cancer endpoints need to be included, including studies of heritable effects and particularly in studies with animals selected for genetic propensity for cardiovascular diseases.

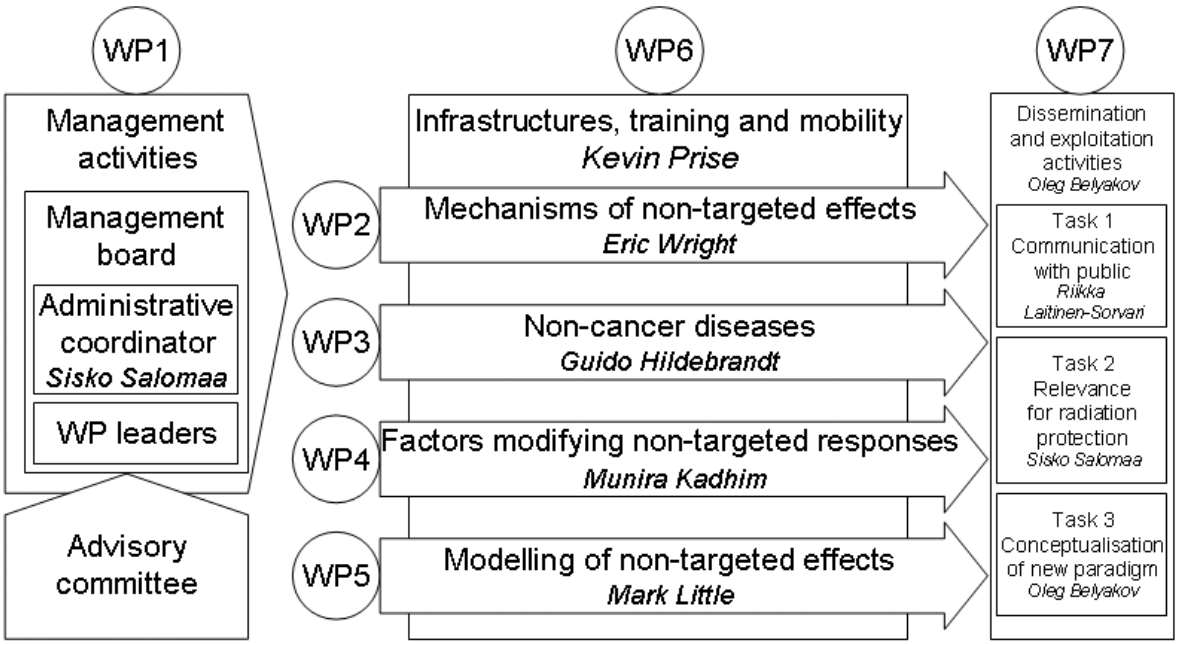
The general objectives of the NOTE project are:

- to investigate the mechanisms of non-targeted effects, in particular, bystander effects, genomic instability and adaptive response;
- to investigate if and how non-targeted effects modulate the cancer risk in the low dose region, and whether they relate to protective or harmful functions;
- to investigate if ionising radiation can cause non-cancer diseases or beneficial effects at low and intermediate doses;
- to investigate individual susceptibility and other factors modifying non-targeted responses;
- to assess the relevance of non-targeted effects for radiation protection and to set the scientific basis for a modern, more realistic, radiation safety system;
- to contribute to the conceptualisation of a new paradigm in radiation biology that would cover both the classical direct (DNA-targeted) and non-targeted (indirect) effects.

2. Project structure

The NOTE research activities are organised in six work packages (Figure 1.). Four work packages (WPs 2-5) are problem-oriented, focussing on major questions relevant for the scientific basis of the system of radiation protection. The integration activities provided by WP6 strengthen the collaboration by supporting the access to infrastructures, mobility and training. WP7 provides dissemination and exploitation activities in the form of workshops and a public website. Managerial activities (WP1) ensure the organisation and structures for decision making, monitoring of progress, knowledge management and efficient flow of information and financing.

Figure 1. The NOTE project structure.



We are studying effects of low doses at exposure levels that are relevant for occupational, environmental and medical exposures. Our experiments include studies performed using microbeams whereby we can go down to the ultimate low dose of one track per cell. Higher dose levels are used in parallel, as it is important to judge whether it is possible to extrapolate the effects on the basis of experimental information obtained at high doses. We are also studying effects of protracted doses by employing unique exposure facilities designed for long-term animal experiments.

WP 2 (Mechanisms of non-targeted effects) is structured around a number of specific questions:

- What is the target for non-targeted effects?
    - What cells produce signals?
    - What is the trigger for the production of signals?
    - What cells respond to signals?
    - What intracellular processes are involved in responding to signals?
- What are the messengers of bystander responses?
- What are the mechanisms of non-targeted effects in tissues?
- What are the mechanisms of genomic instability perpetuation?
- What are the mechanisms of transgenerational instability?

WP 3 (Non-cancer diseases) investigates if non-targeted effects can provide new mechanistic explanations for the development other diseases than cancer, by investigating:

- mechanisms of the induction of cardiovascular diseases in vitro and in vivo;
- inflammatory vs. anti-inflammatory effects;
- role of non-targeted effects in developmental defects.

WP 4 (Factors modifying non-targeted responses) investigates:

- influence of genetic factors on the in vivo production of non-targeted effects;
- impact of genetic predisposition on non-targeted effects response in vitro;
- molecular mechanisms determination that contribute to the distinct radiation responses at low dose radiations in mice with different radiosensitivity and
- role of DNA repair and checkpoint responses.

WP 5 (Modelling of non-targeted responses) approaches low dose effects by mathematical modelling in order to improve the understanding of the potential role of non-targeted effects in the development of different pathologies. In particular the following systems and endpoints will be modelled:

- intracellular and local intercellular communication and damage mechanisms;
- intercellular communication mechanisms in vitro;
- extracellular signalling mechanisms in vivo;
- genomic instability mechanisms in vivo.

WP 6 (Integration activities) provide means for building the European Research Area and connecting with strong research groups in third countries by:

- development and better use of existing radiobiological infrastructures;
- strengthening collaborative RTD activity;
- supporting training and mobility of scientists and
- educating the next generation of European radiobiologists on non-targeted effects.

Finally, WP 7 (Dissemination and exploitation activities) will provide means for communication with public and exploitation of NOTE results by the scientific community and stakeholder groups by organising:

- a public NOTE website;
- a workshop on the conceptualisation of the new paradigm;
- a workshop on the relevance of non-targeted effects for radiation protection.

3. Status of research after the first project year (1.9.2006-31.8.2007)

During the first 12 months, research on mechanisms and factors modifying non-targeted responses has focused on the *in vitro* approach but complementary *in vivo* investigations have also been carried out. The *in vitro* investigation is essential for the initial validation of the biological system used in testing the hypotheses proposed in work packages and their tasks. Towards the end of the second year, there should be sufficient data from both *in vitro* and *in vivo* studies that help in planning for later stages of the project where the focus should be on future *in vivo* studies to address key questions such as the contribution of individual variation, sensitivity of different tissues and hereditary effects and how all these relate to the problems of late health effects

Epidemiological studies could demonstrate that ionising radiation as a long-term dose-dependent effect may induce an impairment of the immune system as well as a persistent inflammatory profile that could increase the risks of both cancer and non-cancer diseases. Therefore, the main objective of the research on non-cancer effects is to study whether low and intermediate doses of ionising radiation can cause non-cancer diseases or beneficial effects in several experimental systems in vitro and in vivo

to provide qualitative and quantitative data for mathematical modelling, to better understand low dose risks.

During the first 12 months a fundamental part of the activities on non-cancer effects was the establishment and validation of suitable models / experimental assays, the generation of mainly preliminary but also already validated experimental data on the endpoints, reviewing the experimental literature, and performing the irradiations of experimental animals for late experimental endpoints to be evaluated during the later stages of the NOTE-project. These experimental activities will provide suitable qualitative and quantitative experimental data to be used and tested by partners working on mathematical modelling of non-targeted effects.

During the first year, there have been a number of review activities concerned with modelling of non-targeted effects, and outline models have been constructed arising from these. In particular, modelling of the bystander effect has been largely focussed on feasibility studies for a model simulating cell communication intended typically for *in vitro* cell cultures where cells are *not* in close contact, so that one can neglect gap-junction intercellular communication (GJIC); this has entailed tight interaction between experimentalists and modellers. Likewise, an outline model for inflammation-mediated cardiovascular disease has been developed.

4. Road map for research on low dose risk

Based on the findings and experience obtained since the beginning of the NOTE project, the Management Board has outlined the strategy for research during the later phases of the NOTE project. This strategy provides a road map for redirection of work within the existing Work Packages and identifies priority areas for research to be addressed in the internal call for proposals in 2008. In particular, the aim is to identify the milestones to be achieved on the way to the new paradigm on radiation biology. The research carried out to date both in NOTE and internationally provides strong evidence that the prevailing DNA paradigm is inadequate in describing the biological effects of ionising radiation and that aspects of non-targeted effects need to be incorporated, yet not discarding the strong knowledge base on DNA effects obtained during the past decades.

While not certain that the research to be performed in NOTE and internationally would justify a final formulation of the new **radiobiological paradigm** within the next few years, the NOTE MB shares the view that the science is mature enough to start the conceptualisation of the new paradigm. A milestone for this will be the international workshop to be arranged by NOTE in Ireland in 13-14 September 2008. The classical DNA paradigm deals with DNA damage, repair and lethality. The new aspects brought about by the non-targeted effects include cell signalling, epigenetic effects and effect of phenotype (tissue microenvironment).

Having the general understanding on how radiation acts on cellular and tissue level, further steps toward the new **radiation risk paradigm** on health effects caused by targeted and non-targeted effects could emerge. The risk paradigm would need supporting evidence from epidemiology and is not foreseen during the lifetime of the NOTE project. The revised risk paradigm in turn could have a major impact in the **radiation protection paradigm**, should there be enough evidence on health risks previously not taken into account (such as cardiovascular diseases) or reliable evidence on thresholds for health effects like cancer due to epigenetic mechanisms. Even before such conclusive evidence becomes available, some degree of precaution may be needed in some areas of radiation protection.

From the radiation protection policy point of view, the NOTE consortium has identified three main questions:

1. Is there a deviation from LNT for cancer risk at low doses, and how much of this can be accounted for by non-targeted effects?
2. Can ionising radiation cause non-cancer diseases at low and intermediate doses?
3. How large are the differences in individual susceptibility to ionising radiation?

In terms of research needed for the formulation of the new radiobiological paradigm, the strategic questions and choices for research direction within NOTE lifetime are:

- What is the relative contribution of targeted and non-targeted effects in space and time after different doses of ionising radiation, with the emphasis in the low dose range?
    - experience gained in the intercellular signalling studies will be evaluated by mid-term of the project
    - the relevance of results obtained in cell culture studies will be evaluated in tissue and animal studies
    - three dimensional tissue models will be used to further investigate the spatial distribution of signalling effects
    - modelling of experimental data will be expanded in space and time
- In addition to effects on DNA, what are the most likely mechanisms capable of leading to health effects at low and intermediate does of ionising radiation?
    - mechanisms of inflammatory effects will be studied *in vivo* and *in vitro*
- Can we demonstrate non-targeted effects in humans exposed to ionising radiation?
    - clastogenic factors in plasma of medically irradiated subjects will be investigated
- Is the variation in non-targeted response observed between individuals and cell lines linked to sex, genetic background, epigenetic effects or phenotype?
    - factors underlying the individual susceptibility will be further investigated

5. Contribution to policy developments and risk assessment

There is broad international agreement among governments that the current system of radiation protection is effective, robust and adequately protects man and the environment. There are, however, scientific challenges that may bring into question various aspects of the current approach, and which may have significant policy, regulatory and operational implications. These challenges include non-targeted effects of radiation and individual susceptibility.

5.1. Non-targeted effects challenge the LNT model and thereby also the concept of dose as surrogate of risk

A basic paradigm in radiobiology is that, after exposure to ionising radiation, the deposition of energy in the cell nucleus and the resulting damage to DNA, the primary target, are responsible for the harmful biological effects of radiation. The radiation-induced changes are thought to be fixed in the first cell division following radiation exposure and health effects are considered to result from clonal proliferation of cells carrying mutations in specific genes. Since the initial damage induced in DNA has been shown to be proportional to a linear-quadratic function of dose, risk is also be considered to be proportional to this function of dose, and frequently (for conservatism) risk is assumed to be a linear function of dose. In this case, risk from multiple exposures is considered to be additive, and risk from high and low LET radiation exposure is assumed to be qualitatively the same. These assumptions are incorporated into the Linear-No-Threshold (LNT) Hypothesis that is used in radiation protection practice. A linear dose response means that every increment of dose and the associated risk can be assessed separately, irrespective of prior or future doses, as long as doses are below the level at which deterministic effects arise. A fixed dose increment is always associated with the same additional risk. Doses received by an individual at different time points can be summed up (cumulative dose) and collective dose can be used to predict risk at the population level. If linearity does not hold at low doses, this would have major implications for radiation protection.

5.2. Non-targeted effects may give new mechanistic explanations and models for the development of diseases other than cancer

Direct damage to DNA and its repair yields a plausible mechanistic explanation for the development of diseases such as cancer and genetic (hereditary) effects, where specific mutations induced in single cells are clonally expanded (UNSCEAR 2000). However, there is emerging evidence in the A-bomb survivors and in other exposed groups that ionising radiation also causes other diseases than cancer. Although much of the evidence is related to relatively high dose radiotherapeutic exposure, some of the groups, e.g., the A-bomb survivors, exhibit excess risk at moderate to low doses. Non-cancer diseases are currently not incorporated in the estimates of risk underlying radiation protection standards. One reason for this view is that there has been no plausible mechanistic explanation how such effects could be induced at low doses where only a few cells are traversed by radiation tracks. Non-targeted effects have challenged the current radiobiological DNA paradigm by showing that radiation can induce biological effects in non-hit cells and in progeny of hit cells, thereby amplifying the number of cells potentially affected and shifting the responses from cellular to tissue level. There is emerging evidence of excess risk of non-cancer late health effects in the Japanese atomic bomb survivor Life Span Study (LSS) cohort and in the Adult Health Study (AHS) subcohort. In particular excess radiation-associated mortality due to circulatory, digestive and respiratory diseases has been observed in this cohort. These excess risks are generally observed at doses of the order 1-1.5 Gy. Excess cardiovascular disease, both heart disease and stroke, has been observed in a number of cohorts of patients after radiotherapy for cancer. Although the mechanisms of cardiovascular and cerebrovascular disease induction are not yet fully understood, it is very clear that inflammation is involved. Elevated levels of interleukin 6 (IL-6) have been observed in the Japanese atomic bomb survivors many years after exposure, as also of TNF-α, IFN-γ and IL-10. Other markers of inflammation such as elevated erythrocyte sedimentation rate and elevated levels of C-reactive protein have also been observed many years after exposure in this cohort. Elevated levels of IL-6 are associated with elevated risk of cardiovascular disease in a number of other (non-radiation exposed) cohorts. Other, more indirect, mechanisms could also be involved. Deficiencies in the immune system may play an indirect role, given the suggested role of infections in cardiovascular disease. T cell and B cell population numbers are known to vary with radiation dose among the Japanese atomic bomb survivors. Another indirect mechanism for the induction of hypertension and cardiovascular disease has been suggested by the known elevation in parathyroid hormone with increasing radiation dose in the atomic bomb survivors. Parathyroid hormone is known to play a role in regulation of blood pressure, with increasing levels of the hormone resulting in increases in blood pressure.

5.3. Individual susceptibility is a challenge for radiation protection

All individuals in a population may not be equally protected. In the near future, new technologies such as DNA chips are likely to offer new possibilities for screening of the genetic predisposition to ionising radiation by detecting variant gene alleles. These gene variant include both well known disease genes like AT and allele variants of polymorphic genes (SNPs, single nucleotide polymorphisms). Like in classical targeted effects, individual susceptibility plays a role in non-targeted effects as well. Reasons for this individual variability need to be investigated.

6. NOTE meetings, workshops and training courses

NOTE organises project meetings yearly. The kick-off meeting of the NOTE project was held in Espoo, Finland on 11-14 September, 2006 and the First NOTE annual meeting was held in Aldemar Knossos Royal Village Hotel, Crete, Greece 17-20 September 2007. The Second NOTE annual meeting will be held on 15-18 September, 2008 in Galway.

A major milestone for the conceptualisation of the new paradigm will be the Paradigm workshop in 13-14 September, 2008. The workshop will consist of lectures on the state of the art of research on non-targeted effects as well as science philosophical consideration on what is meant by a paradigm and when can we say that it has changed. The discussions will be oriented on defining the key features of the classical DNA paradigm and the features of non-targeted effects that add to this existing knowledge. The workshop is expected to define the new radiobiological paradigm**,** i. e. describing how radiation acts on cells and tissues. To pave the road for the future formulation of the radiation risk paradigm, the classical thinking and the new information are defined to explain the development of radiation-induced health effects at different dose levels and at different time intervals (acute - delayed). The stochastic and deterministic nature of the effects will be discussed. For the new radiation risk paradigm, however, supporting evidence from epidemiological studies will be needed. This is particularly true for the non-cancer effects such as cardiovascular diseases.

The NOTE training course "Protocols and pitfalls in the study of non-targeted effects of radiation" was held 15-16 September 2007 just before the 1st NOTE annual meeting (17-20 September 2007) on Crete, Greece. The organisers of the course were Carmel Mothersill and Kevin Prise. A major and endlessly discussed problem in the field of non-targeted radiation effects is the variability of techniques, results and approaches used by different laboratories. Many assays are "lab specific" with poor transferability. This course was aimed at students and post docs working in the field of non-targeted effects of radiation. It aimed to present techniques commonly used to study non-targeted effects with particular emphasis on the practical problems of experimental design and data interpretation. A key component of the course was extensive discussion of specific techniques in small groups led by more senior scientists with hands on experience.

Apart from own meetings, NOTE participates in organisation of other events such as:

The First International Workshop on Systems Radiation Biology took a place on February 14 - 16, 2007 in GSF - National Research Center for Environment and Health, Neuherberg/Munich, Germany. The workshop was intended to improve the cooperation of experimental and theoretical scientists working in the interesting and very inter-disciplinary field of radiation biology of complex biological systems in order to improve the quantitative understanding of relevant health effects of low doses of ionizing radiation on humans. To this end three major research projects join efforts, namely the EU Integrated Projects RISC-RAD ("DNA damage responses, genomic instability and radiation-induced cancer: the problem of risk at low doses") and NOTE ("Non-targeted effects of ionising radiation"), and the US-DOE LowDoseProgramme. This present unsatisfactory situation is characterized by problems of different scientific languages between the disciplines as well as of large differences in research approaches of theorists and experimentalists.

The Second International Systems Radiation Biology Workshop, which was intended to encourage experimental and theoretical scientists working in the emerging field of systems biology to discuss its application to the radiation biology of complex biological systems. The main goal was to improve the quantitative understanding of radiation health effects of low doses of ionizing radiation on humans. To this end three major research programs: NASA, EU-Integrated Projects (NOTE; RISC-RAD) and the US-DOE Low Dose Program, joined efforts to organize this workshop on January 24-26, 2008 in Washington DC (see details at https://www.orau.gov/sysradio2008/default.htm).

The Third Systems Radiation Biology Workshop will be arranged on 12-14 January 2009 in Rovaniemi, Finland, to continue the series of these successful meetings.

Finally, NOTE participated in arranging the Science and Values in Radiological Protection, OECD-NEA workshop in Helsinki, Finland, 15-17 January 2008. Conclusions of the workshop are presented in another paper of the IRPA12 Proceedings (Salomaa et al) More details are available at the workshop website: http://www.nea.fr/html/rp/helsinki08/welcome.html.

7. Conclusion

Please visit the project website http://www.note-ip.org to obtain more information or contact us by e-mail note@stuk.fi.

8. Acknowledgements

The project is supported by the European Commission under the Euratom specific programme for research and training on nuclear energy, 6th Framework Programme. The authors (Management Board of NOTE) wish to thank all consortium members and investigators on innovative and efficient research collaboration.

9. Main publications

Publications of the NOTE projects are available from the NOTE website at http://www.note-ip.org, please follow: Frontpage > Publications and IPR > Papers published. Links to abstracts or full text papers (if a publisher supports open access policy) are provided.

1. Ryan, L.A., Smith, R.W., Seymour, C.B. and Mothersill, C.E. (2008) Dilution of irradiated cell conditioned medium and the bystander effect. *Radiat Res*, **169**:2, 188-96.
2. Ryan, L.A., Seymour, C.B., O'Neill-Mehlenbacher, A. and Mothersill, C.E. (2008) Radiation-induced adaptive response in fish cell lines. *J Environ Radioact*, **99**:4, 739-47
3. Gow, M.D., Seymour, C.B., Byun, S.H. and Mothersill, C.E. (2008) Effect of dose rate on the radiation-induced bystander response. *Phys Med Biol*, **53**:1, 119-32.
4. Friedland, W., Paretzke, H.G., Ballarini, F., Ottolenghi, A., Kreth, G. and Cremer, C. (2008) First steps towards systems radiation biology studies concerned with DNA and chromosome structure within living cells. *Radiat Environ Biophys*, 47:1, 49-61.
5. Shao, C., Folkard, M. and Prise, K.M. (2008) Role of TGF-beta1 and nitric oxide in the bystander response of irradiated glioma cells. *Oncogene*, **27**:4, 434-40.
6. Little, M.P., Heidenreich, W.F., Moolgavkar, S.H., Schollnberger, H. and Thomas, D.C. (2008) Systems biological and mechanistic modelling of radiation-induced cancer. *Radiat Environ Biophys,* **47**:1, 39-47.
7. Little, M.P., Tawn, E.J., Tzoulaki, I., Wakeford, R., Hildebrandt, G., Paris, F., Tapio, S. and Elliott, P. 2008b) A systematic review of epidemiological associations between low and moderate doses of ionizing radiation and late cardiovascular effects, and their possible mechanisms. *Radiat Res*, **169**:1, 99-109.
8. Rodel, F., Hofmann, D., Auer, J., Keilholz, L., Rollinghoff, M., Sauer, R. and Beuscher, H.U. (2008) The anti-inflammatory effect of low-dose radiation therapy involves a diminished CCL20 chemokine expression and granulocyte/endothelial cell adhesion. *Strahlenther Onkol*, **184**:1, 41-7.
9. Liu, Z., Prestwich, W.V., Stewart, R.D., Byun, S.H., Mothersill, C.E., McNeill, F.E. and Seymour, C.B. (2007) Effective target size for the induction of bystander effects in medium transfer experiments. *Radiat Res*, **168**:5, 627-30.
10. O'Neill-Mehlenbacher, A., Kilemade, M., Elliott, A., Mothersill, C. and Seymour, C. (2007) Comparison of direct and bystander effects induced by ionizing radiation in eight fish cell lines. *Int J Radiat Biol*, **83**:9, 593-602.
11. Poon, R.C., Agnihotri, N., Seymour, C. and Mothersill, C. (2007) Bystander effects of ionizing radiation can be modulated by signaling amines. *Environ Res*, **105**:2, 200-11.
12. Rodel, F., Keilholz, L., Herrmann, M., Sauer, R. and Hildebrandt, G. (2007) Radiobiological mechanisms in inflammatory diseases of low-dose radiation therapy. *Int J Radiat Biol*, **83**:6, 357-66.
13. Tartier, L., Gilchrist, S., Burdak-Rothkamm, S., Folkard, M. and Prise, K.M. (2007) Cytoplasmic irradiation induces mitochondrial-dependent 53BP1 protein relocalization in irradiated and bystander cells. *Cancer Res,* **67**:12, 5872-9.
14. Burr, K.L., van Duyn-Goedhart, A., Hickenbotham, P., Monger, K., van Buul, P.P. and Dubrova, Y.E. (2007) The effects of MSH2 deficiency on spontaneous and radiation-induced mutation rates in the mouse germline. *Mutat Res*, **617**:1-2, 147-51.

15. Davies, B.G., Hussain, A., Ring, S.M., Birch, J.M., Eden, T.O., Reeves, M., Dubrova, Y.E. and Taylor, G.M. (2007) New germline mutations in the hypervariable minisatellite CEB1 in the parents of children with leukaemia. *Br J Cancer*, **96**:8, 1265-71.

16. Hatch, T., Derijck, A.A., Black, P.D., van der Heijden, G.W., de Boer, P. and Dubrova, Y.E. (2007) Maternal effects of the scid mutation on radiation-induced transgenerational instability in mice. *Oncogene*, **26**:32, 4720-4.

17. Maguire, P., Mothersill, C., McClean, B., Seymour, C. and Lyng, F.M. (2007) Modulation of radiation responses by pre-exposure to irradiated cell conditioned medium. *Radiat Res*, **167**:4, 485-92.

18. Miccoli, L., Burr, K.L., Hickenbotham, P., Friedberg, E.C., Angulo, J.F. and Dubrova, Y.E. (2007) The combined effects of xeroderma pigmentosum C deficiency and mutagens on mutation rates in the mouse germ line. *Cancer Res*, **67**:10, 4695-9.

19. Mothersill, C., Salbu, B., Heier, L.S., Teien, H.C., Denbeigh, J., Oughton, D., Rosseland, B.O. and Seymour, C.B. (2007) Multiple stressor effects of radiation and metals in salmon (Salmo salar). *J Environ Radioact*, **96**:1-3, 20-31.

20. Mothersill, C., Smith, R.W., Agnihotri, N. and Seymour, C.B. (2007) Characterization of a radiation-induced stress response communicated in vivo between zebrafish. *Environ Sci Technol*, 41:9, 3382-7.

21. Ranza, E., Facoetti, A., Morbini, P., Benericetti, E. and Nano, R. (2007) Exogenous platelet-derived growth factor (PDGF) induces human astrocytoma cell line proliferation. *Anticancer Res*, **27**:4B, 2161-6.

22. Facoetti, A., Ballarini, F., Cherubini, R., Gerardi, S., Nano, R., Ottolenghi, A., Prise, K.M., Trott, K.R. and Zilio, C. (2006) Gamma ray-induced bystander effect in tumour glioblastoma cells: a specific study on cell survival, cytokine release and cytokine receptors. *Radiat Prot Dosimetry*, **122**:1-4, 271-4.

23. Mothersill, C., Bucking, C., Smith, R.W., Agnihotri, N., Oneill, A., Kilemade, M. and Seymour, C.B. (2006) Communication of radiation-induced stress or bystander signals between fish in vivo. *Environ Sci Technol*, **40**:21, 6859-64.

24. Ballarini, F., Alloni, D., Facoetti, A., Mairani, A., Nano, R. and Ottolenghi, A. (2006) Modelling radiation-induced bystander effect and cellular communication. *Radiat Prot Dosimetry*, **122**:1-4, 244-51.

25. Kis, E., Szatmari, T., Keszei, M., Farkas, R., Esik, O., Lumniczky, K., Falus, A. and Safrany, G. (2006) Microarray analysis of radiation response genes in primary human fibroblasts. *Int J Radiat Oncol Biol Phys*, **66**:5, 1506-14.

26. Liu, Z., Mothersill, C.E., McNeill, F.E., Lyng, F.M., Byun, S.H., Seymour, C.B. and Prestwich, W.V. (2006) A dose threshold for a medium transfer bystander effect for a human skin cell line. *Radiat Res*, **166**:1 Pt 1, 19-23.

27. O'Dowd, C., Mothersill, C.E., Cairns, M.T., Austin, B., McClean, B., Lyng, F.M. and Murphy, J.E. (2006) The release of bystander factor(s) from tissue explant cultures of rainbow trout (Onchorhynchus mykiss) after exposure to gamma radiation. *Radiat Res*, **166**:4, 611-7.