MELODI –
Multidisciplinary European Low dose Initiative

4th Draft of Strategic Research Agenda (SRA)

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Revised version of 2nd Draft of MELODI SRA following the 3rd MELODI Workshop 2011 in Rome and the 4th MELODI Workshop 2012 suggestions from MELODI members, DoReMi members and workshop participants as well as other scientists taking into account recent research developments

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1. STATUS OF THE STRATEGIC RESEARCH AGENDA (SRA)

1.1. Introduction

The Multidisciplinary European Low Dose Initiative (MELODI) platform has the overall aim of consolidating the European initiatives on researching and better understanding the health effects of exposure to low dose ionising radiation. It is intended to provide a working framework over at least 20 years.

The European research platform MELODI is supporting the policy goals to improve radiation protection standards in Europe, and to prioritise and coordinate European R&T policies to achieve maximal impact on scientific knowledge and thus to consolidate the current scientific basis for the system of radiation protection (see ICRP recommendations and Basic Safety Standards). This is particularly important for the development of the Europan project OPERRA together with ALLIANCE and NEIRIS and in the context of HORIZON 2020.The Fukushima nuclear accident has highlighted the necessity to consolidate the scientific knowledge on radiation risk at low doses in a way that emergency response measures, especially for the general public in directly effected regions and beyond, can be based on the best scientific evidence that conveys to the public the validity of measures taken in order to simultaneously protect public health and ensure the economic and societal continuity in the country and in Europe.

The purpose of the present document is to present a long-term SRA for the MELODI programme. This should serve to guide the coherent integration of national low dose R&D programmes, and to facilitate the process of preparing EURATOM calls in this field.

This will require development of new and original research lines as well as pursuing on-going research in epidemiology, disease modelling and improving understanding of molecular, cellular and tissue level mechanisms of both cancer and non-cancer effects induced by radiation. Most research to date has concentrated on the induction of cancer but recent developments have indicated that exposed populations are also at risk of developing other non-malignant diseases and there are specific individual responses to radiation. The molecular biology 'revolution' has opened up the possibility of acquiring a new range of biomarkers for diseases of relevance to radiological protection. Combined with dedicated and new methods to measure (tissue) exposure to radiation, this allows to evaluate the health risks to individuals. It is the intention of MELODI to incorporate where appropriate new technologies as well as to integrate and promote existing and developing fields of research in a more holistic approach than has been adopted previously.

The research activities have to be accompanied by the establishment of activities that ensure maintenance and development of competences through adequate education and training activities and access to the necessary infrastructures such as irradiation facilities, epidemiological cohorts, data- and biobanks and analysis platforms.

Key scientific issues that steer the SRA have been identified by the High Level and Expert Group (HLEG), namely:
(1) the shape of the dose-response for cancer
(2) the investigation of individual radiation sensitivity
(3) a consideration of induced non-cancer disease
Plus the cross-cutting:

(1) radiation quality
(2) tissue sensitivity
(3) internal emitters

The current scientific consensus is that the health effects which should be addressed are:

Cancer – including secondary cancers

Cardiovascular disease

Lens opacities

Neurological effects (cognitive effects)

Adverse effects to normal tissue from radiation therapy

The research areas and priorities highlighted by the SRA should provide answers to the following overarching questions:

How robust is the current system of radiation protection and risk assessment?

How can it be improved?

What are the areas of greatest uncertainty in radiation research and radiation protection?

What are the areas of greatest uncertainty in radiation protection?

How to prioritise the questions and to identify research needs to address these questions?

The MELODI SRA focuses on key questions for radiation protection which cannot be solved by a single group or country.

1.2. The European short and mid-term NoE DoReMi (2010-2015)

The answer to the questions posed by the HLEG is expected to come from multidisciplinary low dose research in Europe. To this end, the HLEG recommended creation of a Network of Excellence (NoE) on Low Dose Research towards Multidisciplinary Integration (DoReMi). This started in January 2010 initially with 12 institutional partners (BfS, CEA, CREAL, STUK, IRSN, ISS, SCK-CEN, SU, HPA, HGMU, UNIPV and IC), plus 10 additional partners in 2011 and in 2012 ((UKER, GUF, UROS, UMB, NRPA, NIPH, ENEA, IES, DIT, Erasmus STUK, IRSN, HMGU, CEA, HPA, UNIPV, ISS, SCK-CEN, BfS, SU, CREAL, IC, UKER, GUF, UROS, UMB, NRPA, NIPH, ENEA, IES, DIT, ERASMUS MC, OBU, OBRUN, NRIRR, SURO,NUVIA, AWE,USAAR,LUMC, UBWM, LMU) (thus, 32 partners in 2013). DoReMi is co-ordinated by Prof. Sisko Salomaa (STUK, Finland). DoReMi comprises 7 work packages:

WP1: Coordination and management,

WP2: Structuring MELODI,

WP3: Education and Training,

WP4: Infrastructures,

WP5: Shape of dose response for cancer,

WP6: Individual radiation sensitivity for cancer (and non cancer)

WP7: Non-cancer effects.

1.3. The European longer term initiative MELODI (2010 - >2030)

Within DoReMi WP2 concerns structuring and establishing the operational tool for developing the MELODI platform to ensure longer term commitment (>20 years) to low dose research in Europe. Thus, DoReMi is a transitional initiative providing EU financial support and scientific feasibility studies to facilitate and accelerate the integration process within the MELODI platform.

To achieve this, a broad concerted effort is required to develop a long-term SRA for MELODI. The SRA should be largely based on scientific consensus and integrating new research lines developed in the mid-term transitional research agenda (TRA) of DoReMi. In order to be effective, the SRA requires periodic updating in line with future developments arising from new scientific knowledge and technologies.

2. SCIENTIFIC VISION

2.1. The present situation

The present system of health risk evaluation and radiation protection is based on current scientific knowledge and societal considerations of acceptance. It is steered by the ICRP protection system (ICRP publication 103, 2007) which, for low doses, has evolved around the perceived risk of induced malignancies and, to a lesser extent, heritable effects attributable to radiation. It uses the linear-no-threshold (LNT) assumption derived from high dose radiation effects extrapolated to the low dose region. It is a pragmatic and cautious approach for protection purposes and is essentially in accordance with the views of UNSCEAR and NCRP. It is widely thought that there is no compelling evidence as yet against LNT as a description of radiation effects (see R. Wakeford, Rome 2011). ICRP combine the LNT hypothesis with a judged single value of 2 as a dose and dose rate effectiveness factor (DDREF) for cancer induction. This value is used to lower the dose response slope, to allow for a presumed reduction of cancer risk at low dose. However, there is little reason to use DDREF for radiation protection at this time, and if so, new value need to be derived at the lower doses (see D Preston, Rome, P. O'Neill, 2011). In reality there is no empirical evidence to support a single value and moreover experimental data derived from both human and other mammalian species show a wide range of values dependent on parameters such as tissue or organ involvement and tumour type. The risk per unit dose is weighted for different organs and tissues by factors (W_T) that reflect their perceived relative sensitivities to induced malignancies. Likewise, differing carcinogenicity dependent on radiation quality is addressed by recommended weighting factors (W_R). Radiation risks to children are judged to be 2-3 times higher than those to the population as a whole. It has to be stressed that W_T, W_R, and the consideration of a single dose response relationship for all cancers, all ages and both sexes, are simplifying judgements.

In contrast to the ICRP view, a report from the French Academies (2005) supported the opinion that there is a practical low dose threshold for induction of cancer. Adoption of this view would have significant societal implications, both financial and legal for radiological protection in Europe. Phenomena such as transmissible genomic instability and bystander effects may also modulate cancer risk relative to that predicted from the LNT model. This implies a wider range of candidate risk extrapolation models, and the MELODI programme needs to address not only the risk for cancer but also for the non-cancer effects that to date have not featured in radiation risk evaluation at low doses. Consequently, new approaches are needed combining epidemiology with fundamental mechanistic studies of the processes that drive the radiation-induced health effects and individual susceptibilities. Such information will permit more biologically realistic mathematical models to be developed to extend risk evaluation to dose levels below which direct human data are unavailable.

For high radiation doses (> 1Gy) for which epidemiological studies are particularly significant the radiation protection system is reasonably well established. Nevertheless, uncertainties still exist, in particular for medium (>100 mGy, <1 Gy) and low (<100 mGy) radiation doses and continue to need attention, such as:

shapes of dose response curves for different types of cancers and non-cancer effects;

sensitivity variations dependent on age with possible differences between *in utero* irradiation, infants and older children, and between young and old adults.

individual radiation sensitivity and predisposition to cancers and certain non- cancer effects;

biological effectiveness of different types of radiation;

the dose rate effect, including fractionated exposures;

sensitivity of different cell types and tissues;

non-targeted effects of radiation;

effects of radionuclides and internal contamination;

mixed radiation exposures;

interactions of radiation with chemical agents.

It has been widely recognized that epidemiological studies have limitations for statistical reasons for estimation of radiation risks at low doses (<100mGy) and very low doses (<10mGy). Nevertheless, it is important to extend risk estimation down to environmental exposure levels such as mGy or μGy (see also Smith 2010, Wakeford and Tawn 2010). It is now generally accepted that, as also pointed out by the HLEG report, low dose risk estimations need to be based on an understanding of the mechanisms involved and this is the primary thrust of the MELODI programme.

There are many reasons why knowledge about radiation induced insults from low doses and dose rates remains elusive.
- The effects of low doses are usually much smaller than those for high doses, making it much more difficult to assess risks/effects due to inherent methodological/sensitivity of detection limits.
- Depending on the dose level and condition of radiation, low doses may have just a modulating effect on normal metabolism, which may result in persistent perturbation and damage when concomitant with other stresses.
- Many other parameters (specific tissue reactions) may interfere with or modulate the observable low dose effects because radiation is only one of many environmental insults producing overlapping effects.
- -There is no stringent specificity of ionizing radiation, i.e. no particular health effect has been identified as being unique to radiation.
- -Important experimental prerequisites: The definition of radiation doses and observable biological effects (including health effects) together with determination of the confounding factors has to be extremely precise in order to obtain highly reproducible results with little variability that statistical analysis.
- -Dosimetric issues: To be able to determine the relationship between radiation and the effects, the quantification of the radiation is very important. This quantification includes characterisation of the radiation, because radiation quality and the local distribution of free radicals and damage produced contribute to the biological

outcome. Not all dosimetry issues are as yet solved and improving the dosimetric quantification and characterisation can significantly decrease the uncertainty on the dose effect relationship. Some important issues are:

— Research on micro- and nanodosimetry
— Dosimetry of internal contamination
— Dosimetry of medical exposures
— small animal dosimetry
— Hadron and high LET dosimetry
— Biological dosimetry

- -**Biological endpoints and health** effects

-The biological endpoints determined need to be very well defined: this applies to molecular type of analyses (e.g. 'omics' etc.) and their producibility as well as to classical endpoints such as survival, mutation induction, cell transformation, etc.

- In epidemiological studies the medical definition of the pathologies examined together with the possible confounding factors (gender, age, lifestyle, exposure to stresses other than radiation etc. should be as clearly defined as possible including the use of molecular biomarkers for specific pathological conditions (cancer, non cancer) on physiological states (hormonal state, level of antioxidant, DNA repair, immunological defences, metabolic deficiencies etc.).

2.2. The fundamental molecular interactions associated with ionising radiation (IR) and the processes leading to cancer and non-cancer effects.

Normal life is associated with the production of free radicals. They are involved in most cell/tissue reactions: normal metabolism, intracellular and intercellular signalling, cellular defences: DNA repair immunological defences as well as in inflammatory reactions. Most of these radicals are held at a certain, well equilibrated level (homeostasis), scavenged or inactivated by cellular antioxidants and anti-radical defence systems. However, antioxidant defences are limited. Thus, when overproduced in the course of metabolic stress some may initiate endogenously damage in various cellular constituents including nucleic acids (DNA), proteins and lipids. This is thought to increase the oxidative burden during the course of life, and may indeed constitute precursors to a number of non-cancer diseases such neurodegenerative diseases (Alzheimer's and Parkinson's) and ageing which affects the central nervous system and arteriosclerosis, heart and onset of strokes which affect the cardiovascular system and metabolic diseases such as diabetes, as well as different types of cancers.

Sparsely ionising radiation is generally assumed to exert in living systems direct effects (about 40%) as well as indirect effects (about 60%) due to the production of oxidative free radicals. With densely ionising radiation direct effects are the more prominent, and the majority of the DNA damage is due to the direct effects of radiation with a small contribution from indirect effects. Independent of the ionisation density of the radiation only those radicals formed close to critical targets are important due to the short lifetime of most free radicals in human cells. In any case, ionising radiation induces damage to all cell constituents. In this respect, it is different from most other genotoxic agents: It is somewhat more powerful in cell killing than in mutation or cancer induction (thus, its high therapeutic efficacy). The ability of ionising radiation to induce complex cluster types of lesions (see below) also supports this view. Some lesions may overlap or interact

with damages from other types of stresses (ex: oxidative stress from normal metabolism and with that from ionising radiation, plus chemical pollution (see radon and smoking etc.)).

Interestingly, in terms of overall cellular damage to DNA, ionising radiation at low doses adds relatively few additional isolated oxidative lesions in addition to the plethora of those formed endogenously. However, low dose ionising radiation does induce, in addition to endogenous-like damage, low levels of complex DNA damage detected as clusters of lesions within one or two helical turns of the DNA by a single radiation track. These clusters of damage include double-strand breaks, some of which are complex with additional lesions close to the DSB ends, and complex damage sites which are known to be very difficult to repair and deleterious to cells, although their identification remains to be elucidated. An important issue is the role of the microenvironment of cells when in tissue or in 3D models as it is thought that the low level of clustered damage at low doses against the high background of isolated endogenous damage constitutes the genotoxic lesions initiating mutations and genomic instability and may result in cellular transformation and cancer. Evidence is accumulating, although at high doses of sparsely ionising radiation, that the microenvironment of cells in tissue modifies the damage response; the effects at low dose and on radiation quality remain essentially unknown.

Far less is also known on the induction of oxidative damage to cellular proteins and membranes by ionising radiation and their actual relevance to health effects. However, there is increasing evidence that oxidative damage induced in proteins and membranes (lipids) may considerably modify cellular structures and functions including mitochondrial functions, enzymatic DNA repair and also defences against oxidative damage. Oxidation or free radical-induced changes of proteins and lipids can interfere with important regulatory and cellular signalling processes. Thus, these changes and their possible reversal and/or recovery from them do merit careful analysis with regard to short and long term low dose effects including cancer and non cancer diseases (see M. Radman 2012).

From ionising radiation's interaction with living matter it can thus be inferred that direct action will induce direct and long lasting structural modifications in macromolecules (if these are not replaced or repaired). Whilst the majority of endogenously-induced oxidative damage in DNA is well taken care of by the DNA repair systems, complex damage is difficult to repair and accompanying radiation-induced accumulation of oxidative stress in proteins including chromatin and membranes impairs important general metabolic and DNA damage recognition, repair and signalling functions. Differences in repair in euchromatin versus that occurring in heterochromatin may need to be taken into account (see work of D. van Gent, M. Löbrich). Cells have well developed repair systems, namely base excision repair, which maintain the stability of the genome against endogenously induced oxidative DNA damage. However, antioxidant defences are limited, may be overcome by activity-mediated or environmental radiation or chemically-induced oxidative stress. Additionally, anti-oxidative defences appear to depend on age and life-style

In addition to these effects, oxidative free radicals have been identified as being involved in intercellular signalling and bystander effects between irradiated and unirradiated cells and these communication processes are perturbed by low dose radiation. The relevance of these perturbations to health effects of radiation remains to be resolved.

Interestingly, most normal metabolic cellular functions are based on partially reversible redox and free radical reactions. In fact, cells react very sensitively to even slight perturbances by low

radiation doses or other stresses. Sometimes, depending on the dose, this may have even some stimulatory effects on cellular metabolism (hormesis) strenghthening survival and cellular fitness allowing adaptive radiation responses (i.e. apparent radioresitance after a low dose followed by a high challenging dose) to occur. However, the more persistent perturbances of these are clearly associated with cancers and non-cancer effects. Thus, one of the most important future challenges of low dose research is to establish to what extent ionising radiation perturbs normal cellular metabolism at the cell, tissue and organ level, influences synergistically the DNA damage responses as well as interfers with the equilibrium of normal systemic signalling (homeostasis) of the human body and as a consequence promotes or induces pathological conditions.

With regard to cancer it has become evident in recent years that the process of radiation-induced carcinogenesis involves persistent changes including genetic (somatic mutations) and epigenetic alterations concomitant to genomic instability and changes in the microenvironment of stem and differentiated cells and tissues.

In this respect, it is interesting to note that ionising radiations of different qualities vary considerably in their ability to induce direct structural and indirect radiation effects (oxidative, free radical mediated stress), and also, by definition, the rate by which free radicals and cellular damage are produced (effects of dose rate) may condition short and long term radiation effects.

The local distribution of the free radicals produced in cells and tissues as well as the pre-existing cellular oxidative stress and the available arsenal of antiradical and antioxidant defence systems (under genetic and epigenetic control) will contribute to the determination of the final biological outcome. Therefore, the basic metabolic, proliferative, genetic, epigenetic, immunological, inflammatory, hormonal and physiological status of cells and tissues need to be investigated as an important pre-determinant for low dose radiation-induced insults. The contribution of bystander mediated responses and intercellular signalling pathways should be clarified as well.

It will be of utmost importance to launch research to define quantitatively the levels of oxidative stress in cells, tissues and organs that are part of normal homeostasis and those levels that can be achieved by low ionising radiation exposure and if they may be regarded as precursor conditions to perturb the homeostasis for the development of cancer and non-cancer effects. Additionally, it will be important to determine relevant molecular and structural changes induced uniquely by ionising radiation directly and that are in the long term persistent and contributing to cellular, tissue and organ dysfunction. The roles of different cell types, stem cells, progenitor and germ cells will need to be defined. Knowing that some stem cells are particularly radioresistant with little individual variation research in tissue and cancer specific stem cells will be particularly relevant (M.T. Martin, Rome, 2011).

Finally, the radiation responses need to be analysed as the perturbation of a complex system. All mechanistic analyses should fit into this (see A. Ottolenghi, Rome 2011), and should be linked with systems biology approaches.

2.3. Research Priorities

The research priorities are presented according to the 3 key scientific issues of major concern in radiation research and radiation protection, identified by the HLEG:
- Shapes of dose response curves for cancer
- Non-cancer effects
- Individual radiosensitivity

Some of these issues together with the crosscutting issues radiation quality, tissue responses and internal emitters are already being addressed by DoReMi and are part of the Transitional Research Agenda (TRA) of DoReMi and assocated pilot studies. Molecular Epidemiology may be considered as well as a cross-cutting issue.

A number of questions that are key issues for MELODI has been identified. These have been derived from the HLEG report, the MELODI workshops in Stuttgart in 2009, Paris in 2010, Rome in 2011, Helsinki in 2012, and responses to SRA discussions at the different workshop. The list of questions is extensive and is presented in annex 1 of this SRA, grouped by the 3 key issues of shape of the dose response for cancer, non-cancer effects and individual sensitivity.

A reduction of uncertainties in these areas* of major concern in radiation research and radiation protection is likely to be achieved by establishing links between fundamental mechanistic studies (including most recent technologies such as "omics" and next generation sequencing) and epidemiological studies on suitable cohorts (with sound dosimetric and well-defined medical bases) and focussing on the following items in the forthcoming years:

2.3.1. Dose response relationship for cancer

The priorities are:
- Establishment of sound (molecular) epidemiological studies on suitable cohorts (uranium miners, nuclear workers, patients that receive diagnostic or therapeutic radiation…)
- Identification of relevant pathways involved in low dose radiation-induced cancers, and corresponding molecular biomarkers (using "Omics" and next generation sequencing)
- Acquisition of new information and a better understanding of :
  — the relationship of actual low dose (and/or low dose rate) radiation exposure to the occurrence of specific types of cancer,
  — the effects of different radiation qualities
  — the effects of chronic exposure from external radiation and/or internal contamination
  — the involvement of different cell types in specific cancers
- Development of relevant dosimetric approaches and application of these to quantify the exposure (especially difficult and necessary in case of large cohorts)

Research on suitable human epidemiological cohorts remains a very high priority of research in forthcoming years. Not only promising research on existing retrospective epidemiological cohorts should be continued, but also research on new prospective cohorts should be initiated and include cohorts from cancer patient biobanks. Other reliable cohorts are from Chernobyl, the Mayak workers, uranium mining and other contaminated sites where relevant biomaterial is available and accessible. Prospective and retrospective cohorts for this type of study are also CT scanor Cone Beam CT in children and cohorts from patients treated with radiotherapy in childhood or adulthood. There is already some evidence for this (M.S. Pearce 2012).

Among the well-controlled medical cohorts one prospective cohort to be considered should be that of radiotherapy patients with a good survival prognosis. These are at risk of out-of-field low dose induced second cancers particularly with newer modalities of delivering radiotherapy (IMRT,

Hadron therapy, IGRT). Whilst treatment field doses are reasonably well specified, further effort needs to be given to improving the dosimetry for the out-of-field tissues and organs. Other cohorts such as children exposed to CT scans with possible long term follow-up, population cohorts undergoing screening with X-ray examinations (example: breast cancer screening, colorectal cancer with virtual CT colonoscopy) and occupationally exposed individuals such as interventional cardiologists and aircraft crews are groups where individual dosimetry can be reasonably well defined. A typical cohort could be children born with craniofacial defect, easily receiving up to 10 Cone Beam CT scans during early childhood to allow functional and esthetic defect closure. Regarding A-bomb survivors, Chernobyl and South Urals (Mayak and Techa River Residents) populations good individual dosimetry has been problematic. In the past improvement of retrospective dosimetry led to successive dosimetric systems that allowed improving the assessment of the dose-risk relationship. For most of these groups there is still an urgent need for improvement of retrospective dosimetry and modelling. Suitable modelling will bridge the gaps between epidemiological and radiobiological studies and should allow the quantification of low dose health risks.

The Fukushima Dai-ichi powerplant incident has served to remind us that many nuclear incidents involve, in the most part, people being exposed to low radiation doses. Such incidents provide opportunities for scientific study of radiation-associated disease risk when the immediate consequences have been adequately addressed. MELODI partners are willing to collaborate with Japanese scientists in such studies. It is also apparent that there is value in considering development of recommended protocols for use in post-emergency situations that will optimise the gathering and compilation of radiation disease risk related information.

Maximising the value of epidemiological studies may be aided by incorporation of suitable molecular or cellular biomarkers into studies. The 'omics' approach and next generation DNA sequencing technologies are promising routes to pursue. Attempts to identify suitable biomarkers are a priority alongside dosimetric biomarkers, but it has to be realised that there may not be markers specific to radiation-induced cancers (reviewed by Pernot E. et al. 2012). The direction would then switch to parallel studies of biomarkers of diseases per se and of exposure and thus a 3-way correlation with disease. The development of biomarkers indicative for radiation exposure, biological (early and late) effects, i.e. pre-pathological and pathological states, and radiation susceptibility are likely to constitute important pre-requisites in low dose radiation research. To this must be added consideration of confounding factors by other agents that cause the diseases in question.

Low dose rate, dose fractionation and chronic exposure from external radiation and/or internal contamination are another important priority (see paper by D. Laurier et al. 2012, Radioprotection) The chronic leukaemia Ninshal raises issues that cannot be answered today . Dose levels are very low, below that of natural radiation, but results are puzzling, and there is a real need for continuing research.

Research priorities in somewhat more detail:

2.3.1.1. Studies of the spectrum of damage induced by ionising radiation (dependent on radiation quality)
— at the cellular, tissue and organ level and its relationship to

— damage repairability (identification of pro-repair factors ?)
— activation or inactivation of specific metabolic pathways,
— cell type specific effects

2.3.1.2. Clarification of the mechanism of radiation-induced cancer, in particular the role of:
— genetic,
— epigenetic
— non-targeted effects
— cell-type specific aspects of cancer induction.
- the involvement of mutations (mutational theory),
- the contribution of non-targeted effects and radiation quality,
- the involvement of gene silencing, cell differentiation and stem cell development..

2.3.1.3. Studies on the extent to which the sensitivity to cancer induction may develop during life (e.g.: including dosimetric studies in cohorts such as young children (examples: CT scans for congenital deformities or common exposures to a large group of children such as dental cone beam CT, and screening populations).

Research needs to continue on the dependence on track structure and microdosimetric features of the tracks' spatial distributions of energy deposition events. The induction of specific types of damages to DNA, RNA, proteins and lipids (tandem damage of lipids and sulphur-containing proteins) may allow identification of useful biomarkers of exposures (see Chatgillieloglu, Rome, 2011, Aracely Garca-Garcia et al. 2012*). In particular, methods to identify and quantify complex DNA damaged sites, especially, the ratio between non-dsb clusters relative to complex dsb in vitro and in vivo are required (P. O'Neill, Rome, L'Eccles et al. 2011)). This needs to consider the interplay between the spectrum of damage induced and its repairability and kinetics in modulating the shape of the dose response curve. This feeds into improving the scientific basis of ICRP's W_R values. Biophysical models should be based on experimental evidence and lead to reliable prediction and interplay between experiments and models.*

Furthermore, a greater understanding is needed on the relationships between oxidative stress, DNA damage complexity, DNA repair, chromosomal damage, translocation, DNA damage signalling, perturbed cell cycle regulation, senescence and apoptosis and how the interplay of all of these combine to produce cancer with different radiation qualities at low doses and dose-rates. In addition to DNA repair, the search for prorepair factors should be of particular interest (M.T. Martin, Rome 2011, K. Raj and S. Bouffler, 2012*). Radiation-induced changes in chromatin are tissue-, sex and dose-rate specific. Thus, it will be important to determine the dose-response relationships with regard to possible pathological outcomes in vivo (maybe, biobank materials could also be used for that). Therefore, basic cellular mechanistic studies are required.* Also, the different structure of chromatin in germ cells appears to affect DNA damage processing in germ (stem) cells (C.E. Rübe et al. 2012) and thus heritability of radiation damage. These latter effects may include epigenetic and transgenerational effects (Dickey JS et al. 2011).

Current evidence suggests that both target cell effects and tissue (micro) environmental effects contribute to radiation carcinogenesis. The relative importance of these at differing dose levels remains a research priority. This clearly has a bearing on the relative tissue sensitivities to cancer susceptibility expressed by ICRP in simplified form as tissue weighting factor (w_T) in the basis of epidemiological data. Gender specific tissue weighting factors should be established and taken

into account (M.,H. Müller, Rome). The use of cells, 3D model systems and tissues is likely to be important. Concerning the role of stem cells in carcinogenesis, research should be conducted on radiation-induced changes in the microenvironment of stem cells and their consequences (see M.T. Martin, Rome, 2011). Also, the particular radiation sensitivity of germ cells and embryonic cells at different maturation or developmental stages in relation to fertility problems and congenital malformations need to be assessed.

The contribution of targeted and non targeted effects to cancer induction needs to be clarified (even after the NOTE project). This includes the assessment of delayed genomic instability, transgenerational effects and of low dose hypersensitivity. The role of perturbed immunological functions and inflammatory reactions in radiation-induced cancers has to be assessed as well (see Rödel F et al. 2012).

The extent to which the sensitivity to cancer induction differs depending on developmental stage e.g., in utero, young children, adults, is an important question. In this respect, for example, repeated diagnostic X-rays (e.g., CT-scans) of premature babies and young children as well as the effect of the contrast agents of high atomic number are potentially of concern since it is expected that they are more sensitive to radiation-induced diseases than older children subjected to repeated X-ray examinations (e.g. Cone Beam CT scans in dentistry). Indeed, cumulative exposure of children during early childhood (e.g. to correct conenital deformities) may also present a cocern. A typical example of such risk category may be the pediatric patients with craniofacial anomalies (such as cleft palate), who made easily receive up to 10 CT scans before cleft closure.

2.3.1.4. The use of susceptibility biomarkers should also enable the importance of individual radiation sensitivity to be determined as well as age and developmental specific effects and confounding factors for low dose cancer risk.

2.3.1.5. Bio- and data banking will be essential for analysis and follow-up in this combined epidemiological and fundamental research. It may become necessary to create a dedicated biobanking infrastructure within the MELODI programme

Until the question of appropriate molecular / cellular biomarkers is resolved biobanking of material has to be undertaken. This must be a workpackage in any newly initiated epidemiological study. Also, the extent to which existing biobanks (STORE, GENEPI) can be used needs evaluating.

2.3.1.6. Development and extension of well-designed studies on effects of different types and qualities of radiation, of low dose rate, dose fractionation and chronic exposure from external radiation and/or internal contamination are another important priority.

In fact, extensive research is still needed aimed at understanding the impact of physical parameters such as radiation quality and the dose rate (and/or fractionation) on those aspects of cell responses which could be relevant in risk estimates for cancer induction. It has to be kept in mind that radiation quality and dose-rate can be important tools for testing hypotheses (A. Ottolenghi, Rome) by revealing selective biological responses. In this respect, the effects of dose delivered by internal emitters will need particular attention.

In this research, two aspects will have to be considered: (1) taking into account the particle size of radioactive elements internal (research on nanoparticles) (see for example: Petitot F. et al. 2013) and their particular chemical and radiation toxicity, (2) taking into account the target cells

and tissues with particular focus on the responses of stem cells and early and late progenitor cells.

- It would be most profitable and relevant to focus studies on: tritium and actinides in the nuclear industry; mining industries that cause radium and thorium exposures. Diagnostic, and possibly therapeutic, applications of radiopharmaceuticasl in nuclear medicine are possibly a rich source of experimental studies because planned, monitored and controlled delivery of radiopharmaceuticals gives scope for precise dosimetry follow-up. In this research single cell, 3D and tissue, organ and animal models should be used.

- The identification and the nature and number of 'target' cells at risk for specific cancers in humans are important questions (for example: thyroid cancer). Another example is the glandular tissue in the breast as opposed to the adipose tissue. The amount and distribution of glandular tissue is very variable among women but new techniques may allow patient specific quantification and improved dosimetry. In Europe, breast cancer screening programmes are implemented in most Member States and known to be very well monitored, providing potentially very useful dosimetric data. The target cells are likely to be stem cells or at least relatively early progenitor (little differentiated) cells. In this area studies with short range internal emitters are likely to be of particular value due to their characteristic tissue distribution leading to localised delivery of dose. Of necessity such studies will also consider radiation quality issues.

- Concerning the radiotoxicology of internal emitters research using "omics" and in particular metabolomics recent data suggest that it should be very promising to assess metabolomic signatures identifying contaminated from uncontaminated individuals (see Grison S et al. 2012, J Radiat Res. 53, Metabolomics) and to reveal epigenetic changes associated with irreversible pathological effects, parts of which may be transmissible to next generations (see Soudi M et al. 2013).

2.3.1.7. Animal studies should be undertaken: they generally constitute useful complements for assessing specific mechanistic studies for low dose/low dose rate radiation induced human cancers.

For example, more effort is required to determine whether good animal models can be employed for some cancers, other than AML. Furthermore, appropriate protocols with transplantable tumors should be useful to specify the importance of cellular micro-environmental and non-targeted responses. Furthermore, the induction of genomic instability may be elucidated using "omic" type approaches and epigenetic profiling.

2.3.1.8. Mathematical modelling should help to link the exposure and mechanistic data to the pathological outcome and to assess actual health risks involved.

The information collected in epidemiological studies and in molecular studies on the multiple pathways involved in radiation induced carcinogenesis may then become part of an overall systems biology approach which will allow modelling of biological dose responses for both cancer and, non-cancer diseases.

2.3.1.9. Implementation of an international collaborative working group to better integrate research on childhood leukaemia risks at the European level and beyond.

One major aim of research at low doses and low dose rates is also to provide answers to the questions from the public regarding cancer risks. Several recently published epidemiological studies on childhood leukaemia near nuclear installations raised questions which could not be answered by

current knowledge on the effects of ionising radiation or any other known risk factor. A workshop was organised on this topic in June 2012 under the auspices of MELODI. The goal of the workshop was twofold: a) to learn from past studies and to develop a best possible study design for answering questions on childhood leukaemia incidence close to point sources; b) to identify and define promising directions for future research into the causes and pathogenesis of leukaemia in children at European level and world-wide. The workshop gathered 42 participants from 14 countries and from different disciplines: epidemiology, biology, haematology and genetics, extending widely outside of the radiation protection field. Recommendations were derived from this workshop, regarding the improvement of epidemiological studies and the development of research to better understand the aetiology and mechanisms of childhood leukaemia initiation and development.

One of the major recommendations of the MELODI workshop was the implementation of a collaborative working group on childhood leukaemia risk at the European level. The aim of such working group is to improve the coherence and the interpretation of results of future analyses in Europe, in parallel with the reflexion currently ongoing in the USA under the coordination of the National Research Council (NRC). It should help understanding the variability of results between countries and harmonising methodologies. It would be a step forward to verify the feasibility and pertinence of international pooling analyses. Specific aims of a pooled analysis are: increasing statistical power to allow analyses of leukaemia subtypes, overcoming the problems of boundaries, harmonising data collection and the definition of exposure indicators. Regarding the determination of childhood leukaemia causes, it would provide a basis for the development of multidisciplinary collaborations, with multiple partners on the European level, and with appropriate links to other internationally run projects such as the Childhood Leukaemia International Consortium (CLIC) or the International Childhood Cancer Cohort Consortium (I4C).

2.3.2. Non-cancer effects

Compared with cancer much less information is available on effects of low and medium dose radiation exposures in producing long term consequences such as cardiovascular dysfunction, neurological alterations, lens opacities, or effects on other physiological functions. In order to tackle these important aspects, there is an urgent need for multidisciplinary approaches bringing together radiation biosciences with disciplines such as cardiology, ophthalmology and neurology that to date have had little or no involvement with ionising radiation research. Most urgent issues to resolve are the possible induction of cardiovascular, lens opacities and neurological (cognitive) impairments by low/medium dose ionising radiation. Feasibility studies carried out in the DoReMi project are likely to orient the different lines of future research in these areas.

> 2.3.2.1. Epidemiological and fundamental mechanistic studies should be undertaken in order to determine the dose-effect relationships (absence or presence of thresholds) for the induction of cardiovascular, lens opacities and neurological (cognitive) impairments. *For this, suitable cohorts (some retrospective already existing cohorts, most prospective) with sound dosimetry and medical control have to be identified and/or set up*

It has long been realised that high radiation doses have the potential to cause effects such as circulatory diseases, lens opacities and cognitive impairment but such non-cancer effects at low doses cannot be readily explained by the mutational theory (DNA paradigm) underlying the extrapolation of cancer risk from high to low doses (LNT). Thus, it has been traditionally assumed that the non-cancer effects and diseases show a threshold at doses that are well above the levels

of exposure typically encountered in the public environment, at work or from medical diagnostics. However, some epidemiological evidence as well as various tissue responses and non-targeted effects recently observed at low doses call for new experimental (mechanistic) and epidemiological studies that address the extrapolation issue.

Thus, in addition to the cancer surveillance, non-cancer endpoints have to be included in prospective epidemiological studies proposed above under the dose response sub-heading.

> 2.3.2.2. For each of these pathologies the age and developmental-specific mechanisms involved should be determined. Particular attention has to be put on the involvement of specific tissue, and overall metabolic, hormonal, immunological, inflammatory (tissue micro-environmental) status in the different pathological responses.

Generally speaking, it is likely that the underlying mechanisms involve as starting points (initial events) radical formation and radical (oxidative stress) induced lesions very similar to those implicated in cancer. However, subsequent stages involving different tissues, metabolic, hormonal, immunological, inflammatory and tissue micro-environmental responses are likely to be rather specific and different from those identified for cancer. Thus, it is important to attract new disciplines into the field of radiation research bringing in existing knowledge of mechanisms involved in the development of these conditions.

The observation that inflammatory response is a predisposition to malignancy and may be a risk factor for the development of many other clinical conditions lends support to the hypothesis that radiation injury may predispose to a range of health consequences wider than what was previously thought. Thus, it is important to establish the impact of immunological status on radiation-induced pathological responses that lead to non-cancer effects.

Mechanistic studies on the responses of the tissues involved need to be launched concomitantly in order to identify early predictive markers and clinical endpoints.

> 2.3.2.3. Research on cardiovascular effects should be carried out on low and medium doses of ionising radiation (as a follow up and extension of the previously more high dose oriented EU project CARDIORISK).

Here it is of interest that the etiology of radiation-induced atheriosclerosis is not the same as age-related atherosclerosis, and there is an interaction between elevated cholesterol and radiation - induced development of atheroscleorosis. Coronary artery and microvascular vessels are damaged by radiation, however, it has to be clarified whether the mean heart dose and the dose distribution to major arteries the most relevant parameter to consider (F.A. Stewart, Rome, 2011)

> 2.3.2.4. Research on lens opacities needs to be launched combining, if possible, epidemiological research and mechanistic studies on the dose response relationship including acute and chronic exposures to ionizing radiation.

The development of CT dosimetry tools for brain perfusion CT and other (repeated) brain CT examinations may allow accurate monitoring of this cohort of patients. Moreover, health professionals such as interventional cardiologists, airplane pilots, children living in Co60 contaminated buildings in Taiwan, Chernobyl and other nuclear clean up workers may constitute suitable cohorts. Mechanistic studies need to be launched to understand the response of lens tissues to radiation injury.

2.3.2.5. Research on neurological disorders and cognitive dysfunctions induced by low dose ionising radiation should be undertaken in order to determine low dose and dose-rate related responses.

Impaired IQ caused by irradiation of the foetus, particularly during the critical weeks 8-15, is well known and has led to foetal diagnostic radiology being replaced by ultrasound. Prospective epidemiology is therefore no longer feasible. However considerable interest was generated when a recent study on the effect of low doses of ionizing radiation in infancy concluded cognitive impairment function in adulthood. This suggestion of a second time window during infancy, when diagnostic radiology is still undertaken, opens new aspects for much needed investigation. Also, exposure of premature babies should be of concern.

2.3.2.6. Animal studies are essential complements of all these studies, in particular they are needed for the analysis of low dose acute and chronic effects from external as well as from internal (contamination) exposures.

Given the ethics constraints on research with humans, the use and development of animal models is essential. Lifespan studies in animal models may provide useful complements of information. This is particularly so for studying chronic exposures due to external irradiation or the intake of radionuclides. Recent animal experiments have linked ingestion of low doses of radionuclides to effects on unsuspected biological targets, such as the central nervous system, liver and major organism metabolisms. Chronic uranium exposure at low doses led to molecular and cellular effects on metabolisms of xenobiotics, vitamin D, cholesterol and iron. Behavioural and cognitive effects were also reported after chronic uranium exposure at low doses, in addition to the well-known nephrotoxic effect of uranium. In human populations, uranium has been shown to affect bone metabolism. There is also some evidence that chronic contamination by cesium-137 could affect cardiovascular functions. The extent to which these and other effects are due to the radiation moiety as opposed to chemical toxicity needs to be established.

Radioactive contamination studies should be backed up by investigations on the effects of particle size, distribution and specificity including research on nanoparticles. Furthermore, concerning external irradiation, recent animal studies have reported a high sensitivity of the brain and cognitive impairment after low doses of X-irradiation during the perinatal period, in particular, in neurite outgrowth and in neuron connectivity.

2.3.3. Individual radiation sensitivity

Clinical evidence from diagnostic and therapeutic uses of ionising radiation clearly shows that individuals respond differently to ionising radiation. From a radiation protection point of view, it is thus very important to identify radiation sensitive individuals and to understand the mechanisms involved. However, research in this area is difficult.

Ideally, this should be a consideration (workpackage) within any newly initiated epidemiological study such as those suggested above for investigating cancer dose response. However, just as with the biomarkers for disease/exposure, there is also an urgent requirement for identifying biomarkers, gene markers and phenotypic traits to indicate specific radiation risks in individuals.

To date there are no well established assays, although it is likely that in the current framework of DoReMi such biomarkers will be developed.

In the interim, biobanking of suitable material from relevant epidemiological cohorts is again a necessity. However, at present it is not clear what to store because it is not known what markers

will emerge. Furthermore, there are formidable ethics restrictions placed on this type of research in Europe and of course logistical limitations (especially for children cohorts) on what may be collected from human subjects. For the time being, it seems appropriate to store retrievable DNA (frozen or possibly fixed blood cells) and samples from blood serum or even skin biopsies. Moreover, it will be important that a substantial proportion of the cohort is available for recall in the event that an eventual individual sensitivity biomarker requires some other, as yet unknown, assay that cannot be performed with the banked material. From this, the importance of setting up prospective study cohorts of persons such as children exposed to low radiation doses and with long life expectancy is paramount.

The following issues are of high priority:

2.3.3.1. For the detection of individual sensitivity, it is essential to set up suitable (dosimetrical and medical) cohorts that are well controlled together with appropriate infrastructures allowing concomitant fundamental research (molecular studies) to be carried out using most recent technologies. Research should cover the following items:

    2.3.3.1.1. sensitivity of different cell types (stem cells and progenitor cells) in different types of tissues, redox profiles (oxidative stress), genetic (single nucleotide polymorphisms or SNPs, copy number variations or CNVs, next generation sequencing) and epigenetic profiles, the DNA repair capacity, capacity to undergo radiation-induced death, the immunological, hormonal, inflammatory, general health status of radiation sensitive and resistant individuals, latencies for different pathologies (cancer, non-cancer effects).

2.3.3.2. Using such well-defined cohorts, knowledge on genes and genetic polymorphisms (DNA repair, cell cycle checkpoint genes, oncogenes, genes of DNA and general metabolism, hormonal and immune responses etc.) as well as epigenomic imprints should be sought in order to define their roles in individual low dose radiation responses.

This knowledge can then be used

    2.3.3.2.1. To define sensitive subpopulations in the cohorts and

    2.3.3.2.2. The effects of confounding factors such as age, sex, gender, lifestyle, physiological and reproductive status, and

    2.3.3.2.3. Concomitant exposures to other physical, chemical or infectious agents or from mixed radiation fields as well as

    2.3.3.2.3. The amount of radiation sensitive tissue (example: the amount glandular tissue versus adipose).

Some endpoints have shown promise in the field of markers for individual radiosensitivity e.g., G2 sensitivity, dicentric chromosomes or micronuclei and may need further study. However, to date, all assays have fallen short of being reliable individual predictors. For example, when applied to groups of normal or over-responding radiotherapy patients a bi-phasic profile can be seen but there is a considerable overlap. Applying some newer assays for markers of radiation exposure (H2AX, 53BP1) and specific DNA repair activities (RAD50, MRE11) have shown greater promise for indicating intrinsic individual radiation sensitivity and repair capacity, and this work should be encouraged. Some tests (e.g. MRE11) may even be predictive for long term cancer risks in human. These newer cytological and molecular assays have to be applied on a large scale for validation. It

is possible that an integrated analysis based on a constellation of results from several markers will emerge as the most reliable way to specify an individual's sensitivity. The development of user-friendly indicators of dose and risk would also help to support justification and optimization of medical exposures (E. Vano, Spain.)

At present, it is unclear to what extent inflammatory and immunological factors are involved in individual radiation responses. Likewise, the degree to which non-targeted radiation responses differ between individuals needs to be examined. Ultimately it needs to be established the extent to which individual sensitivity is dependent on genetic background in contrast to the role played by potentially modifiable lifestyle factors and measures such as the amount of radiosensitive tissues (example: the amount of glandular tissue in the breast). From this will emerge the potential usefulness of embarking on programmes of systematic genetic profiling of individuals within cohorts such as radiation workers.

Within the framework of individual radiosensitivity, it is likely that developmental stage is an important factor. Thus differing responses to exposure in utero, in childhood and in adulthood should, where possible, be factored into studies of mechanisms.

With regard to the susceptibility of radiotherapy patients (successively been treated in childhood for a first cancer) to develop second cancers it is of interest to search for possible individual deficiencies in defence capacities (antioxydants, DNA repair, apoptosis, immune defence) against radiation insults.

Inclusion of various functional assays for radiation sensitivity in epidemiological studies will increase statistical power for identifying risk factors in later genome wide association studies. Moreover, additional functional cohorts could come from human longevity studies (cancer susceptibility and radiation response), cancer susceptible individual and radiation therapy patients with aberrant responses.

2.3.3.3. Combined epidemiological and animal model studies should be useful in identifying risk variants.

In-bred laboratory animal models cannot represent the intrinsic variability of a human population. However, they can be useful for validation purposes. Specific endpoints can be examined and specific modifiers can then be further explored using suitable animal models (e.g. for congenital malformation risks or for cancer risks like osteosarcomagenesis (RB1), mammary tumours (Aps) and medulloblastoma (ptch or thyroid rRET-PTC) cancers).). Radiation quality and dose-rate effects should be considered as well.

2.3.4. Hereditary and transgenerational effects

Although hereditary radiation effects have not yet been observed in humans there possible occurrence should be considered. It is likely from lower animal studies that they exist and may be seen at very long term (several generations later) (P. Smeesters, Rome,2011 as postulated earlier by Bernard Dutrillaux, CEA, France) and Sankaranarayan (discussion in Rome, 2011).

> 2.3.4.1. *Transgenerational effects should be explored including expanded simple tandem repeats (ECTR) (tandem mutations in mitochondria and changes in epigenetic profiles in connection with radiation-induced human (and animal) diseases in offspring. Epigenetic profiling is likely to provide relevant molecular biomarkers (miRNA)*

2.3.4.2. In view of possible transmissible genomic instability the transmission of persistent oxidative stress and the bystander mediated induction of transmissible genomic instability should be analysed.

3. NEXT STEPS

3.1. Evolution of research areas to be exploited

It is clear from the above questions relating to key issues that the research cannot be adequately undertaken by only extending already existing fields in radiation research such as radiation physics, dosimetry, radiation chemistry, radiation biology, radiation therapy and diagnostics, radiotoxicology etc. New lines of research have to be developed based on recent achievements arising from areas outside of radiation research to broaden thinking with a view to a new dynamism. For example, there have been in recent years many discoveries on specific metabolic functions and pathways, homeostasis, signalling mechanisms, stem cell biology, cellular stress, proliferation, genetics, epigenetics, systems biology, toxicology, genotoxicology, physiology, pathology, immunology, inflammation research, hormone research, research on cell death (apoptosis, mitotic catastrophy, autophagy), central nervous system, recognition and behavioural effects, embryology, teratology, molecular markers for imaging, effects of nanoparticles (nanotechnology), heredity, transgenerational transmittance, diseases (medical treatments and diagnosis of cancer and non-cancers, …).

3.1.1. Infrastructures

For low dose and low dose rate research, the current provision of suitable infrastructures (cohorts, radiation facilities, data-and biobanks, platforms for high throughput analyses) within Europe is a limiting factor. A strategy for the upgrading of infrastructures should be given a high priority.

- Most important is the establishment of suitable cohorts that allow at the same time molecular and medical follow-up. A number of potentially informative cohorts drawn from industrial and nuclear workers, medically exposed groups and residential radon exposures have been suggested in section 2.3.1 of this document. Initiating studies of prospectively followed-up cohorts is strongly recommended and therefore mechanisms have to be set up to ensure the study subjects' continued availability for research. Harmonisation of the collected data and of the methods of collecting them and dedicated dosimetry has to be strengthened.

  Including molecular and mechanistic studies in the surveillance of followed-up cohorts is essential. This requires suitable cell and tissue banking facilities with a harmonised approach across Europe. In this respect there already exist the STORE and BBMRI infrastructures which MELODI should look to exploit.

  A large proportion of the work within MELODI will require ethics approval. This is a notoriously slow and resource-intensive procedure that can add considerable delays to starting projects. Moreover the multi-institute / multi-national nature of the studies often means that all progress is delayed until the final partner has secured approval. Overriding ethics issues need to be sorted out and settled by consensual interaction with the national ethics committees. MELODI should consider how a Europe-wide infrastructure to facilitate ethics approval could be created.

- Suitable radiation sources able to deliver low and low dose rate irradiation to cells, tissues and whole animals (both external beam irradiators and internal radionuclides) are needed together

with associated laboratory facilities and dosimetry capabilities. DoReMi WP4 has begun work to identify and/or develop such facilities that can be made available to MELODI.

The existing and forthcoming radiation facilities including microbeams, devices for alpha, beta, gamma, x-ray, neutron, proton exposure as well as facilities for low dose rate exposures have been listed by DoReMi and MELODI is asked, wherever necessary, to help in getting access.

- Bio- and databanking appear to be important requisites that have from the start to accompany classical and molecular epidemiplogical research. Obviously, banking will be needed in order to collect and to keep relevant biological samples from retrospective and prospective epidemiological studies as well as from animal studies. This will be an important (permanent) source for present and forthcoming fundamental research using specific, newly developed molecular biomarkers defining radiation damage and exposure as as well as pathological changes and disease. Links to existing facilities supported by national or EU funds (see STORE project) were established through DoReMi and their exploitation pursued. Ensuring free access to these data-and biobanks and the long term maintenance of these will be an important organisational and financial challenge of MELODI.

- Analyses platforms for high throughput 'omics' exist in several institutions in Europe. Access is usually possible via collaborative projects or thoughout direct individual contracting. MELODI should seek support for this type of collaborative and integrative efforts in low dose research and facilitate access to forthcoming high level sequencing activities (next generation sequencing) (often done commercially) in the framework of defined low dose radiation research projects.

3.1.2. Education and training

In recent years, many European member states have lost key competences and are no longer capable of independently retaining their current research activities in radiation sciences, with implications for effectively fulfilling operational and policy needs and obligations. Thus, specific programmes aiming at knowledge management across generations have to be designed in order to achieve sustainable continuity and development. Important aspects to be considered are:

(1) The underlying scientific programmes have to address questions that are attractive to both young scientists and faculties of universities as well as to the management of research organisations.

(2) The attractiveness of the field has to be increased by a multiple approach implemented from summer schools to master degrees, PhD and post-doctoral European research training programmes.

(3) In the long term, such programmes cannot be successful unless they do provide job opportunities to young scientists.

(4) In the present situation, sustainability of such programmes can only be achieved by a long-term commitment of funding bodies.

The MELODI platform does effectively respond to these needs and aims at establishing an integrated approach to education and training of research and teaching at Universities and non-university research organisations. Existing elements of education and training activities in this domain such as the European MSc course should be strengthened, making it compliant with the Bologna Process which creates the European Higher Education Area (EHEA) and is based on the cooperation between ministries, higher education institutions, students and staff from 46 countries, with the participation of international organisations. At present, only a few universities in Europe will have the resources to offer a full educational programme at the basic as well as the

advanced level of subjects such as radiation biology and radiation physics. Thus, an integrated approach is needed.

In line with this, the following steps are to be implemented:

- Audit of radiation courses in Europe (undertaken by DoReMi) to establish a European course (and/or summer school) in radiation biology and radiation protection with conventions with European universities and institutions.
- Identification of stakeholders able to provide long term sustainability.
- Proposition of EU calls directed to education and training that promote new ways of setting up new multidisciplinary interactive courses. These must be Bologna compliant and based on solid conventions with leading universities and research organisations and allow inclusion of most recent research developments in the field of low dose radiation research and the evaluation of radiation health risks.

3.2. Maintaining the SRA

It is essential that the SRA is periodically revised in order to take account of new developments, achievements of research from DoReMi, feedback from the consultation processes and the progressive unrolling of the SRA targets.

Revision should include a statement of achievements in terms of scientific ground covered, the establishment of multidisciplinary and multinational teams and the development of infrastructures. MELODI should organise on a permanent basis a team tasked with this review process and the team should make input, initially yearly, to the MELODI workshops. The SRA is as largely as possible based on scientific consensus from these workshops and scientific consultation including the MELODI and DoReMi Websites.

The Scientific Advisory Committee should review and approve successive revisions of the SRA and pass them to the Governing Board for formal endorsement. At this point it may be circulated to all stakeholders and organisations as a support to the continued integration of national R&D programmes and to the formulation of R&D calls.

Revision should be made at least every year taking into account most recent developments and the input of specific working groups. The timing and frequency of the revision needs to be well adapted to the main stream of budgetary procedures, including the Euratom call process.

3.3. ROADMAP

The discussions on the first draft of the MELODI SRA have been further substantiated in the 2nd MELODI Workshop October 18-20, 2010 in Paris , in the 3rd MELODI workshop November 2-4, Rome (see workshop summary by K. Prise and L. Sabatier) and the 4th MELODI Workshop, 11-14 September 2012, Helsinki (see workshop summary by Sisko Salomaa et al. 2013). In spite of that, it seems still premature to outline already ROADMAP for MELODI at this stage more detailed than that presented by HLEG in January 2009. Part of the projected low dose programme is realised by the DoReMi TRA covering the next years up to 2016 and involves important scientific feasibility studies, putting also into place important aspects of essential infrastructures and new approaches to education and training. From the above SRA it is clear that intrinsically, most prioritized research items will have to follow a more or less preset time scale. Thus, it is evident that the search for suitable biomarkers for defined radiation exposures (internal or external), predictions for sensitivity, the initiation of pathological pathways (biomarkers for pre-pathological states) and for final

pathological outcomes will come first in the research on radiation biology networking. Some of these biomarkers are expected to stimulate molecular epidemiological studies and the establishment of suitable prospective or retrospective cohorts (i.e., prospective cohort of CT scans in children, induction of secondary cancers in out-of-radiotherapy-field sites). This research work will be accompanied by fundamental and mechanistic studies on the specific low dose and low dose-rate radiation effects together with their relationship to perturbation of cellular and tissue homeostasis and the induction of cancers and non-cancers. This fully integrated research will highly rely on the input from non-radiobiological research disciplines such as toxicology, immunology, inflammatory research, physiology, pathology, genetics, epigenetics, cardiology, ophthalmology, neurology etc. (see SRA). It is expected that in the long term a systems biology approach together with well defined epidemiological studies will allow mathematical modelling and the evaluation of low dose health risks.

Taking into account feedback from the MELODI GB, the outcomes of the 2nd, 3rd and 4th MELODI workshop and the input of the MELODI SC, the scientific community and the stakeholders the MELODI Roadmap should then (June 2013) give the forseeable timing of the different research lines as well as a possible financial sustainability programme.

3.4. Major considerations

MELODI needs to promote and ensure:

- multidisciplinary integrated low dose research in Europe
- positive efforts to bring in 'new blood'. These comprise specialists with skills in research areas that previously have not been associated with ionising radiation
- sustainability of infrastructures, education and training
- permanent interaction and communication with stakeholders and the public.

Evidently, the SRA has to be adapted together with the SRA of NEIRIS and ALLIANCE to the modalities of the OPERRA project in the framework of Horizon 2020.

3.5. Consultation

A working group of experts constituted by MELODI will list important domains of low dose research, prioritize scientific questions relevant for low dose radiation risk research and assess the corresponding research needs in the light of present EU funded research and other international programmes. In order to promote a high degree of multidisciplinary integration, covering as wide a range of scientific areas as possible, a large consultation is foreseen of the general scientific community through specific MELODI (and also DoReMi) workshops and expert group meetings (seminars). E-mail contacts, the DoReMi and MELODI Web sites, contacts to the members of the MELODI Scientific Committee and to experts in complementary fields (not yet necessarily involved in radiation research) should be explored.

In fact, DoReMi and MELODi workshops open to a large scientific community should be held to attract new scientific competences, new players and provide a driving force in the field of radiation research and radiation protection. Additionally, links to other relevant European research projects (see ALLIANCE, NEIRIS, EURADOS, STAR, TRIREME, EPI-CT, EPI-RadBio, SOLO, ANDANTE, MULTIBIODOSE, RENIB, BOOSTER, PROCARDIO, CEREBRAD, DARK RISK, RIS-IR and others) will be sought. If necessary, specific working groups will be created to develop strategies to deal with very specific research items and issues (for example, new technological and ethics problems).

For the selection of actual research needs as well as topics for future scientific calls a hierarchy will be established based on priorities and consideration of presently funded projects to pre-defined criteria including relevance, feasibility, sustainability, expected outcomes etc.

A regular survey and mapping of national scientific research (as well as education and training activities) launched by MELODI will be required to identify original and novel research lines relevant to low dose research but as yet not realised. Further, the availability and sustainability of suitable infrastructures, education and training as well as modes of interaction and communication with stakeholders and the public will need to be developed.

Thus, it is recommended that there should be:

- Fully interdisciplinary working groups held to develop, refresh and update based on on-going research and re-appraise the research areas and priorities
- Discussion forums set up to attract fundamental scientists
  (from radiation physics, medical physics, dosimetry, biophysics, radiation chemistry, toxicology, imaging, physiology, immunology, cancer research, DNA repair, genetics, oxidative stress, epigenetics, molecular signalling, developmental research, nanotechnology, inflammatory and immunological research, 'omics', protein research, miRNAs, systems biology, medicine).
- A series of MELODI sponsored mixed forums-conferences-seminars-colloquia should be held on e.g.
  - molecular intra- versus extracellular signalling/ immunologica responses
  - cellular damage/ epigenetics
  - nanotechnology/toxicology/internal emitters
  - 'omics' and systems biology
  - stem cell research/ cancer/non-cancer
  - infrastructures, radiation facilities, omic centres, animal research
  - the establishment of cohorts and their dosimetry
  - training & education, degree courses, regulators, researchers etc

3.6. Establishment of the Scientific Committee (SAC)

The MELODI Governing Board established in 2012 a Scientific Committee (SC) essentially composed of experts with well-founded reputations embracing a wide range of disciplines and competences. These cover both the existing branches of radiation biomedical sciences and the new research areas identified and presented in this SRA as important for radioprotection and radiobiology projects and thus most attractive for the MELODI programme.

In order to ensure regular updating and some continuity in the work on the SRA, some members of the present MELODI SRA working group may also be active members of the Scientific Committee. Of course, the MELODI Governing Board formally invited and appointed the SC members who do not represent their specific institution or country, but are serving the cause of MELODI as individual experts.

4. EXECUTIVE SUMMARY

In line with its main objectives, MELODI proposes this long term strategic research agenda to guide the coherent integration of national low dose R&D programmes, and to facilitate the process of preparing EURATOM calls in this field. The SRA has been built on the initial considerations of the HLEG that formulated the overarching questions on specified key issues. The SRA supplements the transitional research agenda (TRA) composed within the DoReMi network of excellence that considers shorter term research needs and priorities up to 2016. The present SRA develops this further in an as widely as possible consensual manner to structure and establish the operational procedures spanning the long term commitment (>20y) of MELODI to low dose radiation research and radiation protection in Europe together with ALLIANCE and NEIRIS in the framework of the wide ranging european project OPERRA in line with HORIZON2020.

The MELODI SRA thus attempts to define the broader concepts and directions for radiation research and radiation protection (for the forthcoming 20 years and or so) taking account of the needs of national and international stakeholders and, very importantly, the public perceptions and anxieties concerning low dose irradiation.

As a general scheme one may consider radiation-induced events at the level of cells or the whole organism as follows: Energy deposition by different types of radiation in living systems will create perturbations in homeostatic equilibrium (metabolism) as well as reversible or irreversible damage (structural changes) which may be detectable at the molecular level by sensitive physical, chemical and biological methods. Cells and tissues react very sensitively to all types of stresses (including ionising radiation), register the damage induced and initiate multiple intra-and intercellular signalling pathways to establish cellular and tissular defences to either cope with the damage or to eliminate the damaged cells and tissues. In order to relate these reactions to irreversible metabolic or to irreversible pathological reactions It is necessary to identify specific biomarkers linked to radiation exposure as well as to normal, pre-pathological and pathological states. Knowledge of the pathways involved should allow to assess these states by mathematical modelling and by a systems biology approach. In other words, Also, at this level of investigation, it should be possible to identify specific biomarkers that can predict or are precursors of pathological developments towards defined diseases. The overall scientific challenges will be to define the borderline between normal metabolism, normal physiological responses and a disease-prone perturbed metabolism being a precondition of pathology that may be induced by low dose ionising radiation.

Additionally there is a high priority for research aimed at being able to define individual radiosensitivity and, for this, reliable markers and accurate patient specific dosimetry are essential. For health risk assessments, the most important question will be which type of radiation exposure, radiation dose and dose-rate will give rise to a pathological outcome such as cancer and non-cancer effects in the short or long term.

Priorities that need to be addressed concern fundamental mechanistic research ranging from radiation track structure and the deposition of energy in biologically important molecules; the resultant perturbations of homeostasis and the steps in the cellular and tissue metabolic pathways that eventually lead to disease pathologies.

In fact, the main priorities are here the step-wise elucidation of the mechanisms of radiation-induced (oxidative) stress responses and their impact on radiation-induced cancers and non-cancer

effects (diseases). To achieve this a holistic approach is proposed starting with radiation-specific effects, radiation-induced molecular, biological and pathological effects involving a systems biology approach as well as molecular epidemiology and mathematical modelling in order to come up with more solid low-dose health risk assessments. To ascertain low doses and low dose rate health risks combined mechanistic and epidemiological studies appear to be the most promising. The pathologies considered are outlined in this report where the need is stressed for the MELODI platform to involve a constellation of classical and emerging technologies in a highly multidisciplinary approach. Improved dosimetry will be needed to achieve a lower uncertainty on the dose-effect relationships. Elucidating the shapes of low-dose response relationships, resolving the question of thresholds and establishment of dosimetric tools in individuals (also as part of a cohort) is paramount to resolving questions of risk for both populations and individuals. Much is known about radiation-induced cancer in humans and animal models but this needs to be pursued particularly at low doses. More recently, the scientific community has realised that low radiation-induced health effects range well beyond cancer. Thus, also priority non-cancer areas such as cardiovascular, ophthalmic and neurological need to be brought into focus. The SRA notes that this will require input from disciplines, clinicians and scientists where there has been little or no prior involvement in radiation research and from radiology departments having large database of medical exposures, where new dosimetry tools could estimate the required organ doses with a sufficient accuracy.

This SRA represents a point fairly early on in the MELODI programme, indeed prior to the complete research outcomes from the DoReMi network. The programme is steadily evolving; some lines of enquiry emerge showing great promise whilst it becomes apparent that others are less likely to yield answers to the key issues. Thus, it is essential to keep the SRA under permanent review and periodic reassessment and revision. Revision is necessary to take account (as much as possible) not only of specific research achievements but also the feedback from a wide ranging consultation with the scientific community and in particular from the MELODI workshops and seminars, and the Scientific Committee as well as with stakeholders and the public.

The SRA will play an important role in tracking most relevant and recent scientific developments and defining the joint research strategies on radiation research and radiation protection in Europe in line with HORIZON 2020.

5. REFERENCES

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6. Annexes

6.1 Annex 1: Questions identified as being key issues for MELODI

These are grouped according to the three key issues specified by the HLEG and have been collated from the HLEG report and discussions at the MELODI workshops in Stuttgart, September 2009 and in Paris 2010 and responses to the first draft of the SRA discussion document received after the 2nd International MELODI workshop in Paris.

1. Shape of the dose-response curves for cancer
- Mechanisms
  - What is the dependence on track structure, spatial distribution of energy deposition events? – interplay between spectrum of damage induced and its repairability in modulating the shape of the dose response curve.
  - What is the dependence on dose rate and LET?
  - What are the molecular biomarkers that can be validated and used in molecular epidemiology to define pathological impact and disease? –cancer and non-cancer effects

- Are molecular biomarkers available or may suitable biomarkers be developed for radiation-induced carcinogenesis (molecular signatures) in animals and humans and as biological dosimetry of human exposure?
- What is the implication of irradiation of stem cells in carcinogenesis?
- Can the processes underlying radiation-induced carcinogenesis be modelled for different types of cancers?
- To what extent does the sensitivity to cancer induction differ for exposures during various developmental stages (e.g. *in utero*, young children, adults)?
- What is the effect of radiation quality and the sensitivity for different tissues for radiation-induced carcinogenesis and disease?
- What are the mechanisms underlying the appearance of secondary cancers or out-of field low dose radiation effects in humans?
- Can good animal models be developed to analyse radiation-induced cancers other than acute myeloid leukaemia or are good animal models available?
- What is the impact of non-targeted effects on radiation-induced carcinogenesis? - cellular signalling at low dose and low dose rate; adaptive responses to radiation
- What is the relationship between oxidative stress, DNA damage complexity, chromosomal damage, translocation, DNA damage signalling, perturbed cell cycle regulation, senescence, apoptosis and the induction of cancer (and non cancer effects) by radiation?
- What is the role of epigenetic effects including chromatin remodelling on health effects induced by radiations of different quality?
- What is the impact of immunological status (systemic factors) on radiation-induced pathological responses (inflammation, cancer, non-cancer)?

- Dosimetry
  - How can the information on dosimetry and biokinetics of internal emitters be improved to understand radiation-induced short and long term effects?
  - What are the most important radionuclides to focus on (scoping of internal emitter studies) to gain better understanding of their short term radiotoxicity and long term effects (cancer and other pathologies)?

- Omics and systems biology
  - How can research based on 'omics' contribute to a systems biology approach to processes underlying radiation-induced carcinogenesis and non cancer effects? - Involvement in homeostasis?
  - How can 'omic' approaches enhance our understanding of the effects of radionuclides?

- Epidemiology
  - What are the cohorts that can be used for molecular epidemiological approaches to understand low dose radiation effects (cancer, non cancer)?
  - Is it possible to launch an epidemiological study on low dose induced second cancers?
  - Is it possible to launch an epidemiological study on out-of field low dose radiation effects in humans?
  - Can existing biobanks (STORE, GENEPI) be used in molecular epidemiological studies?
  - Can specific epidemiological studies be conducted to reveal and analyse specific radiation responsive cancer prone tissues?
  - Can feasibility studies be performed on non-cancer effects (cardiovascular, lens opacities, neurological effects)?

2. Non-cancer effects

The system of radiological protection is mainly based on excess risk of cancer induced by ionizing radiation. The main data on stochastic effects have been derived from situations with a very short exposure at a high dose rate, like Hiroshima and Nagasaki populations. Much less information is available on effects of internal exposures or long term consequences on non-cancer effects such as cardiovascular dysfunction, neurological alterations, lens opacities, or effects on other physiological functions.

In order to tackle these important asoects there is an urgent need for multidisciplinary approaches: cardiology, neurological aspects, toxicology, dosimetry, radioecology, embryology, bioinformatics and biomathematics, pharmacokinetics...

- Mechanisms
  - What are the mechanisms involved in radiation-induced lens opacities?
  - What are the mechanisms involved in radiation-induced cardiovascular effects?
  - What are the mechanisms involved in radiation-induced effects on the central nervous system (neurogenesis) and behavioural changes?
  - Could neurodegerative diseases be accelerated by low dose ionising radiation? (G. Obe, Rome)
  - Are these mechanisms consistent with stochastic or deterministic dose responses?
  - What are the mechanisms involved in radiation-induced effects on the digestive system?
  - What are the mechanisms involved in radiation-induced effects on reproduction and trans-generational effects?
  - What are the mechanisms involved in radiation-induced effects on the immune system (inflammation, immunodeficiency)?
  - How can systemic effects be distinguished from organ specific effects?
  - What is the impact of non-targeted effects?
  - What is the impact of radiation quality, dose and dose rate, acute and chronic exposure?
  - What are the age, gender, population and temporal effects?
  - What is the possible impact of synergistic and interactive effects with other agents?

Concerning the mechanisms involved in tissue responses it has to be noted that for many years great effort has been focused on cell level responses to radiation (e.g. study of DNA mechanisms) which have contributed to an understanding of low dose effects and individual radiosensitivity. However, this has not greatly increased our understanding of low dose responses that may involve other processes than repair. Certainly, non-targeted effects are to a large extent independent of repair processes, and tissue or system level responses such as the development of cardiovascular effects almost certainly seem less dependent on DNA damage and repair than on changes in cell-to-cell microenvironment and intra-and intercellular signalling. To better understand tissue responses, the key questions are:

- To what extent are in vitro experiments on single cell types relevant in predicting responses of more complex tissues and organs to low doses, e.g. are the biological responses (radiation sensitivities) uniform amongst different cell types and between tissues?
- Is there a long term adverse tissue response at low doses in tissues other than cardiovascular/cerebrovascular tissue and lens (bone, brain etc.)?
- Are the risks of impairment of system level response adequately known at low doses (e.g. immune competence, cognitive ability, reproductive capacity, osteogenic regeneration?

– Does the known genetic predisposition to cancer risk extend to non cancer risk of tissue level responses at low doses? If so, which biological pathways are influenced by these genetic factors?

- Epidemiology
  - Do confounding factors: diet, smoking and many other lifestyle factors, plus genetic and epigenetic factors, multi-stress exposures contribute to non-cancer effects?
  - What are the main non cancer diseases to be considered after low dose radiation exposure?

Are there suitable cohorts available? (out-of-field exposures in radiation therapy, CT scans, nuclear medicine patients, interventional cardiologists, dentists, staff preparing radiopharmaceuticals (PET imaging), workers exposed to alpha emitters (Mayak), uranium miners and others (fluorspar), aircrews).

3. *Individual radiation sensitivity*

An "overriding priority" is for the research to include ethical considerations.

- Mechanisms
  - What is the evidence that individual sensitivity plays a significant role towards cancer and non-cancer pathologies through modulating radiation response to exposures at low dose and dose rates? - Links to cancer predisposition.
  - Are there genetic and/or epigenetic modifiers/biomarkers available that allow determination (monitor, predict) of individual sensitivity to radiation, cancer and disease development?
  - Which mammalian and non-mammalian systems should be able to validate candidate biomarkers related to individual radiation sensitivity?
  - To what extent are inflammatory and immunological factors involved in individual radiation responses?
  - To what extent do non-targeted radiation responses differ in different individuals?
  - What are the factors involved in individual sensitivity and dependent on genetic background, age, gender and lifestyle?
  - Can a multilevel approach using cells in culture, tissue cultures, non mammalian and mammalian models help to analyse individual sensitivity?
  - Can an 'omics' approach help to elucidate individual sensitivity and be used to develop a systems biology approach?
  - Can omics help to define tissue weighing factors?
  - How do stem cell and progenitor cell biology contribute to individual radiation sensitivity and tissue responses?
  - Do genetic or epigenetic modifiers of radiation responses affect individual radiation responses similarly at low and high LET radiation?
  - Is individual radiation sensitivity dose rate dependent?
  - Can risk assessments for individuals be developed on the basis of molecular indicators for cancer and disease? – leading to genetic profiling of individuals?
  - Are mechanisms and factors governing cancer susceptibility independent of dose rate and radiation quality, or are there differences in the degree to which risk modifiers contribute to individual risk at different dose rates and radiation qualities?
  - Can the magnitude of individual sensitivities be quantitatively assessed and compared?

- Can non-mammalian and animal models contribute to a better understanding of the mechanisms involved in individual sensitivity?
- **Epidemiology**
  - Are there cohorts available or which can be set up that allow establishment of direct links between molecular experimental studies and epidemiological studies ("molecular epidemiology") on individual sensitivity?
  - Are there cohorts available or may be set up to allow modelling of individual sensitivity responses?
  - Can populations at risk be identified and distinguished by biological markers?
  - Can realistic cohorts be designed including low dose exposures and protracted exposure scenarios (medical imaging cohorts, nuclear workers, flight crews) that allow detection of individual sensitivity by available biomarkers?
  - Can biomarkers, gene markers and phenotypic traits indicate specific radiation risks in human individuals? -Ethical problems to be considered

MELODI Statement

(18 November 2010)

on a

Short- to medium-term research agenda for R&T projects to improve
the scientific basis for radiation protection in Europe

The following statement of the MELODI governing board provides information on re- search priorities, which are currently being refined into a first edition of the Strategic Research Agenda of MELODI, elaborated on the basis of the concepts developed by HLEG, of the transitional research agenda of the Network of Excellence DoReMi, of the proceedings of the 1st MELODI workshop (28-29 September 2009, Stuttgart), and of the 2nd MELODI workshop (18-20 October 2010, Paris).

The research calls by EURATOM for R&T projects (Research and Training) have
been led by the policy goals to improve radiation protection standards in Europe, and to prioritise and focus European research efforts to achieve maximal impact on scientific knowledge and avoid duplication of research, with a view to consolidating the current scientific basis for the system of radiation protection, as recommended by ICRP and defined in the Basic Safety Standards. These goals are fully supported by MELODI.

The present radiation protection system is mainly based on scientific knowledge from epidemiological studies, which have played an important role in assessing the magnitude of radiation risk in the dose range down to about 100 mSv. Epidemiological studies continue to contribute to low dose risk research and particularly on risks of low dose rate chronic exposures, risks from internal emitters and non-cancer risks.

However, further refinement of low dose risk estimates will necessitate the close association of epidemiological with experimental mechanistic studies. For example, by using suitable molecular and cellular biomarkers, the value of future epidemiological studies (molecular epidemiology) for radiation risk research is expected to be maximised. To achieve maximal value, robust and reliable biomarkers are required for exposure, for effects on the cellular and tissue level and, most importantly, for radiation-associated diseases. The research should always include a realistic assessment of the associated uncertainties.

Experimental evidence suggests that both effects in target cell and effects and responses in the tissue environment (microenvironment) contribute to radiation-induced disease. The relative importance of these effects for different radiation qualities, at different dose levels and exposure conditions (acute, chronic, fractionated) in relation to different diseases is currently not sufficiently understood. Experimental work can best contribute by seeking dose levels where disease-associated effects are or are not observed, either in target cells, in the tissue environment or in the interacting system of both (tissue, organ or organism).

The experimental and epidemiological studies require a commitment to collect and sustainable archiving of biological materials and data.

Mathematical and computational modelling of experimental data will allow a better understanding of radiation track structure and mechanisms of radiation effects at the level of the DNA, other intracellular targets, at the level of target cells and the tissue environment. These modelling efforts together with those using animal and epidemiological data including systems biology

approaches will provide further insights into biological effectiveness effects of radiation quality. According to MELODI, priorities for forthcoming and long-term future research should take into account the need to investigate effects of ionising radiation of different qualities on radiation-induced cancer and non-cancer diseases as well as on individual variation of radiation risks. All efforts should include a careful dosimetric approach.

The long-term priorities include the following areas:

(1) for radiation-induced cancers and non cancer effects

- Identification, establishment and continued follow-up of suitable cohorts of radiation exposed people for epidemiological studies related to cancer and non-cancer effects.
- Identification, development and validation of biomarkers for radiation expo sure, effects and disease.
- Continuing development of suitable whole animal as well as human cellular models (including somatic stem cells) for radiation carcinogenesis and non- cancer diseases which bear clear relationships to human diseases.

(2) for radiation-induced cancer

- Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to carcinogenesis, This involves the under- standing of the relationship between early and late effects, targeted and non- targeted effects as well as the role of delayed genetic instability.
- Identification of the nature and number of target cells at risk for a specific cancer in humans.

(3) for radiation induced non cancer effects

- Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to cardio-vascular effects.
- Identification of the nature of target cells at risk for specific non-cancer effects in humans.
- Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to cerebro-vascular disease and cognitive function.

(4) for individual and general health and radiation protection issues

- Understanding the impact of inter-individual variation of radiation risks in relation to cancer and non-cancer effects, and how this might impact on dose response relationships in populations.
- Clarification of the contribution of radiation effects in target cells as well as radiation effects and responses in the tissue environment and interaction between both target cell and tissue environment at different dose levels to the development of radiation-associated diseases.
- Examination of the impact of low dose and low dose rate radiation effects on immune function.
- Understanding of the effect of age-at-exposure on radiation risk.
- Better understanding of the risks of internal emitters following internal contamination with radionuclides.

MELODI is currently in the process of structuring all these priorities within a Strategic Research Agenda (SRA). In view of the most recent developments, MELODI recommends that short- to medium-term priorities (funding period 2011/2012) should be given to:

➢ Quantification of the role of ionising radiation in cardio-vascular and cerebrovascular effects after low dose (< 500 mSv) irradiation.

➢ Development of suitable biomarkers for exposure (immediate post radiation as well as long term after exposure), for cellular and tissue effects and for radiation associated leukaemia, solid cancers and non-cancer effects. The biomarkers should be usable for molecular epidemiological studies of cancer risk below a cumulative dose of 100 mSv and for non-cancer risk studies below 500 mSv, respectively.

➢ Clarification of the role of effects in target cells and in the tissue environment in a dose range with clear focus on low doses. This includes the development of suitable tissue, organ and animal models for the identification of tar- get cells and the interaction between target cells and tissue environment as well as the utilisation of stem cell approaches.

➢ Identification and analysis of suitable epidemiological cohorts if available with archived biomaterial to improve low dose radiation risk assessment by reducing uncertainties especially for the age- and gender-dependency of radiation risk and including those uncertainties contributed by exposure assessment. These may include cohorts exposed to internal contaminations.

MELODI Statement
(October 2011)
on a

Short- to medium-term research agenda for R&D projects to improve
the scientific basis for radiation protection in Europe

The following statement of the MELODI governing board provides information on research priorities, which are currently being refined into an extended Strategic Research Agenda of MELODI, elaborated on the basis of the concepts developed by HLEG, of the transitional research agenda of the Network of Excellence DoReMi, of the proceedings of the 1st MELODI workshop (28-29 September 2009, Stuttgart), and of the 2nd MELODI workshop (18-20 October 2010, Paris). This executive statement of the MELODI governing board updates and consolidates the MELODI statement from November 2010.

The research calls by EURATOM for R&T projects have been led by the policy goals to improve radiation protection standards in Europe, and to prioritise and focus European research efforts to achieve maximal impact on scientific knowledge and avoid duplication of research, with a view to consolidating the current scientific basis for the system of radiation protection, as recommended by ICRP and defined in the Basic Safety Standards. These goals are fully supported by MELODI.

The Fukushima nuclear accident has highlighted the necessity to consolidate the scientific knowledge on radiation risk at low doses in a way that emergency response measures especially for the general public in directly effected regions and beyond can be based on the best scientific evidence that conveys to the public the validity of measures taken in order to simultaneously protect public health and ensure the economic and societal continuity in the country. K.

MELODI recommends the introduction of research projects specifically addressing the situation in Fukushima where Europe could join Japanese efforts to develop suitable cohorts for the purpose of long term research. This could be of interest for cancer, non-cancer, and may be other projects. Apart from demonstrating the solidarity with the Japanese people, this could be a further incentive for European R&D teams to join efforts with Japanese teams.

The present radiation protection system is mainly based on scientific knowledge from epidemiological studies, which have played an important role in assessing the magnitude of radiation risk in the dose range down to about 100 mSv. Epidemiological studies continue to contribute to low dose risk research and particularly assessments of risks from low dose rate chronic exposures and from internal emitters, and non-cancer risks. However, further refinement of low dose risk estimates will necessitate the close association of epidemiological with experimental mechanistic studies. For example, by using suitable molecular and cellular biomarkers, the value of future epidemiological studies (molecular epidemiology) for radiation risk research is expected to be maximised. To achieve maximal value, validated and reliable biomarkers are required for exposure, for effects on the cellular and tissue level, individual radiation sensitivity and, most importantly, for in-situ stages of radiation-associated diseases. Any research should include a realistic assessment of the associated uncertainties.

Experimental evidence suggests that both effects in target cells and effects and responses in the tissue environment (microenvironment) contribute to radiation-induced disease. The relative

importance of these effects for different radiation qualities, at different dose levels and exposure conditions (acute, chronic, fractionated) in relation to different diseases is currently not sufficiently understood. Experimental work can best contribute by seeking dose levels where disease-associated effects are or are not observed, either in target cells, in the tissue environment or in the interacting system of both (tissue, organ or organism).

The experimental and epidemiological studies require a commitment to collect and sustain archiving of biological materials and data.

Mathematical and computational modelling of experimental data will allow a better understanding of radiation track structure and mechanisms of radiation effects at the level of the DNA, other intracellular targets, at the level of target cells and the tissue environment. In this context, MELODI recommends to include the development of omics-based analysis capabilities in a scope of future multidisciplinary research projects. The modelling efforts together with those using animal and epidemiological data including systems biology approaches will provide further insights into radiation quality effects on biological effectiveness.

According to MELODI, priorities for forthcoming and long-term future research should take into account the need to investigate effects of ionising radiation of different qualities on radiation-induced cancer and non-cancer effects as well as on individual variation of radiation risks. All efforts should include a careful dosimetric approach. The long-term priorities include the following areas:

(1) for radiation-induced cancers and non cancer diseases

       Identification, establishment and continued follow-up of suitable cohorts of radiation exposed people for epidemiological studies related to cancer and non-cancer effects

       Identification, development and validation of biomarkers for radiation exposure, effects and disease.

       Continuing development of suitable whole animal as well as human cellular models (including somatic stem cells) for radiation carcinogenesis and non-cancer effects which bear clear relationships to human diseases.

(2) for radiation-induced cancer

       Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to carcinogenesis. This involves the understanding of the relationship between early and late effects, targeted and non-targeted effects as well as the role of delayed genetic instability.

       Identification of the nature and number of target cells at risk for a specific cancer in humans.

(3) for radiation induced non cancer diseases

       Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to non cancer effects such as cardio- and cerebro-vascular disease, cataract of the eye and impaired cognitive function.

       Identification of the nature of target cells at risk for specific non-cancer effects in humans.

(4) for individual and general health and radiation protection issues

       Understanding the impact of inter-individual variation of radiation risks in relation to cancer and non-cancer effects, and how this might impact on dose response relationships in populations.

       Clarification of the contribution of radiation effects in target cells as well as radiation effects and responses in the tissue environment and interaction between both target cell

and tissue environment at different dose levels to the development of radiation-associated diseases.

Examination of the impact of low dose and low dose rate radiation effects on immune function.

Understanding of the effect of age-at-exposure on radiation risk.

Better understanding of the risks of internal emitters following internal contamination with radionuclides.

As an ongoing process MELODI develops and periodically updates the long-term Strategic Research Agenda (SRA) and makes use of the yearly executive statements to recommend short- to medium-term priorities (funding period 2012/2013). In the years to come priority should be given to:

Quantification of the role of ionising radiation in the development of non cancer disease such as cardio- and cerebro-vascular effects, cataract of the eye and impaired cognitive function after low dose (< 500 mSv) irradiation.

Development of suitable biomarkers for exposure, cellular and tissue effects as well as for in situ stages of diseases. The biomarkers should be usable for molecular epidemiological studies.

Clarification of the role of effects in target cells and in the tissue environment in a dose range with clear focus on low doses. This may include in vivo detection systems as well as suitable in vitro systems.

Identification and analysis of suitable epidemiological cohorts if available with archived biomaterial to improve low dose radiation risk assessment by reducing uncertainties especially for the age- and gender-dependency of radiation risk and including those uncertainties contributed by exposure assessment. Suitable data and biomaterial banks should be established with a clear focus on the possibilities to share data, material and knowledge within the scientific and wider radioprotection community.

Development of guidelines for short, medium and long-term exposure and health risk monitoring in case of a major nuclear accident in Europe. This is to ensure the public that all possible scientific efforts are undertaken to record, document and analyse exposed populations in the scientifically best available way to generate information about health consequences from possible accidents in future in a comprehensive manner.

6.4 Annex 4

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MELODI Statement*

(March 2013)

on a

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Short- to medium-term research agenda for (I) R&D projects to improve the scientific basis for radiation protection in Europe and (II) on the building of an integrated research platform for the promotion and administration of research in radiation protection in Europe in the wider context of the Horizon 2020 initiative of the European Commission

MELODI is an European Platform dedicated to low dose radiation risk research. In April 2009 five partners (BfS, CEA, IRSN, ISS and STUK) previously involved in the High Level Expert Group (HLEG) signed a Letter of Intent, in which they jointly marked their intention to progressively integrate their R&T programmes in low dose radiation risk research with similar R&T programmes funded by the European commission. MELODI intends to built a platform for radiation protection research in EUROPE to promote the idea of joint programming and integrated funding for R&T. A key point of the evolving platform is the openness of MELODI to all national R&T organisations and regulatory bodies as well as stakeholders in Europe interested, willing and capable to sustainably engage in such an integration process. Based on this intention and under guidance of the initial signatories MELODI was founded in 2010 as a registered association with 15 members. The purpose of MELODI is:

- to develop a strategic research agenda (SRA) and to propose R&T priorities for Europe in its field of competence
- to seek the views of stakeholders on the priorities for research, keep them informed on progress made, and to contribute to the dissemination of knowledge.
- to interface with international partners like WHO and IAEA.
- to organize open MELODI scientific workshops.

Up to now, the MELODI workshops are a driving force for MELODI to review the state of knowledge on low dose radiation effects at the international level, with the focus on identifying open questions and uncertainties relevant to radiation protection. The workshops are used to develop and continuously refine the SRA for low dose research in Europe and roadmaps for the key research fields, to scope the research area and to prioritise topics of immediate and medium term action for each of the key research fields. As an immediate output of the workshops MELODI summarizes in yearly statements what has been achieved and in which direction the field of low dose research should evolve. The recommendations of the MELODI statements were used and integrated in the launch of EU-calls for research proposals in prioritised topics.

Until end of 2012 MELODI has a total of 22 members and organised 4 open workshops, has developed a SRA and updated it on a yearly bases und published yearly statements on research priorities in the field of low dose radiation research.

The following statement of the MELODI governing board provides information on research priorities, which are currently being refined into an extended Strategic Research Agenda of MELODI, elaborated on the basis of the concepts developed by HLEG, of the transitional research agenda of the Network of Excellence DoReMi and of the proceedings of the annual workshops (1st MELODI workshop, 28-29 September 2009, Stuttgart; 2nd MELODI workshop, 18-20 October 2010;

3rd MELODI workshop, 2-3 November 2011 Rome; 4th MELODI workshop, 11-14 September 2012, Helsinki). This executive statement of the MELODI governing board updates and consolidates the MELODI statement from October 2011.

The research calls by EURATOM for R&T projects have been led by the policy goals to improve radiation protection standards in Europe, and to prioritise and focus European research efforts to achieve maximal impact on scientific knowledge and avoid duplication of research, with a view to consolidate the current scientific basis for the system of radiation protection, as recommended by ICRP and defined in the Basic Safety Standards. These goals are fully supported by MELODI.

With the Horizon 2020 initiative, the European Commission will disband the traditional framework programmes, and will introduce new management modes for research and training within EURATOM. While the administrative processes will be reorganised, radiation protection will remain a key EURATOM objective. Joint and/or coordinated research activities, in particular in the fields of effects and risks from low doses from industrial, medical or environmental exposures, of radioecology and of radiological and nuclear emergency preparedness will be further supported by EURATOM to provide a pan-European basis for a robust, equitable and socially acceptable system of radiation protection.

In this regard, MELODI together with ALLIANCE and NERIS will act as a focal point to promote research in the field of radiation protection in Europe and its member states. It is in the interest of the scientific community to ensure cost-efficiency in managing future research and training programmes and to foster high-performance research in radiation protection in Europe. This implies the promotion of interdisciplinary collaboration by attracting scientists from other disciplines and research areas to the field of radiation protection and the development of innovative mechanisms for 'joint programming' within the wider field of radiation protection and between national and European research and training programmes.

MELODI will prepare, in close collaboration with ALLIANCE, NERIS and others the implementation of the radiation protection part of the EURATOM research and training programme. To achieve this goal, a new umbrella organisation will have to be set up, with appropriate governance structures, statutes and procedures. In order to achieve this goal, MELODI itself is also strengthening its governance structures, statutes and procedures. The ongoing development of the Strategic Research Agenda (SRA) in the field of low dose research in parallel with similar activities in radioecology and nuclear emergency preparedness, the implementation of structures and procedures for priority settings and the establishment of a strong, cost –efficient management structure will lay the foundation for future research and training programmes in radiation protection in Europe.

The Fukushima nuclear accident has highlighted again the necessity to consolidate the scientific knowledge on radiation risk at low doses in a way that emergency response measures especially for the general public in directly affected regions and beyond can be based on the best scientific evidence that conveys to the public the validity of measures taken in order to simultaneously protect public health and the environment, and ensure the economic and societal continuity in the country.

MELODI recommends the introduction of research projects specifically addressing the long lasting situations in Fukushima and Chernobyl where Europe could join national and international efforts to develop suitable cohorts for the purpose of long term research. This could be of interest for cancer and non-cancer somatic effects as well as for other health effects, in particular of impaired

cognitive function. Apart from demonstrating the solidarity with the people affected by Fukushima and Chernobyl, this could be a further incentive for European R&D teams to join efforts with teams from affected countries.

European R&D activities in this research area have to be backed up by complementary organisational dispositions ensuring maintenance and development of competences by adequate education& training activities and access to suitable infrastructures, i.e. irradiation facilities, epidemiological cohorts, data-and biobanks and analysis platforms

The present radiation protection system is mainly based on scientific knowledge from epidemiological studies, which have played an important role in assessing the magnitude of radiation risk in the dose range down to about 100 mSv. Low dose radiation risk may involve natural radiation exposures as well as industrial and medical exposures (including therapeutic out of field exposures a well as diagnostic exposures)

Conjoint epidemiological and mechanistic studies are proposed to further improve evaluation of radiation risks and overall radiation protection. Epidemiological studies continue to contribute to low dose risk research and particularly assessments of risks from low dose rate chronic exposures, risks from internal emitters and non-cancer risks. However, further refinement of low dose risk estimates will necessitate the close association of epidemiological with experimental mechanistic studies. For example, by using suitable molecular and cellular biomarkers, the value of future epidemiological studies (molecular epidemiology) for radiation risk research is expected to be maximised for prognosis in accidental situations as well as in diagnosis in medical exposures. To achieve maximal value, validated and reliable biomarkers are required for exposure, for effects on the cellular and tissue level, individual radiation sensitivity and, most importantly, for different consecutive stages in the development (in-situ stages) of radiation-associated diseases. Any research should include a realistic assessment of the associated uncertainties.

Experimental evidence suggests that both effects in target cells and effects and responses in the tissue environment (microenvironment) contribute to radiation-induced disease. The relative importance of these effects for different radiation qualities, at different dose levels and exposure conditions (acute, chronic, fractionated) in relation to different diseases is currently not sufficiently understood. Experimental work can best contribute by seeking dose levels where disease-associated effects are or are not observed, either in target cells, in the tissue environment or in the interacting system of both (tissue, organ or organism). Recent findings indicate that radiation effects do not only include potent genetic but also epigenetic and immunological control mechanisms that are of particular importance for cell and tissue specific effects, individual radiation sensitivity and trans-generational responses. The role of tissue and cancer specific stem cells in radiation responses needs to be clarified as well. Careful analysis of cellular and tissular regulation pathways should allow to apply systems biology approaches for risk evaluation purposes.

The experimental and epidemiological studies require a commitment to collect and to sustainably archive biological materials and data data and to assure their actual utilization..

Mathematical and computational modelling of experimental data will allow a better understanding of the radiation track and energy transfer as well as the mechanisms of radiation effects (1) at the genomic level (including genome-wide analysis studies (GWAS), transcriptome, spliceosome and epigenome, (2) at other intracellular targets such as the mitochondria, (3) at the level of target cells and (4) at the tissue environment. In this context, MELODI recommends to enhance the development of omics-based analysis capabilities (including microarrays and next-generation sequencing) in a scope of future multidisciplinary research projects. The combination of modelling

efforts and animal, cellular and epidemiological data applying systems biology approaches will provide further insights into biological effectiveness of radiation quality.

According to MELODI, priorities for forthcoming and long-term future research should take into account the need to investigate effects of ionising radiation of different qualities, i.e. high-LET radiation on radiation-induced cancer and non-cancer diseases as well as on individual variation of radiation risks. All efforts should include a careful dosimetric approach. The long-term priorities include the following areas:

(1) for direct effects on radiation-induced diseases

- Identification, establishment and continued follow-up of suitable cohorts of radiation exposed populations for epidemiological studies related to cancer and non-cancer effects. A re-assessment of dose (uncertainties) is highly recommended
- Identification of the synergistic effect of combined exposure with environmental pollutants, to better match the real situation of an exposed population.
- Identification, development and validation of biomarkers for radiation exposure, effects and disease.
- Continuing development of suitable whole animal as well as human cellular models (including somatic stem cells) for radiation carcinogenesis and non-cancer diseases which bear clear relationships to human diseases.
- Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to human pathologies. This involves the understanding of the relationship between early and late effects, targeted and non-targeted effects as well as the role of delayed genetic instability.
- Identification of the nature and number of target cells at risk for specific radiation related diseases in humans.
- Examination of the impact of low dose and low dose rate radiation effects on pathways/processes contributing to non-cancer disease such as cardio- and cerebro-vascular disease, cataract and impaired cognitive function.
- Development of suitable whole animal cohorts for long-term follow-up for induction of non-cancer diseases

(2) for individual and general health and radiation protection issues

- Understanding the impact of inter-individual variation of radiation risks in relation to cancer and non-cancer diseases, and how this might impact on dose response relationships in populations.
- Clarification of the contribution of radiation effects in target cells as well as radiation effects and responses in the tissue environment and interaction between both target cell and tissue environment at different dose levels to the development of radiation-associated diseases.
- Examination of the impact of low dose and low dose rate radiation effects on immune function.
- Understanding the effect of age-at-exposure on radiation risk, from unborn child to adult ageing stage.
- Understanding of the dependence of radiation risk on gender
- Analysis of the role of trans-generational effects and heritable radiation effects
- Better understanding of the risks of internal emitters following internal contamination with radionuclides, paying attention to the type of decay, the intake pathways, chemical speciation of the radioelement (and its daughters) in the tissue or body fluids and the

biodistribution. Comparison of the theoretical impact using biological effectiveness data with the experimental data.

As an ongoing process, MELODI develops and periodically updates the long-term SRA and makes use of the yearly MELODI executive statements to recommend short- to medium-term priorities. These remain in principle unchanged to the statement 2011. In the years to come priority should be given to:

- Quantification of the role of ionising radiation in the development of non cancer disease such as cardio- and cerebro-vascular disease, eye lens opacities and impaired cognitive function after low dose (< 500 mSv) irradiation.

- Development of suitable biomarkers for exposure, cellular and tissue effects as well as for in situ stages of diseases. The biomarkers should be usable for molecular epidemiological studies.

- Clarification of the role of effects in target cells and in the tissue environment (including stem cells and cells of the immune system) in a dose range with clear focus on low doses and low dose rates. This may include *in vivo* detection systems as well as suitable *in vitro* systems.

- Identification and analysis of suitable epidemiological cohorts if available with archived biomaterial to improve low dose radiation risk assessment by reducing uncertainties especially for the age- and gender-dependency of radiation risk. Uncertainties due to the exposure assessment should be clearly announced. Suitable data and biomaterial banks should be established with a clear focus on the possibilities to share data, material and knowledge within the scientific and wider radioprotection community.

- Development of guidelines for short, medium and long-term exposure and health risk monitoring in case of a major nuclear accident in Europe. This is to ensure the public that all possible scientific efforts are undertaken to record, document and analyse exposed populations in the scientifically best available way to generate in a comprehensive manner information about health consequences from possible accidents in the future.

Efforts in 2013/2014 should focus on the establishment of a new organisational platform to promote and administer radiation protection research in frame of the Horizon 2020 initiative of the European Commission. The new structure should be tested in a first call in 2014/2015. In the meantime, funding of low dose research in Europe should be ensured by external calls initiated by the NoE DoReMi along the roadmap laid out by the TRA of DoReMi.

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Approaches to be considered

Classical
- Cytogenetics (Multi-FISH, chromosome painting) radiation chemistry, biochemistry, radiation sources, microirradiation,

Emerging
- Transcriptomics, Proteomics, Metabolomics, Epigenomics, transcriptomics
- Systems Biology (interdisciplibary studies on molecular pathways)
- development of exposure and disease specific biomarkers
- inactivation of specific genes (miRNA, epigenomic silencing…)
- QT-PCR

New techniques:
- Molecular Imaging
- new radiation devices: microirradiation, synchroton, heavy ions, conformational radiation therapeutic devices, radiation pharmacology, immunoradiology

- Nanostring nCounter
- High throughput sequencing
- Reproduction - hereditary transmission
- 2 and 3D electrophoresis
- mass spectrometry electrospray, HPLC, chromatography,
- nanotechnology
- genetic an epigenetic imprinting,
- Molecular imaging
- MRI
- computer assisted tomography and cone beam CT
- emerging (3D) X-ray technology

Epidemiology
Classical and molecular Epidemiology
- whole populations and specific populations.
- retrospective and prospective cohorts.

Taking into account the limits/advantages of the different existing cohorts in terms of size, duration of follow-up, data quality and completeness, and availability of additional information, no single cohort could provide a satisfactory dose response relationship adjusted for smoking and other occupational carcinogens. However, the pooling of the available data using common study protocols should provide sufficient statistical power. The discrepancies in biomonitoring and medical surveillance between countries are an issue and should be addressed in the protocol as well as the authorisations of national ethical committees and local trade unions. The population of workers involved in the nuclear fuel cycle could provide a very good opportunity to evaluate the possibility to construct a combined cohort (compatibility of databases) with a precise reconstruction of past exposures to insoluble uranium oxides and other exposures (availability of data, job exposure matrix construction),

including the feasibility of biological sampling and biomarkers testing (legal and logistic procedures), and the collection of additional information (hypertension, serum cholesterol levels….) from the occupational medical files. Its particular interest would be to test the feasibility to collect blood samples directly from exposed workers, including those, who have been exposed in early years of nuclear industry to relatively high levels of radiation, from both intake of radionuclides and external radiation and are still alive. These workers would be considered of high priority for biological sampling and specific follow-up ((both retrospective and prospective) with the aim at launching epidemiological studies involving approaches from molecular biology and toxicology. Such studies have a potential to address short-and long-term pathologies of cancerous and non cancerous nature.

- mathematical modelling
- Reliable medical assessment and follow-up of suitable cohorts (short and long term pathologies)
- Genetic and transgenerational studies in mice and humans