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BOOK OF ABSTRACTS

47° ANNUAL MEETING OF THE EUROPEAN RADIATION RESEARCH SOCIETY

XX MEETING OF THE ITALIAN RADIATION RESEARCH SOCIETY

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OPENING LECTURE

Heavy ions in therapy and space

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Research in the field of biological effects of heavy charged particles is needed for both heavy-ion therapy (hadrontherapy) and protection from the exposure to galactic cosmic radiation in long-term manned space missions. Although the exposure conditions (e.g. high- vs. low-dose rate) are different in therapy and space, it is clear that a substantial overlap exists in several research topics, such as individual radiosensitivity, mixed radiation fields, and tissue degenerative effects. Late effects of heavy ions are arguably the main health risk for human space exploration, and with the increasing number of cancer patients treated by heavy-ion therapy, including young adults and children, this issue is now becoming the main source of uncertainty for the success of hadrontherapy as well. Reducing uncertainty in both cancer and noncancer late risk estimates is therefore the first priority in heavy-ion radiobiology. In addition, researchers involved either in experimental studies on space radiation protection or heavy-ion therapy often use the same accelerator facilities. Several heavy-ion therapy facilities are now under construction or planned in Europe, USA, and Japan. Beamtime will be available at these facilities for clinical radiobiology and basic heavy-ion effects experimental research, as already happens since several years at the HIMAC in Japan. The NASA Space Radiation Laboratory (NSRL) in Brookhaven (Long Island, NY) provides beams of very heavy ions at energies around 1 GeV/n which are of specific interest for space radiobiology. In Europe, these very high energy beams are available at GSI in Germany, where the new Facility for Antiprotons and Ion Research (FAIR) is currently under construction. It is foreseeable that the availability of beamtime and the presence of many dedicated research programs will lead to great improvements in our knowledge of biological effects of heavy ions in the coming few years.

KEYNOTE SPEAKERS

KEYNOTE SPEAKER

Space Radiation Health Risks for Exploration Missions

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Exploration mission safety assurance requires the understanding of radiation risks and limiting risks to acceptable levels, however the large uncertainties in risk predictions are an impediment to mission design. Predicting the health risks from galactic cosmic ray (GCR) exposures carries large uncertainties due to the qualitatively distinct microscopic energy deposition and early biochemistry of heavy ions compared to low linear energy transfer (LET) radiation, such as X-ray or gamma-rays. Radiobiology research with heavy ions has identified important qualitative differences that suggest conventional approaches based on scaling to epidemiology data for gamma-rays using quality factors have inherent limitations [1,2]. We describe an alternate approach using the direct application of data from animal studies with heavy ions and fission neutrons [2]. GCR risk estimates suggest fatal cancer risks for long-term space missions could approach 20% fatality [3]. This high efficiency of heavy ions in causing solid cancers has several components. High LET radiation causes non-targeted effects (NTE), which dramatically increases risk at low dose. The quality of heavy ion tumors is also distinct with more aggressive tumors, shorter latency, and decrease immune cell infiltration into tumor volumes. This later aspect suggests a possible role for synergistic risks from space radiation and immune changes due to microgravity.
Space radiation risks for non-cancer effects are diverse in nature. The risk of acute radiation syndromes from solar particle events is readily avoided using passive shielding and alert dosimetry. There is a significant probability of vision impairing cataracts with short latency (<3 years), and cognitive and memory detriments during a mission. We review results from heavy ion accelerator-based animal experiments with heavy ions on risks to cognition that suggest a potentially significant risk for a Mars mission [4,5], and discuss modeling approaches to make quantitative estimates of cognitive risks.

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5. *Cucinotta FA, Cacao E. Life Sci Space Res 25, 129-135, 2019.*

KEYNOTE SPEAKER

Monte Carlo simulation of radio-induced DNA damage

Carmen Villagrasa ***(on behalf of the IRSN Rosiris Team)***

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Keywords: Monte Carlo Simulation, radio-induced DNA damage, Geant4-DNA

Increasing knowledge of the mechanisms of cell damage formation induced by ionising radiation has been a fundamental research area in radiation protection for more than 30 years. Indeed, this knowledge can then be used to improve the assessment of the subsequent risk at different types of exposure (internal, external, different particles, energies) and at different dose levels.
Microdosimetry first and nanodosimetry second, are two formalisms that, by investigating the energy deposition generated by irradiation at the cellular and intracellular scale, contribute to this research. Although both approaches can be used experimentally, the great advances in computational capabilities in recent years have made simulation, and more particularly Monte Carlo (MC) simulation, the method of choice for this type of research. Thus, using the experimental data and the knowledge acquired on the different stages of the interaction between radiation and biological matter, this method can be used to calculate the damage at the level of a cell population, particularly the damage produced to the DNA which will greatly condition the fate of the cells.
In this presentation, the objective is to give an overview of the work done at IRSN concerning the simulation of DNA damage. Based on the use of the Geant4-DNA code[1, 2], our damage simulation chain[3] pays particular attention to the geometrical models of DNA as we have shown that taking into account a realistic representation of the hetero and euchromatin domains influences the number and complexity of damages[4,5]. In addition, an increasingly complex simulation of the chemical step is needed to improve the results and to be able to address specific issues such as the effects of flash radiotherapy or the use of metallic nanoparticles in radiotherapy. To this end, the latest developments of the Geant4-DNA collaboration implemented in our calculation chain will be presented. Finally, current developments concerning the extension of the simulation chain to take into account variability at the level of an irradiated cell population (extension in scale)[6] and DNA damage repair mechanisms (extension in time) will also be discussed.

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KEYNOTE SPEAKER

The role of extracellular vesicles in mediating acute and late radiation effects in the bone marrow

Katalin Lumniczky, Ilona Csordás, Tünde Szatmári, Rita Hargitai, Dávid Kis, Eszter Szarka, Géza Sáfrány

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Extracellular vesicles (EVs) are membrane-bound structures released by the cells in the extracellular compartment. EVs are very heterogeneous in size and their internal cargo. The major role of EVs is intercellular communication, which is achieved by molecular mediators carried by EVs able to modify the function and/or fate of recipient cells. These mediators can be nucleic acids (mRNAs and diverse miRNAs as well as other small or long non-coding RNAs, DNA), proteins, lipids and small molecular weight metabolites, which by being protected by the double membrane layer preserve their integrity and biological activity in the extracellular space. This complex cargo allows for the transmission of “information packets”, which is a much more efficient way of signalling between cells than transmission of individual “information units”.
Bone marrow is a particularly radiosensitive organ, where radiation damage of the stem cell compartment can be significantly modulated by signals received from the microenvironment. Thus, intercellular signalling is a key mechanism in modulating radiation damage in the bone marrow. A growing number of evidence shows that EVs play an important role in the manifestation of ionizing radiation-induced acute and late bone marrow damage. This presentation aims to give an overview on how ionizing radiation influences EV release and uptake by the different cellular subpopulations in the bone marrow. We will review how EVs by transmitting radiation-induced signals to non-targeted cells within the hematopoietic system are able to increase the level of radiation damage, or in contrary, mitigate certain aspects of radiation damage. We will shortly refer to the role of EVs in radiation leukemogenesis as well.

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KEYNOTE SPEAKER

Describing and predicting radiation effects with parameter poor modeling

Friedrich, T. *1
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Keywords: relative biological effectiveness, radiation therapy, immunotherapy, modelling

A quantitative understanding of radiation effects essential in both radiation therapy and radiation protection. Various approaches of effect modelling attempt to describe and predict radiation effects upon exposure and may reflect mechanistic insights on various levels, e.g. on the conversion of DNA damage into cellular or tissue effects. In the presentation, general strategies of effect models will be reviewed in particular for models with a low number of free parameters, which largely ignore the complexity of biological processes by taking into account effectively the most essential processes. Here, two examples will be given: First, the Local Effect Model (LEM) is used to predict the relative biological effectiveness of high LET radiation. Its capabilities and limitations have been investigated using the Particle Irradiation Data Ensemble (PIDE) which is a data collection of in-vitro experiments after ion vs photon irradiation [1, 2]. To go beyond model tests against experimental data of one type, consistency of the model assumptions were challenged by applying the LEM concept to different endpoints with the same set of model parameters. As a second example, a model for the anti-tumor effects of the combination of radiation and immunotherapy is introduced [3]. The model reflects the radiation mediated immune activation upon the administration of immune checkpoint blockers and succeeds to describe tumor growth curves for a broad set of in-vivo data. It is used to predict suitable exposure conditions for establishing an abscopal effect, which is experimentally testable. In summary, parameter poor models can allow robust effect predictions and associated uncertainty estimates.

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[2]T. Pfuhl et al., Comprehensive comparison of local effect model IV predictions with the particle irradiation data ensemble, Med Phys. 2022;49:714–726
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KEYNOTE SPEAKER

Variability in cellular and individual response to radiation

Andrzej Wojcik

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Keywords: DNA damage and repair, radiation risk, radiation sensitivity, individual variation, genetics, modifiable risk factors

Cells of different origin often differ in their response to radiation. The underlying mechanisms of variability include differences in DNA damage signalling and repair, oxidative stress, state of differentiation or propensity to undergo apoptosis. Cells of the same type isolated from individuals also differ in radiation response but the underlying mechanisms are less understood. Genetic factors are commonly thought to be a substantial contributor but apart from a small number of rare monogenic diseases such as Ataxia Telangiectasia (AT) or Nijmegen Breakage Syndrome (NBS), the inheritance of variably responsive phenotypes among a population of healthy individuals does not follow a classical Mendelian inheritance pattern suggesting that it is not based on mutations or polymorphisms in single genes. Rather, a radiosensitive phenotype can be considered to be a multi-factorial, complex trait, that is based on the inheritance of an unknown number of low-penetrance risk alleles (1). Apart from the genetic component, the reaction of cells and individuals to radiation is influenced by environmental factors and by a third component - chance. Chance is understood as stochastic molecular variation that can influence on phenotypic variation (2). The interesting question is what is the impact of the three components on the response of a cell or an individual to radiation.

We have investigated the response of peripheral blood lymphocytes (PBL) of two donors to radiations of different qualities during 3 seasons of one year. At each season, 3 weekly replicates were carried out. The analysed endpoints were mRNA levels of selected genes and the frequency of chromosomal aberrations. The aim was to assess the interindividual and seasonal variability of response. Could a pattern of response be identified when mean values were calculated from all repeats? We also compared the intra-, and inter-cellular variability of radiation-induced 53BP1 foci in U2OS cells.

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Finally, we tested if patients who undergo radiotherapy two times for cancers of different locations develop similar levels of normal tissue toxicities that would reflect intrinsic sensitivity traits. The results of the experiments will be presented and discussed in the light of prospects of finding biomarkers of individual response to radiation.

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KEYNOTE SPEAKER

Targeting radiobiological hypoxia to improve radiotherapy response

Ester M. Hammond

Oxford Institute for Radiation Oncology, University of Oxford, Oxford, OX3 7DQ, UK

Key to the DNA damage induced by radiation is the presence of oxygen. In conditions of low oxygen (radiobiological hypoxia) significantly less DNA damage is induced by radiation leading to therapy resistance and disease progression. Regions of hypoxia occur in most solid tumours and, although the degree of hypoxia (how little oxygen) varies, these include areas of radiobiological hypoxia. The focus of my work is the mechanistic investigation of the response to radiobiological hypoxia with a view to identifying therapeutic strategies to improve radiotherapy response. In particular, we have focused on the DNA damage response induced in radiobiological hypoxia which is unusual as it is dependent on replication stress and occurs in the absence of detectable DNA damage. Recently, we have implicated the unfolded protein response as part of the response to hypoxia-induced replication stress. An overview of these studies will be presented.

KEYNOTE SPEAKER

FLASH Radiotherapy: The start of a dose-rate revolution?

Prise, K.M.[1]

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Keywords: Dose-rate, Flash radiotherapy, Laser accelerated beams, Spatial radiotherapy

Our understanding of the impact of dose-rate in radiobiology and radiotherapy have been well defined for many years, particularly when considering the effect of reducing dose-rate relative to standard therapeutic dose-rates of around 1-2 Gy/min. The recent observation of FLASH radiation effects has re-focused interest in the role of high dose-rate exposures typically of ~ 100 Gy/s. Overall, the potential benefits of FLASH are related to the protection of normal tissues within the body. Although already the subject of clinical trials, the mechanisms underpinning FLASH responses are not yet fully defined and there are significant gaps in our understanding[1]. With this, there is a resurgence of interest in much higher dose-rates than the current FLASH regime to test whether novel biological responses will be apparent. A major focus is developing the potential of laser-based sources for the production of electrons, X-rays and charged particles. With rapid technological developments, dose-rates for protons, at near clinical energies (60-100 MeV), of 10^9Gy/s have been achieved for radiobiology studies[2] and with electrons and gamma-rays there is the potential to reach extreme dose-rates > 10^{13}Gy/s[3]. In these regimes, there are major gaps in our understanding, not only of biological response, but of the physical and chemical processes that are likely to be involved.
Ultimately, the concept of FLASH and dose-rate effects are likely to be highly influenced by the spatial distribution of dose, at the tissue level, an expanding area of research. Defining both temporal and spatial effects will lead to a greater understanding of how cells, tissues and organism integrate radiation dose and open new opportunities for optimized radiation-based therapies.

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KEYNOTE SPEAKER

Health and safety hazards in static magnetic field – work with MRI scanners versus work with MRI patients

Karpowicz, J.

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Keywords: electromagnetic exposure, workers safety, exposure evaluation, non-ionizing exposure

Safety and health hazards associated with workers' exposure to static magnetic field (SMF) belong to priority bioelectromagnetic research. The characteristics of hazards experienced by workers near various types of MRI scanners (such as vertigo or "flying objects") - studied experimentally in the context of development of MRI medical diagnostics and scanners design – will be summarized [1].
During approximately 30 years of using MRI diagnostics in Poland, due to changes in a design of scanners (0.1T-7T) and accessories used in MRI units, and in a performance of medical diagnostics – it has been observed the trend of a significant (10-fold) increase in the level of actual and cumulative (daily/annual) SMF exposure. Significant dependence of these parameters from the design (ergonomy) of scanners, organization of radiographers work and the understanding of the nature of SMF hazards by the managers of MRI unit was observed. The exposimetric studies showed that the cumulative exposure of radiographers during 10-20 years reaches the level at which published epidemiological studies have found an increased risk of developing arterial hypertension in people with long-term exposure to SMF. The SMF which impact may be sufficient to cause the noticeable biophysical effects induced in the body of the worker moving next to MRI magnet (such as vertigo) have been found near 1.5-7T scanners.
In epidemiological studies focused on the long-term exposure to SMF, the exposure evaluation needs attention for the construction of scanners, work organization and type of executed diagnostics (because the significant fraction of radiographers' work is executed with attention to patients needs, near any type of MRI scanner - it was found that radiographers' exposure to SMF near medium, high and very high-field scanners (1.5T-7T) may have similar parameters, despite differences in levels of diagnostic SMF applied to patients).

[1] Selected co-authored articles: Int. J Occupational Safety and Ergonomics 2006; Environmentalist 2007; Bioelectromagnetics 2013; Pol J Radiology 2012; Electromagnetic Biol. Med. 2013; Magnetic Resonance Materials in Physics, Biol. Med., 2017; Int. J. Env. Res. and Public Health 2022.
Funding: the National Programme "Improvement of safety and working conditions" (the state services for the ministry of labour in Poland – task 2.SP.10).

INVITED SPEAKERS

INVITED SPEAKER

Molecular mechanism for synthetic torpor-induced radioprotection: HIBRAD preliminary results

Hitrec T.[1], Tinganelli W.[2], Negrini M.[3], Babbi G.[4], Squarcio F.[5], Simoniello P.[6], Cenacchi G.[7], Grillini M.[8], Papa V.[9], D'Errico A.[10], Morganti A.[11], Romani F.[12], Compagnone M.[13], Marchesano V.[14], Helm A.[15], Piscitiello E.[16], Occhinegro A.[17], Taddei L.[18], Luppi M.[19], Amici R.[20], Sioli M.[21, 22], Zoccoli A.[23,24], Durante M.[25,26], Cerri M.[27,28]*

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Keywords: hibernation, radioprotection, synthetic torpor, immunohistochemistry, RNAseq,

Cosmic radiation represents an important threat to the health of astronauts during long space flights [1]. So far, the only strategy to counteract such a threat is the use of passive or active shielding, but radiation shields are expensive and cannot provide a full protection from the damage [2]. Radiations are also used in current radiotherapy. While radiation is very effective in killing cancer cells, the therapeutic dose must be weighed against the possible damage to healthy tissue, that limits the efficacy of radiotherapy.
A peculiar biological condition that enhances radioprotection is hibernation/torpor [3]. Torpor is an hypometabolic state used by many mammals such as bears, squirrels, hamsters, mice and many others to save energy in harsh conditions [4].
Although already known in the past [5], the idea to exploit hibernation-induced radioprotection for interplanetary travels [6, 7] or for medical purposes [8] was renewed by recent studies showing new ways to effectively induce a safe and reversible state

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similar to hibernation in non-hibernators [9-12]. Such a state can be referred to as synthetic torpor [8, 13].
As a first step on the path to the unravelling of the molecular mechanism mediating hibernation-induced radioprotection, we conducted an experiment funded by INFN: project HIBRAD. In this experiments, non-hibernating animals (rats) were exposed to 3Gy of X-rays in synthetic torpor or in euthermic conditions. To observe the early molecular response, organs were sampled 4 hours after the irradiation. Tissue damage was evaluated by immunohistochemistry and gene expression by RNAseq.
Here we report preliminary results from HIBRAD, showing that animals irradiated in synthetic torpor don't show sign of liver damage, compared with control animals. Moreover, we have identified a small set of genes that are expressed only in the animals irradiated in synthetic torpor, among which ETKN1 and CISH, possibly responsible for the resistance to the radiation damage.

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INVITED SPEAKER

Space: one small step for an immune cell, one giant leap for the human immune system

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Keywords: immune dysfunction, radiation, microgravity, psychological stress, simulation models

Since the first human journeyed into outer space in 1961, boundaries were pushed and it became possible to visit space for longer periods of time. However, sending humans further into space and extending mission durations challenges the current capabilities of space medicine. When leaving the Earth's surface, the human body experiences **big environmental challenges** caused by an extreme environment. These challenges, either physical (cosmic radiation and altered gravity levels) or psychological (stress) in nature, disrupt the body's homeostasis resulting in adverse health effects.

One of the important spaceflight associated health problems which is considered particularly important for the success of long-term, exploratory-type human space missions is **immune dysfunction**. Physical and psychological space stressors have been shown to weaken the immune defense capacity, e.g. cell skeleton alterations and lowered immune cell numbers. This weakening in combination with an increased microbial virulence, growth and resistance in space increases the **health risks** to astronauts. At the moment, however, the exact effects of the induced immune dysfunction are unknown and more research is needed to evaluate how different spaceflight stressors can affect the immune system.

To this aim we are using both in-flight experiments on board the international space station as well as space-analogue models (e.g. Antarctic expeditions, bed rest), as a platform to analyze T cell behavior. Furthermore, *in vitro* and *in vivo* ground-based experiments using space simulating models are also currently being performed at SCK CEN with a specific focus on further elucidating the signaling pathways that control immune cell alterations induced by space flight stressors.

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INVITED SPEAKER

Radiation Chemistry based modeling investigations of the FLASH mechanism

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Radiation Chemistry, FLASH mechanism, Track Structure, Biophysical Modeling

The mysterious differential effectiveness of ultra-high dose rate (UHDR) irradiations, returning a protective effect on normal tissues for same antitumor efficacy as compared to conventional dose rates, the so-called FLASH effect, observed in numerous preclinical experiments, triggered in the last 3-4 years an exponentially growing number of biophysical modeling works attempting to investigate and explain it from the mechanistic point of view.
Since it was appearing that such a phenomenon should imply several physical, chemical and biological stages of the radiation action, different spatio-temporal scales were considered and analyzed in these modeling approaches.
An overview of these investigations will be concisely reported, with a focus on the ongoing joint efforts of GSI and TIFPA in this context, especially in the attempt of combining different scales.
In particular, radiation chemical based approaches, employing TRAXCHEM [1-2], the GSI radiation chemical track structure code and its specific extensions, allowing to go from the physical stage to the homogeneous chemical stage will be mentioned and a novel dedicated extension of the Generalized Stochastic Microdosimetric model (GSM2)[3-4] for UHDR regime, aiming at combining the DNA damage and repair kinetics with the chemical stages on several levels. Impact of LET [5] and dose delivery features will be discussed as well.

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INVITED SPEAKER

Low doses ionizing radiation and vascular diseases

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Keywords: low doses, ionizing radiation, vascular system, experimental studies

The relationship between high dose ionising radiation and cardiovascular diseases is well established. In contrast, after moderate doses (0,5-5Gy) the relationship is suggestive and weak after low doses (<0,5 Gy). However evidence is emerging after low-level exposure to ionizing radiation that the risk of diseases of the circulatory system could increase and is one of the most important matters currently facing radiological protection (Gillies et al, 2017). In the present framework recommended by the ICRP, no account is taken of any risk of CVD (e.g., heart disease and stroke) consequent to exposure to low doses or low doserates. Epidemiological studies, mainly occupational ones, suggest that the risk of CVD following low-level exposure is increased, but the results are not always consistent and causative associations cannot yet be made because of unresolved interpretational issues. Another difficulty for epidemiologists is the lack of data related to the biological mechanisms associated with the observed findings. This is complicated by uncertainty on what are the doses to the relevant organs/tissues (e.g., brain, heart) that increase the incidence of CVD. A better understanding of the underlying biological and molecular mechanisms is needed. If one proves that there is an increased risk of CVD following low-dose exposure, it may have a considerable impact on current low-dose health risk estimates.
Experimental studies on cardiovascular system indicate different responses based on the target, the dose and the dose-rate. The response of low doses on macrovascular or microvascular diseases is different based on cellular and molecular microenvironnement. For instance in the context of atherosclerosis, a chronic inflammatory disease, low doses modulate inflammatory profile and decreases the lesion sizes depending of the dose-rate in mice models (Mancuso et al, 2015; Legallic et al, 2015; Ebrahimian et al, 2017). Whereas in the context of ischemia low doses increase post-ischemic nevascularisation (Heissig et al, 2002; Ministro et al, 2016). All of the current data suggests that all depends on the target and that more epidemiological and experimental research is required before final conclusions.

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6Low-dose ionizing radiation induces therapeutic neovascularization in a pre-clinical model of hindlimb ischemia.A Ministro , P Oliveira , R Nunes , A Dos Santos Rocha , A Correia , T Carvalho , J Rino , P Faísca , J Becker , J Goyri-O'Neill , F Pina , E Poli , B Silva-Santos , F Pinto , M Mareel , K Serre, S Constantino Rosa Santos 2017 Jun 1;113(7):783-794.

INVITED SPEAKER

From mutation induction to secondary cancer risk predictions in particle therapy: modelling through systematic data collection and Microdosimetric Kinetic Model

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Keywords: Mutation induction, particle therapy, Microdosimetric Kinetic Model, secondary cancer

Purpose: Since the early years, particle therapy treatments have been associated with concerns for late toxicities, especially secondary cancer risk (SCR). Nowadays, this concern is related especially to patients for whom long-term survival is expected (e.g. breast cancer, paediatrics). We present a modelling analysis aiming at improving our understanding of the RBE for mutation induction (RBE_M) for different particle species.
Methods: We built a database collecting all available RBE data for mutation induction (in vitro HPRT mutation assay) from literature (105 entries, distributed among 4 cell lines and 14 particle species). Statistical and modelling analysis were applied to the data. The latter was performed by applying the microdosimetric kinetic model (MKM) to describe the mutagenesis in analogy to the lethal lesion induction. Exploiting the formal similarity between mutation induction and tumor induction, the new MKM formalism has been used in an updated version of the Schneider approach to evaluate the excess absolute risk (EAR) of secondary cancer in case of particle therapy. Using this new framework, a case of lymphoma has been simulated using the Topas Monte Carlo code, to evaluate the EAR following a treatment with protons.
Results: A correlation analysis between RBE for survival (RBE_S) and RBE_M reveals significant correlation between these two quantities. The correlation is stronger when looking at a subset of data based on e.g. cell line and particle species. We also show that the MKM can be successfully employed to describe RBE_M, obtaining comparably

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good agreement with the experimental data. The EAR for the organs at risk has been also evaluated within the MKM-based framework, showing the impact of variable LET and the intra-fraction correlation between survival and tumor induction.
Conclusions: We show that RBE_S and RBE_M are strongly related. Together with the successful application of the MKM, in analogy to the RBE_S, RBE_M and EAR can be readily implemented into TPS evaluations. This might contribute to a more accurate estimation of secondary cancer risk in particle therapy.

INVITED SPEAKER

Carbon ion radiobiology: new insights in the molecular and cellular mechanisms of tumour control

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Keywords: carbon ions, hadrontherapy, radiobiology

Presently, carbon ions represent the ions with the optimal combination of physical and radiobiological features for achieving the best results in terms of local control for radioresistant and hypoxic tumours while sparing of the surrounding normal tissues. By the end of 2021, more than 40.000 patients have been treated with C-ions with very encouraging outcomes, according to the Particle therapy co-operative Group (PTCOG) and several new clinical facilities are also being built or are planned for construction worldwide (https://www.ptcog.ch/). Concurrently with the growth of the clinical activity, we are witnessing a significant rise in research studies that are focusing on a deeper understanding of the molecular mechanisms underlying cellular and tissue responses to the targeted and non-targeted effects of carbon ions in order to better exploit their radiobiological features and identify the tumors that can benefit from this treatment the most. In this talk, the current knowledge of the cellular and molecular mechanisms of action of carbon ions for the treatment of cancer will be compared to that of X-rays, with a focus on the newly emerging topics of heavy ion radiobiology such as signalling pathway activation, microenvironment response, and immunomodulatory properties. Finally, a brief digression on experimental models that are not conventional for radiobiology will be critically presented in view of potential useful applications for the investigation of radiation effects.

INVITED SPEAKER

Dosimetry for FLASH radiotherapy: challenges and recent developments

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Keywords: FLASH radiotherapy, dosimetry, beam monitoring, high dose-rates

FLASH radiotherapy (RT) is attracting a significant interest since the first investigations carried out in 2014, demonstrated by the increasing number of related publications [1]. Several preclinical studies worldwide have demonstrated that ultra-high dose rate (UHDR) beams produce an improvement of normal tissue sparing while maintaining high tumor control probability compared to conventional dose-rate RT (FLASH effect). However, to fully understand the mechanisms and the biological processes, reliable beam monitoring and dosimetry technologies must be developed and new protocols are needed [2]. This is crucial to support the first clinical trials and for the clinical translation of FLASH RT. Currently used detectors saturate at these extreme regimes, therefore the optimization of already established technologies as well as the investigation of novel radiation detection and dosimetry methods are required [3]. The main challenges coming from the peculiar beam parameters characterizing UHDR beams for FLASH RT will be discussed. A status of the current technology will be provided, including recent developments for established detectors and novel approaches currently under investigation with a view to predict future directions in terms of dosimetry approaches and practical procedures for the clinical translation of FLASH RT.

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INVITED SPEAKER

FLASH radiotherapy – towards clinical implementation

Crister Ceberg

Lund University

The current interest in ultra-high dose rate irradiation stems mainly from its well documented radiobiological sparing effect, compared to conventional dose rates, which has been thoroughly established in many cultured cancer cell lines in vitro, as well as in several different normal tissues in vivo.
Although the size of the effect seems to depend on various conditions, for instance, including dose-rate parameters and environmental factors, there is also recent evidence for a differential sparing of normal tissue, as compared to the anti-tumour effect, which in theory may be used to widen the therapeutic window in radiotherapy.
While encouraging data continue to accumulate, one of the present challenges in FLASH radiotherapy concerns how this widened window may best be exploited in clinical applications, alongside other differentially sparing treatment techniques that are already available, such as target conformity and dose fractionation.
In this lecture, an overview of the FLASH project at Skåne University Hospital and Lund University will be presented, including preclinical in vitro and in vivo experiments, veterinary clinical trials, as well as the planned route towards a future clinical implementation, including novel technical solutions for ion chamber-based dosimetry, treatment planning, and motion management.

INVITED SPEAKER

There might be much more to EMF/RF-EMF exposures than just the electromagnetic sensitivity

Leszczynski, D.

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People respond differently to environmental factors that induce biological effects. Some people tan easily, some get skin burns and, for the rest of people, the tan/burn susceptibility lies somewhere in-between tan and burn. The same is e.g. with pollens. Some suffer, some don't, even when pollens are very abundant. Such examples could be given very, very many. To every environmental factor, or pollutant, natural or man-made, there is always a group of more sensitive individuals. Currently, one of the hotly debated man-made environmental factors are electromagnetic fields.

Part of the population considers themselves as sensitive to the man-made electromagnetic radiation (EMF) – electromagnetic sensitive (EHS). Sensitivity is characterized by a broad variety of non-specific symptoms that the sensitive people claim to experience when exposed to EMF. While the experienced symptoms are currently considered as a real life impairment, the factor causing these symptoms remains unclear.

It is logical to consider that the EHS exists. However, the research was unable to reliably establish causality link between EMF exposures and EHS because the psychology-driven provocation studies, asking about subjective feelings, are too crude and prone to bias. In order to gain objective insight into how EMF exposures affect human body it is necessary to perform studies that examine biochemical effects of EMF exposures in human volunteers. Surprisingly, such research is missing. Most of biochemical effects are known from animal and in vitro studies that not necessarily apply to humans in real life exposures.

Human studies are urgently needed because the problem of individual sensitivity to EMF might be much larger than the EHS alone.

The International Agency for Research on Cancer (IARC) has classified EMF as possible human carcinogen. When the classification of the RF-EMF was performed, important part of the evidence was provided by studies where animals were co-exposed to RF-EMF and some other known carcinogens.

Could EMF/RF-EMF exposures potentiate harmful effects of chemicals that humans are exposed to? It is a possibility but we know nothing in this matter because research has not been done.

ORAL PRESENTATIONS

Astronaut radiation protection and space radiation research.

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Space radiation can be the single limiting factor of human and robotic space exploration. The space radiation environment is complex, with different qualities and quantities of radiation. It can have detrimental effects to human health, as well as hardware on board of spacecrafts. As the European Space Agency (ESA) enters a new era of space missions beyond Low Earth Orbit, to the Moon and beyond, new measures need to be taken to safeguard astronauts and protect spacecrafts on deep space missions. ESA's "Science in Space Environment" (SciSpacE) programme is dedicated to facilitating state of the art science in space and on Earth, to enable human and robotic space exploration, as well as to deliver solutions to problems back on Earth. SciSpacE offers an extensive programme for radiation research, with the use of European irradiation facilities, as well as current (the International Space Station) and future (Gateway — the Moon orbiting station, the Moon's surface) platforms. In this presentation, we will elaborate on the complex space radiation environment and operational constrains of current and future space missions. Current astronaut radiation protection strategies and future challenges will be described. We will present ESA-supported current and future opportunities for radiation research and discuss the ESA's objectives of science in and for space.

Towards the definition of the ASI roadmap for space life sciences in human space exploration and prospectives on radiation-risk mitigation

Vittorio Cotronei (ASI), Marta Del Bianco (ASI), Francesca Ferranti (ASI), Silvia Mari (ASI), Claudia Pacelli (ASI), Sara Piccirillo (ASI), Valerio Vagelli (ASI)

Human space exploration is one of the most effective drivers for scientific research and technological innovation. In view of the steadily growing international relevance of human space exploration beyond Low Earth Orbit, the Italian Space Agency (ASI) is continuously reinforcing the network of the national scientific community and industrial stakeholders for the success of future Moon and Mars exploration missions. This requires tackling unsolved issues related to the effects of long-duration space flights and developing the required, enabling technologies.

ASI has activated four working groups with the participation of national experts from the scientific community on the macro-areas of space life sciences: integrated physiology, microbiology, biological systems for life support, and radiations. The groups have analyzed the current scenario of space life science research and have identified the most relevant objectives and key issues to enhance the national contribution and competitiveness towards enabling human deep space exploration in collaboration with international partners and agencies.

In this contribution, we will present and discuss the results produced by the working groups, with emphasis on radiation risk mitigation and development of countermeasures for human space exploration.

Biological damage induced by space radiation: calculation of astronauts' doses and RBE for cell death and chromosome aberrations

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Keyword: space radiation, RBE, radiobiology, Monte Carlo simulations.

In this work, the BIANCA (BIophysical ANalysis of Cell death and chromosome Aberrations) biophysical model was extended to heavy ions up to Fe, with the goal of evaluating the biological damage induced in astronauts exposed to space radiation. Specifically, two radiobiological databases were generated, the first one describing Human Skin Fibroblast (HSF) cell survival, and the second one describing the induction of lymphocyte dicentric chromosomes, as a function of ion type ($1 \leq Z \leq 26$) and LET, as well as dose.
Using an interface between BIANCA and the FLUKA Monte Carlo transport code, astronaut doses and the corresponding relative biological effectiveness (RBE) values for Galactic Cosmic Rays (GCR) in deep space were calculated, under different shielding conditions. To compare the results with cancer and non-cancer dose limits for astronauts, the RBE calculated for HSF cell survival was used to estimate the equivalent dose for deterministic effects (Gy-Eq), whereas the RBE for lymphocyte dicentrics was used to estimate the equivalent and the effective dose for stochastic effects (Sv). Concerning the deterministic limits, for 650 days in free space at solar minimum (as representative of a Mars mission) the RBE-weighted doses for HSF cell survival were lower than the career limit recommended by NCRP report no. 132 for skin. For 365 days, the simulation values were lower than the 1-year limits, both for skin and for blood forming organs (BFO). Concerning stochastic effects, the equivalent doses calculated using lymphocyte dicentrics were similar to those calculated using the Q values recommended by ICRP. Furthermore, for a 650-day mission the results were always lower than the career limit recommended by ICRP (1 Sv), which has been adopted by ESA and RSA; the comparison with the NCRP limits adopted by NASA was more complex, since they are age- and sex-dependent.
Following this work BIANCA, when interfaced to a MC transport code like FLUKA, can now predict RBE values for cell death and lymphocyte dicentrics following GCR exposure, taking into account the ion type (up to Fe-ions), LET and dose.

Adenosine Agonist treatment shows protective effects to C-ion irradiation

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The challenging part of radiation therapy is needed to successfully eradicate cancer and, at the same time, not harm the healthy tissue or cells. Radiation is also still the primary concern for space travelers, such as astronauts who are at risk of being exposed to heavy-ion radiation, affecting their health. Therefore, it is essential to find a reasonable way to protect the healthy tissue from radiation. We treated rats with adenosine agonist 5'-monophosphate monohydrate (5'-AMP) i.p., immediately after whole-body irradiation with 8 Gy or 2 Gy of Carbon (C-) ions and then housed them in a cold room (16^{o}C) for 6 hours. The body temperature of the 5'-AMP-treated rats decreased compared to those treated with saline injection.
The animals treated with 5'-AMP showed higher survival following 8 Gy of C-ions than when treated with saline. Furthermore, the histology results showed that one week after 2 Gy of C-ion irradiation, activated microglia in the brain and apoptotic cells in the liver of 5'-AMP treated rats showed fewer numbers than in saline-treated animals after irradiation. Additionally, *in vitro* experiments using rats' retinal pigmentosum cells (RPE-J) showed that 5'-AMP treatment in combination with hypoxia or lower temperature immediately after 2 Gy of C-ions leads to delayed DNA repair suppresses the radiation-induced mitotic catastrophe. Thus, results suggested that using 5'-AMP together with hypoxia or low temperatures may increase cell resistance to radiation damage.

Solar modulation modeling of the galactic proton flux measured by the AMS02 and PAMELA experiments

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Keywords: galactic cosmic ray solar modulation, solar physics, space radiation

The flux of Galactic Cosmic Rays near Earth is not representative of the Local Interstellar Spectrum at energies below ~30 GeV due to a variety of physical processes arising in their propagation through the heliosphere. The changes in the GCR intensities and energy spectra are related to the solar activity, and are referred to as CR solar modulation.
A numerical modulation model to study the transport of galactic protons in the heliosphere is presented.

The model was applied to the 27-day averaged galactic proton and light nuclei flux recently released by the PAMELA and AMS02 experiments, covering overall an extended time period from mid-2006 to mid-2019. The time evolution of the model parameters and their relationship with solar activity proxies is shown. Correlations, similarities and differences of solar modulation of different nuclei species, as p, He, Li, B, C, are discussed. As we will discuss, our data-driven approach, based on the availability of new precision measurements, leads to new insights on the solar modulation phenomena.

ERFNet Data Hub: a new access for the European space radiation research

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Human long-term, deep space exploration missions are becoming a reality in the 21st century [1]: the NASA's Artemis program and the launch of the Lunar Gateway will lead humanity forward to the Moon and prepare us for the next giant leap, the exploration of Mars. Nevertheless, space radiation may place astronauts at significant risk for radiation sickness, increased lifetime risk of tumors, degenerative diseases, thus representing the main potential showstopper for safe human exploration of the Solar system [2]. The scientific competences required to properly tackle these issues are broad and interdisciplinary. The tools and strategies needed to further consolidate the radiation research efforts are often still not well known to the wide scientific community and the relevant knowledge is often scattered and difficult to reach especially to the non-expert user.
The European Radiation Facility Network - Data Hub (ERFNet-DH) project, funded and coordinated by ESA and implemented by ALTEC (Turin, Italy), aims at providing a solution to the above challenges. The ERFNet-DH focuses on processing and managing data of space radiation research missions. It also provides a service that promotes data sharing and cooperating among the different experts of the international scientific community, with the aim to support space radiation research.
In particular, the ERFNet-DH will offer the possibility to:

- collect, process, store and distribute radiation data from present and past space missions
- provide a data hub for physics, biology and medical research groups interested in working with space and space-related radiation data
- provide operational support to ESA's radiation payloads on the Lunar Gateway
- support the coordination of ESA's radiation research and applications activities
- perform data analysis and run numerical simulations
- be the boost for the development of European Radiation Risk Model.
-

All the details about the ERFNet-DH design and development will be presented.

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The nucleoside analogue 6-thio-2'-deoxyguanosine (6-thio-dG) radiosensitize breast cancer cells to ionizing radiation exposure

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Data obtained in our laboratory suggest that telomere damaging agents are also capable of sensitizing tumor cells to ionizing radiation (IR) exposure. In recent years, the nucleoside analogue 6-thio-2'-deoxyguanosine (6-thio-dG) has been shown to inhibit the proliferation of telomerase-positive cancer cells through its incorporation into telomeres and subsequent induction of DNA damage, cell cycle arrest and cell death. These effects were investigated in several tumor models, such as lung cancer, glioma and melanoma both *in vitro* and *in vivo*, but to date no data are available in *breast cancer cell lines.*
In this work we characterized the response to 6-thio-dG in breast cancer lines and tested the efficacy of the compound in combination with IR in MCF7 cells.
In particular, the anti-proliferative capacity of the nucleoside analogue was studied in the three telomerase-positive breast cancer lines and in primary human fibroblasts (HFFF2, telomerase negative). Furthermore, effect on cell cycle modulation and induction of genomic and telomeric DNA damage (in the first seven days from treatment), were assessed. These experiments let us to identify 6-thio-dG concentrations and treatment duration capable of inducing telomere dysfunction without drastically affect cell proliferation and plating efficiency in order to set up conditions for 6-thio-dG and IR combined treatments. Surviving fraction experiments indicate that 6-thio-dG is able to synergistically increase the sensitivity to IR in breast adenocarcinoma cells. In addition, molecular cytogenetic analysis and experiments on three-dimensional breast cancer models are in progress to further validate the results obtained. Data so far collected confirm telomere targeting as a promising radiosensitizing strategy.

DNA Damage induced by Ionizing radiation activates the innate immune response: study and characterization of the cGAS-STING pathway.

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Keywords: Ionizing radiation, immune system, cGAS, micronuclei.

Ionizing radiation (IR) has been shown to modulate a variety of immune response processes both *in vitro* and *in vivo*. IR-mediated immune system modulation is a complex phenomenon in which several players (such as stress sensors and cytokines) take part regulating immune and inflammatory response. Among them, the cyclic -GMP-AMP synthase (cGAS), a free cytosolic DNA sensor, is capable of recognizing DNA fragments that, after treatment with IR, could be observed as micronuclei (MNi)[1]. Activation of cGAS causes GAMP dependent-STING activation and promotes phosphorylation and translocation into the nucleus of transcription factors (such as IRF3 and IRF7) that induce innate immune responses and type I interferon (IFN)[2]. In the present work the induction of cGAS-positive MNi was evaluated in immortalized human keratinocytes (HaCaT) exposed to X-rays (250 Kev; 0.5, 1 and 2Gy) and fixed 24-120h after treatment, to determine the dose-response and activation kinetic. Data obtained showed that the highest induction of cGAS-positive MNi was observed after 48h from X-rays irradiation and despite a dose-dependent increase in the number of total MN, cGAS-positive MNi increase after 0.5 Gy reaching a plateau in the dose range of 1-2 Gy. Pathway activation kinetic was obtained by analyzing the cGAS-positive MN frequency at 3, 6, 9, 24, 48, 72, 96 and 120 hours after exposure to 1 Gy of X-rays. In addition, type I IFN gene expression was verified by RT-qPCR and ISG15 induction, a ubiquitin-like protein transcriptionally induce by type I IFN, was analyzed by western blot. In addition, literature data suggested instability of nuclear lamina in MNi leading to membrane rupture and allowing DNA fragment recognition by cGAS. It would be important to characterize MNi lamina integrity, to study the relationship between DNA damage and innate immune system activation and to provide useful information for the role of radiation exposure in immune response.

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Innovative Evaluation Method on Biodistribution of a New [^{64}Cu] Chelating Agent in a Murine Model Based on Radiomics

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In vivo experiments using positron emission tomography (PET) to evaluate the efficacy of the dose on the biodistribution of new radiolabeled chelators are a necessary step of translational research.
In this paper we present an innovative method based on radiomics to process and analyze data extracted from images obtained from a microPET/CT study conducted on nude Balb/c mice treated with 7MBq of a novel [^{64}Cu] chelator. The scans of the mice were acquired at three different time points, specifically 1 hour, 4 hours and 24 hours after inoculation of the ^{64}Cu-labeled compound (EC/β+ 61.5%; $t_{1/2}$ = 12.701 h).
In particular, an image segmentation was conducted on PET studies as follows: all images were registered using a 3D whole-body Digimouse atlas; features extraction was performed from seven organs to compare time-course [^{64}Cu]chelator uptake values. Statistical analysis showed a different in vivo biodistribution of the ^{64}Culabeled chelator over time, with a significant variation of many features between groups observed in bladder and liver (greater than 60% and 50%, respectively).
Due to the importance of the biodistribution of radioactive substances in preclinical studies, a method transferable to human PET studies such as the one proposed based on radiomics, never used in the preclinical setting to date, it may be useful to improve future results in the context of in vivo studies in which radionuclides for biomedical purposes will be used.
Keywords: radiomics; micro-PET/CT; mouse imaging; atlas; radiopharmaceuticals

ORAL PRESENTATION

First in vitro experiments and microdosimetric evaluations to investigate if the Neutron Capture reactions are effective in degrading β-amyloid aggregates

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Keywords: NCT, AD, beta amyloid, nanoparticles, microdosimetry

NECTAR (NEutron Capture Enhanced Treatment of neurotoxic Amyloid aggRegates) project, funded by the European Commission, aims to study the efficacy of neutron capture reaction on B-10 and Gd-157 in the degradation of β-amyloid (Aβ) protein aggregates involved in Alzheimer's disease (AD).

AD is a neurodegenerative disorder that affects a wide range of the world population. Although this pathology was discovered in the early 1900s, the triggers factors are still unclear. A key role is attributed to the Aβ protein which accumulates in the extra-cellular matrix of the brain compromising the proper functioning of the nervous system cells.
Nowaday the only drug capable of interfering in the AD accumulation process is *aducanumab*[1], a very expensive monoclonal antibody that still requires years of use in patients to prove its effectiveness even in the long term. It is therefore necessary to conduct further research on performing AD treatments. A study has shown the effectiveness of X radiation in damaging the Aβ aggregates: both in *vitro*[2] and in *vivo*[3] experiments were conducted, the first case showed not relevant changing in the protein structure, instead of the latter characterized by a relevant Aβ burden reduction. Finally, a pilot study[4,5] on a small cohort of patients showed how different CT scans caused an improvement in terms of their cognitive and behavioural sphere.
The NECTAR project proposes an innovative idea: to exploit the selectivity of neutron capture reactions on B-10 and Gd-157 in order to degrade protein structures. The range of secondary particles coupled well with the aggregates size, allowing them to deposit

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their energy locally, saving healthy tissue. This process will be combined with the action of gamma rays, produced by the same neutron capture reactions, which act over a long distance causing a neuroinflammatory response of the glia cells which should promote a further clearance of Aβ aggregates.
The talk will present the preliminary results achieved. In particular, the first in *vitro* experiments carried out at the nuclear reactor of the University of Pavia and the first Monte Carlo simulations conducted at a microdosimetric scale.
In addition, studies will be focused on simulations concerning the irradiation effects on AD transgenic mice in view of the NECTAR developments which involves in *vivo* activity.

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Proton-Boron-Fluorine Enhanced Protontherapy: first proof-of-principle experiments with monochromatic low-energy and clinical high-energy proton beams

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Keywords: protontherapy; Proton-Boron-Fluorine Enhanced Protontherapy (PBFEPT); F-BPA; low-energy protons; alpha particles; clonogenic survival; micronuclei; tandem accelerator; clinical beam.

The ability of the Proton-Boron Capture Therapy (PBCT)[1] approach to enhance proton biological effectiveness, has been recently demonstrated using the ^{11}B carrier sodium mercaptododecaborate (BSH)[2-4]. The rationale resides in the highly DNA-damaging α-particles generated by the nuclear p+^{11}B→3α (pB) reaction, whose cross-section peaks as protons slow down across the tumour-conformed Spread-Out Bragg Peak (SOBP). Here, a novel data are presented using another binary strategy based on the reaction p+^{19}F→α+^{16}O (pF), which presents a resonance around 2000 keV[5]. Compared to the pB process, the peak of the energy spectrum of the generated α-particles is shifted to higher values (up to 13 MeV, versus 4 MeV for pB) thus generating secondary tracks able to traverse multiple cells. Moreover, a much more densely ionizing ion is produced (^{16}O). ^{19}F-labelled p-boronophenylalanine (F-BPA), whose in vitro internalization in human cancer pancreatic cell line (PANC-1) was recently measured[6], was used as ^{11}B-^{19}F carrier. Three different cell lines (DU-145 prostate cancer, PANC-1 pancreatic cancer and MCF-10A normal breast epithelial cells) were irradiated at a 3-MV tandem accelerator (CIRCE laboratory, Caserta, Italy) with proton energies close to either the

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pB or pF reaction cross-section maximum (~700 keV and ~2000 keV, respectively). To investigate the clinical usefulness of the combined pB-pF approach, DU-145 and PANC-1 were also irradiated along a clinical proton SOBP (energy range: 131.5-164.8 MeV) at CNAO (Pavia, Italy). Clonogenic survival and micronucleus induction were measured in cells irradiated in presence of F-BPA (120 ppm of ^{11}B) suggesting ^{11}B-^{19}F synergic mediated radiosensitization.

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ORAL PRESENTATION

Proton boron capture therapy induces cell death and mitophagy in a heterotopic glioblastoma model

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Keywords: PBCT, glioblastoma, radioresistance

The need to improve radiobiological effectiveness of charged particles have increased the interest to exploit protons and boron nuclear thermal reaction to generate three alpha particles, a phenomenon biologically known as Proton Boron Capture Therapy (PBCT) [1][2][3]. This strategy may hold important therapeutic benefits to treat radioresistant and inoperable brain tumors, including the high-grade glioma, defined glioblastoma (GBM). This work shows a first PBCT preclinical evaluation in a heterotopic GBM mouse model aimed at analyzing pathophysiological and molecular effects in response to this innovative treatment modality. We first validated the reliability of our GBM mouse model by the ultrasound and photoacoustic multimodal imaging technique, allowing the assessment of the tumor oxygenation over the tumor growth.

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Blood oxygenation and hemoglobin were found significantly reduced over time in tumor masses. Reduced levels of ^{18}F-2-deoxy-2-fluoroglucose (FDG) uptake after PBCT treatment were detected by micro positron emission tomography-assisted scanning. Further pathological analyses were performed to examine biological effects such as apoptosis, mitotic index, proliferation and autophagy. Interestingly, mitophagy and caspases levels were observed significantly higher in PBCT compared to proton irradiated GBM. Finally, RNA-seq revealed several deregulated gene and molecular pathways involved in major types of radiation-induced cell death and survival effects after PBCT treatment. Further studies will be fundamental to validate specific biological mechanisms emerged from omics analysis. Moreover, gene and molecular signatures in combination with biomarkers and radiomics data from preclinical imaging may reveal fundamental mechanisms underlying PBCT response leading to a more personalized targeted radiotherapy.

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Characterizing the performance of a proton tomography system for x-ray CT cross-calibration

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Keywords: proton therapy, proton tomography, proton imaging, stopping power

Purpose: INFN research projects have recently studied the feasibility of proton Computed Tomography (pCT) as a possible clinical method to cross-calibrate x-rays CT (xCT), aiming at improving the dose computation accuracy in proton therapy treatment plans[a]. Herein, we report on the performance of our pCT apparatus.

Methods: The pCT system was tested with both a Sedentex-CT Image Quality and an Electron-Density (ED) phantoms. A filtered backprojection algorithm[b], taking into account the protons' most likely path, was applied to reconstruct the phantoms' Relative Stopping Power (RSP) 3D maps. The edge test device inside the Sedentex-CT phantom allowed investigating the spatial resolution of the apparatus. The pCT RSP values of the ED phantom inserts were compared with multilayer ionization chamber measurements to estimate the system accuracy[a].
Results: Proton tomographies of the Sedentex-CT phantom were analyzed, resulting in a spatial resolution of about 0.67 lp/mm (Fig.1). Direct measurements of RSP values of the ED phantom inserts showed a mean absolute percentage error of 0.74%. Finally, statistical analysis suggested a minimum obtainable noise magnitude of about 0.005 for RSP.
Conclusions: The obtained performances allow designing a first clinical application for our pCT system. To this purpose, the RSP maps of a set of stabilized, heterogeneous, biological phantoms have been acquired and will be compared voxel-by-voxel with the

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corresponding xCT images, obtaining a cross-calibrated xCT calibration curve[c]. Once validated, this procedure might be offered to proton therapy centers not equipped with a pCT system, representing a new and direct calibration method to be adopted in treatment planning (Fig.2).

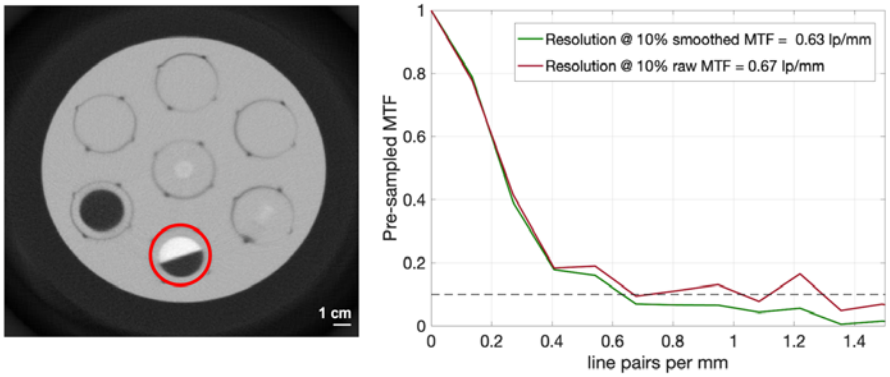

Fig.1: Left: pCT of Sedentex-CT phantom. The red circle highlights the edge image used for pre-sampled Modulation Transfer Function (MTF) estimation. Right: raw and smoothed MTF with associated estimation of spatial resolution at 10% MTF (dashed line).

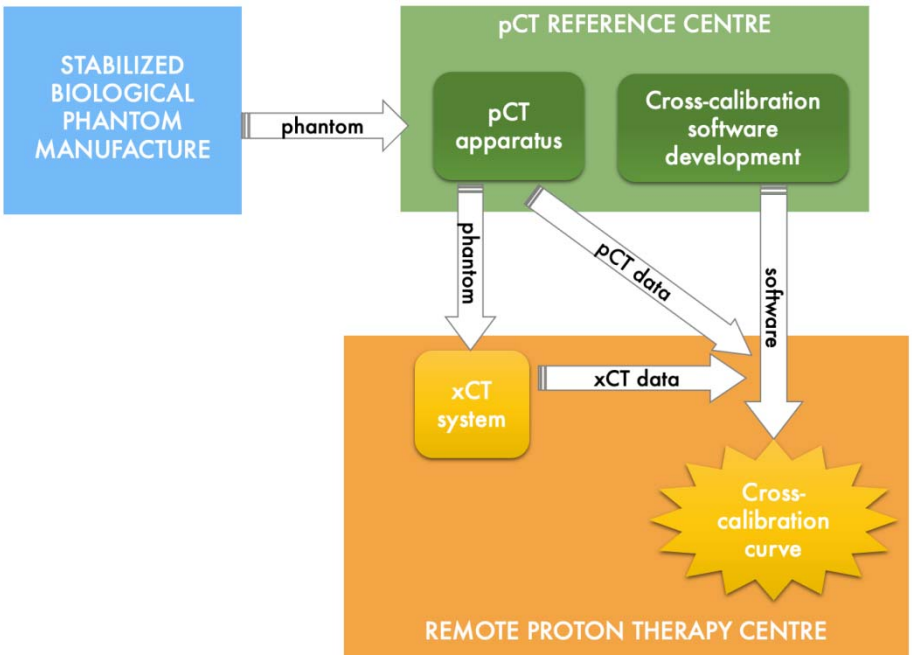

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Fig.2: A possible flowchart of pCT clinical implementation. After the production of a stabilized biological phantom, the reference proton therapy (PT) center will acquire the pCTs. A remote PT center might ship both the phantom and the corresponding pCT data with the developed cross-calibration software. Once phantom's xCT data have been acquired, the remote center could obtain its own cross-calibration curve.

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Gamma radiation for Cultural Heritage preservation at Calliope Facility (ENEA Casaccia R.C., Rome, Italy)

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Keywords: gamma radiation, Cultural Heritage, preservation, side-effects.

Ionizing radiation treatments for preservation of Cultural Heritage artifacts attacked by bio-deteriogen agents (insects, microfungi, moulds) present several advantages over classical procedures, due to their biocide effects[1]. Nevertheless, in various countries, some resistance to ionizing radiation processing is still shown by Cultural Heritage operator communities. The reason for this mistrust is often due to the incorrect knowledge of the physical-chemical modifications (side-effects) induced by ionizing radiation on the irradiated materials. For these reasons, recently further efforts and experimental tests were performed with the aim of providing reliable results, obtained by different experimental techniques widely accepted and recognized, to describe the side-effects occurrence. Moreover, a very important issue is related to the irradiation parameters optimization for the minimization of the radiation-induced modifications on the artifacts, guaranteeing their safeguard and giving reliable and standardized procedures.
At the Calliope ^{60}Co gamma irradiation facility (ENEA Casaccia R. C., Rome, Italy) several studies were performed on cellulose-based substrates by means of different experimental techniques (infrared spectroscopy, Electron Spin Resonance spectroscopy, colorimetric analyses, polymerization degree evaluation) [2, 3]. In this research, the optimal irradiation conditions (in term of irradiation dose and dose rate, environmental atmosphere) were also investigated.
A fundamental part of research activities has covered the study of radiation induced modification over time, performing accelerated and artificial ageing tests, and to verify the synergy between radiation and temperature.

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ORAL PRESENTATION

Dosimetric characterization of a new resin-coated silicon carbide detector

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Keywords: dosimetry, silicon carbide detector, resin process

The research in the development of new detectors for relative dosimetry has been very active in the last two decades. The physical characteristics of Silicon Carbide (SiC), such as wide bandgap, ultra-low leakage current, high electron saturation velocity, almost near tissue-equivalence and high radiation resistance, have attracted the attention of the interested scientific community [1-9]. Moreover, SiC based devices present dose rate independent response and linearity with energy in a wide dynamic range [10, 11]. These features make SiC also suitable for dosimetric applications with both conventional and high intensity beams. In this work a new generation of SiC device based on p-n junction technology was investigated for dosimetric applications. The detector was manufactured in the context of a collaboration between INFN (Istituto Nazionale di Fisica Nucleare, Italy), IMM-CNR (Microelectronic and Microsystems Institute) and STM (ST-Microelectronics, Catania). The adopted detector, built by using new technological processes, presents a detection area of 1 cm^2 and is embedded in epoxy resin in order to make it waterproof. The study aimed at evaluating the potential use of the SiC detector as a relative dosimeter, in accordance with the dosimetric protocols in force (IAEA TRS-398) [12]. The detector response was tested in water with x-ray and electron beam. The released absolute dose during each experimental session

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was evaluated using a standard ionization chamber. In this work the performance and the preliminary characterization of this new detector will be presented and discussed.

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BNCT treatment verification by on-line prompt gamma measurement using CdZnTe (CZT) detectors

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Boron Neutron Capture Therapy is a hadrontherapy based on the reaction 10B(n,α)7Li induced by low energy neutrons. Thanks to the short ranges in tissues of the high LET secondaries the lethal damages affect only tumour DNA sparing the nearby healthy cells. To properly exploit its selectivity, real time knowledge of B10 concentrations and the thermal neutron flux distribution must be known. Today the quantities are measured independently and through indirect methods. This leads to a lack of precision in dose estimation [1].
The B10 concentration and the thermal flux are coupled by the reaction rate of B10 neutron captures. The intensity of 478 keV γ ray emitted after the B10 reaction is a direct measurement of this rate and thus it equals to an on-line monitoring of the B10 dose giving a real time treatment verification [2]. The energy is pretty close to that of PET, nonetheless the single γ emission requires an equipment closer to SPECT scanners. In addition, the huge (n+γ) background characterising BNCT represents an important difference between the still missing detector and those of Nuclear Medicine [3].
Recently, the Physics Department of Pavia University has acquired four modules of the “DoseCapture” system developed by due2lab s.r.l. for BNCT-SPECT [4]. Each module is based on an array of 4 Frisch Grid CdZnTe (CZT) detectors coupled to a squared hole Pb collimator. To reduce activation of the Cd113 naturally occurring in the alloy, Li6 enriched neutron shields surround five out of six sides of the array. A fast digital electronics complete the system.
The modules have been tested using the highly thermalised collimated neutron beam of the Prompt Gamma Neutron Activation Analysis facility housed in the research nuclear reactor of the LENA laboratory of Pavia University. The presentation will report the results of this first measuring campaign which positively supports the development of a B10 dose verification system using CZT γ detectors and tomographic imaging.

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How cell biology and radiation physics met to boost radiotherapy

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In close collaboration with Prof S. Lucas a,d A-C. Heuskin, since 15 years, studies are performed to increase the efficacy of radiotherapy using hadrons instead of photons. Indeed, the use of high energy charged particles like protons or carbon ions presents clear benefits thanks to their depth dose profile and high energy transfer. Currently, research topics include the study of the mechanisms responsible of high LET hypersensitivity and the use of proton irradiation in combination with DNA repair inhibitors to induce synthetic lethality. We also developed a unique nanohybrid compound, composed of gold nanoparticles coupled to targeting antibodies, being able to act as radiosensitizing agents for radiation therapy. We demonstrated for the first time that the enhancing effects of these nanoparticles is not due to “physical” effects but to the fact that the gold nanoparticles induced an inhibition of the antioxidant defenses within the targeted cells. Thioredoxin reductase is the main target of this effect. In addition, the responses of different types of cancer cells as well as cell of tumor microenvironment (TME) after exposure to different radiation modalities as well as to chemotherapy and targeted therapies are being investigated
More recently, the group started to assess the impact of the different types of radiation on the immunogenicity and immunomodulatory capacity of cancer cells and other cells of the TME. For instance, it was determined that unlike X-rays, proton irradiations with high linear energy transfer (LET) partially reprogramed macrophages and promoted a pro-inflammatory phenotype. This discovery provides insight into mechanisms that can regulate the eradication of cancer cells by the immune system. Another project has been investigating the regulation of the expression of PD-L1 by RT. The results showed that X-ray irradiation as well as charged particle irradiation increased the gene expression and cell surface abundance of PD-L1 in multiple cancer cell lines. Using chemical inhibitors and siRNA, it was demonstrated that this mechanism involves the activation of the DNA damage response pathway and relies on the transcriptional activity of IRF1, activated upon irradiation. Furthermore, charged particle radiation increases PD-L1 abundance more strongly than X-ray and this effect seems to be potentiated by an increasing LET at doses that are equally lethal to cancer cells). These results provide further evidence that different radiation modalities at equivalent doses induce different immune-modulatory pathways.
All together, these projects aim at enhancing the efficacy of radiotherapy by new developing radio-sensitization mechanisms coupled to evidence of the underlying mechanisms.

Early detection of subclinical left ventricular dysfunction by speckle-tracking echocardiography after radiation therapy for breast cancer: results from the MEDIRAD EARLY-HEART study

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Keywords: breast cancer; radiotherapy; strain imaging; MEDIRAD EARLY-HEART cohort

Background: Radiotherapy (RT) for breast cancer (BC) can lead to an excess risk of cardiovascular (CV) diseases arising many years after treatment[2,3]. Early detection of subclinical CV changes post-RT could prove beneficial for asymptomatic patients by investigating early subclinical left ventricle (LV) dysfunction.
Objective: Within the European MEDIRAD project, the multicenter EARLY-HEART prospective cohort aimed to assess the impact of RT-related cardiac exposure on subclinical LV dysfunction occurring 6 months and 24 months after RT based on measurements of myocardial function and wall deformation by speckle-tracking echocardiography[4].
Methods: The study included chemotherapy naïve BC women aged 40-75 years treated adjuvant RT. Multi-atlas based segmentation was used to determine whole heart and LV doses (Dmean for mean dose)[5,6,7]. Echocardiography was performed at baseline, 6 and 24 months after RT. Subclinical LV dysfunction was defined with two approaches: a relative decrease in global longitudinal strain (GLS) >15% compared to baseline value (GLS-based LV dysfunction)[8]; a decline in LV ejection fraction ≥10% from baseline to a final value less than 53% post-RT (EF-based LV dysfunction)[9].

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Results: The analyzed population included 186 BC women (mean age 57.5±7.9 years, 64% left-sided BC). Six and 24 months after RT, 14% and 13% of patients respectively presented GLS-based LV dysfunction and 4% and 13% respectively with EF-based LV dysfunction. Significant dose-response relationships were observed with the risk of GLS-based LV dysfunction, 6 months after RT (Odds Ratio OR=1.74 (1.20-2.61) for Dmean whole heart, OR=1.46 (1.17-1.87) for Dmean LV), and remained significant in the same range 24 months after RT. For EF-based LV dysfunction, there was no association with cardiac doses at 6 months, but a significant dose-response relationship was observed at 24 months: OR=1.50 (1.20–1.87) for Dmean whole heart, OR=1.91 (1.31–2.77) for Dmean LV.
Conclusion: These results highlighted that all cardiac doses were strongly associated with the occurrence of subclinical LV dysfunction arising 6 and 24 months after BC RT. Whether measurements of GLS at baseline and 6 months after RT combined with cardiac doses can early predict efficiently subclinical events occurring 24 months after RT remains to be investigated.

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Development of new radioprotective substances: An untold story.

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Keywords: gamma irradiation, in vivo studies, radioprotective agents

Purpose: The classification of chemical radioprotectant includes compounds with the capacity to mitigate the biological effects of ionizing radiation. The research conducted in this regard tries to discover and develop non-toxic active chemical compounds able to protect the affected individuals from various types of post-radiation damage. The present work evaluates a group of 1-(2-hydroxyethyl)piperazine derivatives as potential and affordable radioprotective agents.
Material and methods: Novel compounds were synthesized based on 1-(2-hydroxyethyl)piperazine derivatives and characterized using nuclear magnetic resonance and high-resolution mass spectrometry, followed by cytotoxicity and radioprotective evaluation *in vitro* (i.e. cell lines) and *in vivo* (i.e. an animal experimental model).
Results: In the present study, 19 compounds were tested, with compounds 3e and 8 showing a radioprotective effect that, although comparable with the other molecules tested, displayed a greater tolerance at higher concentrations *in vitro*. The derivate 3e prolonged mouse survival. There was also a significant lymphocyte count increment on day 30, erythrocytes on day 7, and platelets on day 12 after irradiation. On the other hand, compound 8 resulted in greater radioprotective and survival values in the tested mice after 30 days of irradiation. It must be noted that this compound is structurally different from the other derivatives, as it contains no aromatic moiety, has low toxicity, and it is highly soluble.
Conclusion: The overall design of this study included in silico study, in-house synthesis, physicochemical analysis, *in vitro/vivo* toxicity testing, and radioprotective effect evaluation. The most promising compounds were selected based on their toxicological and physicochemical profile, which suggested an interesting structural activity-relationship in the improved radioprotective effect of the tested compounds. Taken together, the present study reports the synthesis and biological characterization of a novel series of (4-alkylpiperazin-1-yl)-3-phenoxypropan-2-ol compound derivatives as promising radioprotective agents.

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Microbeam radiation therapy induces a sparing effect of normal tissue cells through increased expression of ROS scavengers

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Microbeam radiation therapy (MRT) employs spatially fractionated radiation with 25 - 100 µm peak and 400 - 800 µm valley regions. The dose in peak regions reaches up to several hundred Gy, whereas the dose in valley regions stays below the tissue tolerance dose. One hypothesis of an increased therapeutic window of MRT is the influence of intercellular communication.
The influence of intercellular communication after BB and MRT was analyzed by clonogenic cell survival, DNA damage repair, ROS production, and ROS gene expression were evaluated in tumor cell lines A549 and LN18 and normal tissue cell line MRC5.
MRT showed a reduced cell survival in A549 ($p < 0.01$) and LN18 ($p < 0.05$) and a higher cell survival in MRC5 ($p < 0.01$) compared to BB. In all three cell lines a significant increase in DNA double strand breaks was observed 30 min after MRT compared to BB (A549 $p < 0.0001$, LN18 $p < 0.0001$, MRC5 $p < 0.001$). However, 24 h after MRT the significantly higher damage remained in A549 and LN18 ($p < 0.0001$), whereas no significant difference was detected in MRC5 cells. The Amplex Red assay for measuring ROS production showed a significantly higher H2O2 production in LN18 and MRC5 cell lines 24 h after MRT compared to BB (LN18 $p < 0.05$, MRC5 $p < 0.001$). On RNA level, a significant increase in ROS scavengers SOD2 ($p < 0.05$) and PER3 ($p < 0.01$) was detected after MRT compared to BB. However, in the tumor cell line A549, PER3 was significantly upregulated after BB compared to MRT ($p < 0.05$), but no change was detected in LN18.
Our results demonstrate a normal tissue sparing after MRT *in vitro*. MRT caused less DNA double strand breaks and led to an increased survival of normal tissue cells and a reduced survival of tumor cells with a possible relation to an increased expression of ROS scavengers compared to BB. This diverse effect widens the therapeutic window of MRT, making it a promising novel radiotherapy approach.

Single cell profiling identifies a differential transcriptomic response to photon versus proton irradiation in salivary gland organoids

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Keywords: organoids, stem cells, proton therapy, scRNA-seq, ATAC-seq

An increasing number of head and neck cancer patients is being treated with proton therapy. Besides its physical advantages over conventional photon-based radiotherapy, little is known about the biological response to protons of healthy salivary gland tissue.
To investigate and compare the potential differences of photon and proton irradiation, we used a salivary gland organoid model and performed ATAC and single-cell RNA sequencing to identify transcriptomic changes at the single cell level. Our analysis showed the presence of a population of epithelial stem cells (EpSC) enriched in gene expression related to tissue and organ development. Interestingly, photon and proton irradiation (IR) led to a similar reduction of the EpSC population 2 days after IR. However, proton IR samples maintained a higher self-renewal capacity compared to photons, measured as secondary organoid forming efficiency (OFE). In line with this observation, we found that several transcription factors, highly upregulated in the EpSC population of non-irradiated organoids, were significantly downregulated upon photon IR but not protons. Furthermore, while photon IR induced a higher expression of genes related to stress and cell death, proton IR led to an enrichment of cell cycle related genes, such as *CDKN1A, CDKN2B* and *JUNB,* and tissue development related genes such as *SOX9* and *EPCAM.* Lastly, in both EpSC and salivary duct cells, we observed a deregulation of genes related to mitochondrial function upon photons compared to protons. This correlated with a slower recovery of the mitochondrial membrane potential detected by flow cytometry at later timepoints. Mitochondria play a crucial role in the maintenance of stem cell self-renewal and fate decision, and their dysfunction in the long term may affect salivary gland EpSC function. Our study suggests that photons lead to a higher EpSC functional decline compared to protons that provide new possibilities for future therapy improvements.

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Ionizing radiation effect on the extracellular vesicle cargo

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Keywords: Ionizing radiation, Extracellular vesicle, miRNA, RNA-binding protein

Background: Although ionizing radiation (IR) induced bystander effect (RIBE) is long-identified, the exact mechanisms behind the phenomenon have not been fully explored. Extracellular vesicles (EV) are able to induce RIBE, possibly due to their IR-altered cargo. miRNAs as key gene expression regulators carried by EVs, participate in the development of RIBE, but their incorporation methods into vesicles are not yet fully understood. In this study we examined and compared alterations in miRNA profile, and proteins packaging miRNAs into EVs after IR, in bone marrow cells (BMC) and bone marrow-derived EVs (BMC-EVs).
Materials and methods: Male CBA mice were total body irradiated with 0.1 and 3Gy and BMCs and BMC-EVs were isolated after 24h. 14 miRNAs were analyzed by qPCR. The concentration of miRNA packaging hnRNPA2B1 and hnRNPQ proteins were analyzed by Western Blot (WB), their cellular localization was investigated by confocal microscopy. Bioinformatic tools were used to calculate the effects of altered miRNA expression on signaling pathways, and to link RNA binding proteins to miRNAs.
Results: In BMCs the expression of 6 out of 14 miRNAs, while in BMC-EVs 9 out of 14 miRNAs was significantly altered by IR. Changes of miRNA profiles in EVs did not follow the pattern observed in BMC cells, indicating the existence of selective miRNA sorting. Pathway enrichment analysis revealed that differentially expressed miRNAs in EVs were involved in several cellular pathways, such as radiation-response related signaling pathways, support the role of EVs in RIBE. hnRNP protein levels were also modified both in BMC and EV after IR: A2B1 was increased in BMC while decreased in EVs, Q was not altered in BMC, but was elevated in EVs. Changes in the level of particular miRNAs carrying recognition motifs for hnRNP proteins followed the quantitative changes of their respective binding partner protein in EVs. For example miR-93, the most significantly decreased miRNA in EV, binds to hnRNPA2B1, which was also decreased in EVs.
Conclusion: Different miRNA profiles in BMC and BMC-EVs, along with a strong correlation between the level of hnRNP proteins and the concentration of their binding miRNAs in EV, support the existence of selective miRNA packaging mechanisms. Our results also indicate, that IR affects mechanism of miRNA sorting and incorporation into EVs.
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ORAL PRESENTATION

Development of a IORT Treatment Planning System using a GPU-based fast Monte Carlo

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Keywords: IORT, GPU, MonteCarlo, FLASH, TPS

Intra Operative Radiation Therapy (IORT) is a technique that, after the surgical tumor removal, delivers a dose of ionizing radiation (4-12 MeV electrons beam) directly to the surgery bed. During IORT treatments the beam is passively collimated by means of a PMMA hollow tube and whenever needed, temporarily beam modifiers are used to protect the healthy tissues surrounding the target. The use of high intensity pulses of electrons makes IORT the current best candidate for the first implementation of the FLASH effect into clinic. An important IORT limitation is the lack of a Treatment Planning System (TPS) capable of coping with the very limited amount of time available after the surgery (~ 1 min) to obtain both the new imaging of the surgical field, which has undergone substantial morphological modification, and the TPS computation. In this contribution, exploiting the new 3D real-time echographic imaging acquisition provided

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by the SIT company[1], we investigate the efficiency achievable in IORT and IORT-FLASH breast cancer treatment using a GPU-based (Graphics Processing Unit) fast Monte Carlo (MC) called FRED (Fast particle thErapy Dose evaluator)[2,3] for treatment planning. The FRED MC has been developed to allow a fast optimization of the TPS while keeping the dose release accuracy typical of a MC tool. Using FRED we have simulated in detail the LIAC HWL mobile IORT accelerator produced by SIT, and we have developed an optimization tool that, starting from the CT imaging, explores different treatment configurations. We have then combined the FRED simulation with a simple modeling of the FLASH effect. The tumour coverage and the dose absorbed by the organs at risk have been compared, carrying out a quantitative analysis adopting Dose Volume Histograms. The results demonstrate the potential of FRED as a tool for treatment planning and of the FLASH effect in IORT treatments.

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A systematic review of radiation biomarkers for the advancement of human space exploration.

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Keywords: cosmic radiation; spaceflight; biomarkers; gene expression; radiation effects; signal transduction; radiation exposure; radiation susceptibility; radiation sensitivity

Radiation biomarkers are an emerging and rapidly developing area of research, with potential applications in predicting individual radiation susceptibility, predicting severity of normal tissue injury among patients, assessing and monitoring of tumor response to radiation therapy as well as in estimating dose to accidentally radiation-exposed individuals. In this study we utilize the knowledge gathered from various fields about radiation response-related biomolecules to identify biomarkers for space radiation in view of long-term human space exploration. This comprehensive search for radiation biomarkers was divided into 'biomarkers of radiation exposure' and 'biomarkers of radiation susceptibility/sensitivity' based on time parameters and complexity of the topic (i.e. molecular influencers post-radiation exposure and molecules controlling susceptibility/ sensitivity prior and post-radiation). The strategy for the current study is adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and 2020 statement (1) and a guide for conducting a systematic review and meta-analysis (2). The search plan not only considers publications (study articles, literature reviews, systematic reviews, and meta-analyses) but also takes databases, repositories, and registers containing relevant information into account. Biomolecules such as genes, transcripts (coding and noncoding), proteins, as well as epigenetic and possible epitranscriptomic modifications related to radiation response events are all taken into consideration. This research presents a state-of-the art report on scientific evidence on biomarkers of response to ionizing radiation. The compiled list of recommended biomarkers could undoubtedly improve crew health risk assessment in order to better forecast the health implications of ionizing radiation exposure in astronauts and as a potential tool for biodosimetry.

Abstract acknowledgements: SCK CEN - European Space Agency (ESA) - BIOMOON proposal: ESA RFP/3-17004

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ORAL PRESENTATION

NECTAR: A NEW APPROCH TO ADDRESS ALZHEIMER'S TREATMENT

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Keywords: Microglial cells, low LET, high LET, inflammation

Approximately 50 million people globally suffer from Alzheimer's disease (AD) and no effective cure exists for this neurodegenerative disorder up to now. The NEutron Capture-enhanced Treatment of neurotoxic Amyloid aggRegates project (NECTAR) aims to develop, test and prove the feasibility, safety and effectiveness of a novel and revolutionary approach based on Capture-Enhanced Neutron Irradiation (CENI) for damaging and subsequent disintegration of amyloid-beta (Aβ) aggregates. The boron neutron capture technology (BNCT) radiation field consists of a mixture of qualities and, thus, the radiobiological effect of BNCT treatment consists of components with different linear energy transfer (LET) characteristics. In this study we are Modelling the mixed beam of the BNCT reaction on human microglial HMC3 cells using different proportions of alfa particles (high LET) and X-rays (low LET) to evaluate and quantify possible neurotoxic and inflammatory effects induced by this treatment. HMC3 cells were irradiated with different doses of α- (0.5-2.0 Gy), X-ray-radiation (0.5-2.0 Gy) alone as well as mixed radiation (0.5 α + 0.5 X; 1.0 α + 1.0 X; 1.5 α + 0.5 X; 0.5 α + 1.5 X Gy). We characterized the formation of DNA double strand breaks in cells exposed to various irradiation modalities by quantifying γ-H2AX foci. Corresponding experiments exhibited a significant decrease in cell viability and survival of HMC3 cells after exposure to more than 1.0 Gy of α and X-ray alone or mixed beam. Gene expression of two cytokines, IL-6 and IL-1β, was measured by qRT-PCR at 3 and 6 hrs after irradiation. We observed an increase in gene expression of IL-1β in cells irradiated with α-particles and mixed irradiation 6 hrs post-irradiation; no significant changes in cells irradiated with X-rays alone were observed. Studies will be extended to neuronal cells as well.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 964934.

ORAL PRESENTATION

The "Stealth-Bomber" paradigm: a conceptualization of the tumor cell response to carbon ion exposure

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Keywords: Carbon ions, ROS distribution, Tumor cells, Bomber effect, Stealth effect

Carbon ion (C-ion) radiotherapy has physical and biological advantages over conventional radiotherapy due to high energy deposits at the end of their course (Bragg peak). Photon irradiation induces uniform production of reactive oxygen species (ROS) in cells while C-ions exhibit a huge ionization density in individual tracks causing a localized ROS production at the nanometric scale.

Based on our experimental data and Monte-Carlo simulations, we proposed the paradigm of the "stealth-bomber" to conceptualize two opposing processes responsible for the biological superiority of C-ions over photons.

The "bomber" effect encompasses most of the deleterious properties of C-ions on cancer cells at the molecular and cellular levels. It is triggered when the biological targets, such as DNA or organelles, are on the trajectories of C-ions. In this case, the ROS clustered in the tracks are responsible for: complex and irreparable DNA damage; increased levels of oxidized proteins; the absence of dependency on telomere length, and the absence of dependency on intracellular oxygen concentration for the induction of cell death. The consequence, at an equivalent physical dose of photons, is a higher cell killing, specifically on cancer stem cells, by a p53-independent and ceramide-dependent mechanism.

The "stealth" effect symbolizes the property of C-ions to deceive the cellular defenses. Indeed, the absence of significant ROS production outside the C-ion tracks does not allow the achievement of a decisive ROS threshold necessary to activate survival pathways and defense mechanisms. This is objectified by: the decrease in the detection of DNA lesions and the activation of their repair; the non-activation of proliferative and invasive pathways; the absence of stabilization of the HIF-1α transcription factor and the non-activation of its numerous targets; the specific regulation of key effectors of the proteostasis network.

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Altogether, our results led us to propose this new paradigm, setting ROS spatial distribution at the nanometric scale as a highly relevant point, to explain the differential cellular responses to C-ion and X-ray irradiations. It also strongly suggests that hadrontherapy with C-ions will always display a much better efficacy relative to the most advanced conventional radiotherapy technology.

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Tritiated micro-particles: modelling *in-vitro* exposure and biological effects

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Keywords: Tritium, sub-cellular dosimetry, radiation-induced effects

In the next future, due to development studies on deuterium-tritium fusion reactors as well as to the decommissioning of old nuclear power facilities, the tritium release in the environment is expected to increase. New impact mitigation strategies, combined to a better understanding of tritium impact on health and environment, are therefore needed. In this context, the recently-concluded European multidisciplinary project TRANSAT[1] (Transversal Action for Tritium, 2017-2022) has contributed to improve the knowledge on tritium management in fission and fusion facilities and on the impact of specific relevant tritiated products, such as micrometric steel and cement particles, on environment and human health.
In this framework, we aimed at investigating the possible biological damage induced by radiation emitted by such tritiated particles at the cellular and subcellular level. Modeling is essential for the reconstruction of dose levels associated to common toxicological indicators of contamination with radioactive particles, as particle concentration. We thus created a software replica of the setup used for *in-vitro* experiments, with the bronchial epithelium BEAS-2B cell line exposed to tritiated steel and cement particles. We characterized the radiation field associated to the particle presence in the proximity of cells, to estimate, in particular, the dose absorbed by cell nuclei, the main critical target for radiation action.
The dosimetric reconstruction has allowed us to make predictions on the radiation-induced DNA damage, as a well-recognized indicator of the biological effectiveness of these tritiated products. Dosimetric results will be presented and discussed seeking for a better interpretation of the outcome of experimental in-vitro genotoxicity assays, to advance in informing dose-response curves and help in the challenge of building the bridge between radiobiological damage and risk for relevant specific exposure scenarios.

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Cellular effects of long-term radiation exposure on aquatic biota within the Chernobyl exclusion zone

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Keywords: Chernobyl exclusion zone, radioactive contamination, aquatic biota, chromosomal aberrations, hematological effects

The effects of chronic irradiation of aquatic biota in water bodies within the Chernobyl exclusion zone (CEZ) during 1998-2021 were studied. The absorbed dose rate for hydrobionts from water bodies of the CEZ registered in a range from 0.2 to 430 μGy/h and in the reference lakes - up to 0.08 μGy/h. It is determined that the rate of chromosomal aberrations in the root meristem tissues of aquatic plants in the most contaminated lakes on average in 2-3 times, and in cells of the pond snail embryos in 4-6 times exceeding the spontaneous mutagenesis level, inherent to aquatic organisms. During the period of studies, a tendency to decrease of chromosomal aberration level in mollusks from all lakes of the exclusion zone was registered. The probabilistic prediction of the chromosomal aberration rate for gastropod snails in lakes of the CEZ have shown that spontaneous mutagenesis level (2.0-2.5%) can be reach in the most contaminated lakes in 2060-s-2070-s. Analysis of leukograms of fish peripheral blood showed the decrease of lymphocyte cells, as well as the increase in the number of granulocytic cells with increase of radiation dose rate. Along with changes in leukograms an increased level of morphological damages of erythrocytes (structural and proliferation abnormalities) was determined, which is generally for pray fish in 4-12 times and for predatory fish in 7-15 times higher than in fish from reference lakes. High number of erythrocytes with structural and proliferation abnormalities in blood of fish from lakes with high levels of radioactive contamination allows us to assume that the qualitative indexes of red cells in blood of fish are more sensitive to chronic radiation impact in comparison with the elements of white blood. A variety of forms of pathological changes in the structure of blood cells, mainly erythrocytes, may indicate low resistance of cytogenetic apparatus of fish in the face of considerable mutagenicity and genotoxicity of environment. In this situation the ionizing radiation causes damage to the lipid structures of biological membranes (e. g. lysosomes) and violation of their barrier functions that ensure compartmentalization in the cell. This leads to disruption of spatial isolation of enzymes to their substrates and release enzymes to further destruction of macromolecules and intracellular structures. As a result, there are changes not only in the cytoskeleton, but also in functioning of all the organelles in the cell.

Effects of particle irradiation and oxidative stress response on survival and differentiation process of primary human stem cells

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Keywords: Hadrontherapy, Adipose-derived stem cells, Oxidative stress, Nrf2

Radiation therapy has made significant advancements in the past years and is considered as an indispensable tool in cancer treatment. Its main challenge is to destroy cancer cells without the depletion of healthy tissues. This is achieved by understanding the radioresistance mechanisms of cancer cells as well as normal stem cells upon ionizing radiation exposure. Stem cells possess the ability to regenerate themselves and differentiate into specialized cells. These two mechanisms are more or less regulated in order to limit abnormal expansion and lineage imbalance. Recently promising radiation therapy techniques such as hadrontherapy are increasing worldwide. A great concern is the late effects of these radiation qualities since accelerated particles produce complex DNA damage not only in the tumor but also touch the surrounding healthy tissue exposed to low dose levels. Furthermore, examining the biological plausibility of low-dose irradiation is an essential factor to follow up oxidative stress response that may impact proliferation and differentiation processes of normal stem cells. For this reason, we have carried out a study that compares different radiation qualities (X-ray, proton, and carbon ion) on adipose-derived stem cells (ADSC) in presence and absence of nuclear factor erythroid 2-related factor 2 (Nrf2), the cryoprotective controller against oxidative stress. Our results suggested an enhanced cell killing effect when these cells were subjected to carbon-ion irradiation in absence of Nrf2, and to a lesser degree when exposed to X-ray and proton.

We are now analyzing the stemness properties of the exposed cells before and after irradiation as well as their adipogenic and osteogenic abilities by FACS. This study conducts a mechanistic understanding of stem cell response to irradiation which leads to improving knowledge about the overall radiosensitivity of human stem cells.

3D liver biological scaffolds: a promising approach for the evaluation of low and high LET radiation effects on radioresistant cell lines

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Keywords: 3D liver bioscaffolds, low and high LET, mucosal melanoma, pancreatic carcinoma

Introduction

Compared to 2D cell cultures, ECM-derived bioscaffolds (Figure 1) retain specific growth factors that facilitate cell adhesion, tissue integration, remodeling and differentiation. Additionally, these scaffolds are porous allowing the transport of oxygen and nutrients to the seeded cells and ensuring the waste metabolites' physiological output.

Gynecological mucosal melanomas (MMs) and pancreatic cancer (PaC) are aggressive tumors with a dismal prognosis. Preclinical and clinical data showed that both tumors display intrinsic resistance to conventional radiotherapy (RT), while C-ion RT could be valuable for their treatment.

Materials and methods

MM HMV-II and PaC PANC-1 cells were seeded in decellularized biological scaffolds and irradiated with 2 Gy and 4 Gy of photons (X-ray) or C-ions. Afterwards, every seven days for four weeks, control and irradiated scaffolds were formalin-fixed, embedded in

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paraffin and sectioned. Their histological specimens were stained with Hematoxylin and
Eosin, Periodic acid-Schiff, Masson's trichrome, Alcian blue and Picrosirius red, and revised independently by two anatomopathological experts.

Results

MM and PaC cell lines repopulated the scaffolds in a histological-coherent way and were able to grow into depth, without dedifferentiation. Images of the sections indicate significant different morphological features, according to the type of RT and the characteristics of each cell line, starting from >7 days' time point. In particular, C-ions exhibited more severe effects on PANC-1 cells in terms of early molecular alteration (binucleated cells, increased nuclei size, cytoplasm disaggregation, diapedesis). Melanin production of HMV-II cells was premature in the scaffolds treated with X-ray compared to C-ions.

Conclusions

Hepatic scaffolds provide a fertile 3D environment for tumor cell growth and proliferation and represent a promising experimental approach for more comprehensive radiobiological studies, even in the case of long-term studies.

Figure 1: Hepatic biological scaffold.

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Double fraction of synchrotron Microbeam Radiation Therapy improves local control and triggers regression of locoregional metastasis in murine melanoma.

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Keywords: Microbeam radiotherapy, immune response, abscopal effect

Experimental synchrotron X-ray-generated microbeam radiation therapy (MRT) is an innovative model of cancer radiotherapy with an excellent therapeutic ratio, but optimization of the irradiation protocols, as well as assessment of metastatic spread, are needed to go ahead toward clinical implementation. Here, we demonstrated that: (i) two 396-Gy peak-dose fractions of MRT are more effective than one in attenuating tumor growth in the B16-F10 melanoma mouse model; (ii) both single dose MRT and broad beam irradiation accelerated the formation of metastasis in superficial cervical lymph nodes but remarkably the second MRT fraction triggered a very pronounced regression of locoregional metastasis that lasted for 5 weeks. This observed reduction cannot be explained by direct exposure of cervical lymph nodes to low-dose scattered radiation, therefore we hypothesized the presence of abscopal effects. In search for factors that generated this anti-tumor/anti-metastatic response, we measured plasma concentrations of 34 cytokines in cohorts of mice that received either one or two MRT fractions. Neutrophil and T cell-attracting chemokines CXCL5, CXCL12 and CCL22 were significantly increased two days after the second MRT irradiation, indicating that delayed melanoma growth and metastasis progression in animals treated with two MRT fractions could be a consequence of increased recruitment of anti-tumor neutrophils and T cells. Indeed, we demonstrated elevated infiltration of neutrophils and activated T-cells in the tumors following the second MRT.

Our study indicates the approach for an optimal MRT regimen that promotes local and locoregional tumor control with the potential to manage distant metastasis, a most common cause of death even after successful treatment of the primary melanoma.

FLASH with carbon ions: tumor control, normal tissue sparing and distal metastasis in a mouse osteosarcoma model

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Compared to conventional dose-rate irradiation, ultra-high dose-rate irradiation can substantially widen the radiation therapy window, allowing the radiotherapist to spare the healthy tissues while controlling the tumor with the same efficacy. Nowadays, this effect is known as the FLASH effect, and it is a breakthrough in radiotherapy.
Although the normal tissue sparing at ultra high dose-rate has been demonstrated with electrons, photons, and protons, so far, evidence with heavy ions is limited to in vitro cell experiments.
We present the first in vivo results with high-energy 12C- ions delivered at an ultra-high dose rate.

In our results, irradiation with an ultra-high dose rate of carbon ions was able to control murine osteosarcoma in the posterior limb of C3H/He mice the same as with conventional dose-rate irradiation.

Moreover, FLASH irradiation decreases normal tissue toxicity and significantly reduces lung metastasis compared to conventional dose-rate irradiation and sham-irradiated animals.

FLASH irradiation reduces radiation-induced skin and lung toxicity while being as efficient as conventional irradiation in antitumor response

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Purpose:
FLASH radiotherapy (FLASH-RT) is a new technique, involving treatment of tumours at ultra-high dose rates, which has been shown to reduce normal tissue from radiation-induced toxicity, whilst equalling the anti-tumour effect of conventional dose rate radiotherapy (CONV-RT). Here, we performed a dose-response study in mice comparing the effect of FLASH-RT versus CONV-RT on skin and lung toxicity as well as tumour response in a lung cancer xenograft model.
Experimental Design:
Two human tumours xenografted in CD1-nude mice and one syngeneic tumour xenografted in C57BL/6J mice were used for the comparative determination of the antitumor response. Mice were treated using a 6 MeV electron linear accelerator once xenografts reached 80 mm^3 with FLASH-RT or CONV-RT, both at a single dose of 20 Gy or at a fractionated dose of 30 Gy (3 x 10 Gy). Acute and late radiation effects were quantified in healthy C57BL/6J mice by skin toxicity scoring, lung CT-scan imaging and histopathological analysis, after hemithorax irradiation in the dose range of 10 to 30 Gy in a single fraction.
Results:
We found that FLASH-RT and CONV-RT showed similar efficacy with regards to growth delay/control of lung cancer cells transplanted into immunocompromised and immunocompetent mice. No differences were observed between the treatments with single dose of 20 Gy and fractionated dose of 30 Gy, for both, FLASH and CONV irradiations. No macroscopic signs of cutaneous lesions were observed after 30 Gy hemithorax FLASH-RT, although we observed hair depigmentation restricted to the irradiated area. In contrast, mice exposed to 20 or 25 Gy CONV-RT developed severe cutaneous lesions and earlier hair depigmentation. Both, lung CT-scan imaging and histopathological analysis, demonstrated lower inflammation after FLASH-RT compared to CONV-RT.
Conclusions:
In this study, the results showed that FLASH-RT reduces radiation-induced skin and lung toxicity, while showing equivalent tumour response as CONV-RT.

Towards a holistic approach to radiation protection of biota: the role of non-targeted effects

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The "non-targeted effects" of ionizing radiation including bystander effects and genomic instability predominate after low dose exposures and dominate response outcomes. These effects are unique in that no classic mutagenic event occurs in the cell showing the effect. In the case of bystander effects, cells which were not in the field affected by the radiation show high levels of mutations, chromosome aberrations, ROS and membrane signaling changes (horizontal transmission of mutations and information which may be damaging) while in the case of genomic instability, generations of cells derived from an irradiated progenitor appear normal but then lethal and non-lethal mutations appear in distant progeny (vertical transmission). The phenomena are characterized by high yields of mutations and distant occurrence of events both in space and time. This precludes a mutator phenotype or other conventional explanation and appears to indicate a generalized form of ROS mediated stress induced mutatgenesis which is well documented in bacteria. The nature of the signal travelling between irradiated and unirradiated cells and organisms is currently unknown but our recent experiments suggest that there may be a physical component such as a vibration wave involved. UV photon mediated transmission has also been documented and the latter mechanisms can induce the release of exosomes which by themselves can induce bystander effects when added to recipient cells . This presentation will discuss the phenomenology of non-targeted effects both in vitro and in vivo, including recent data suggesting that excitation decay-induced photons in the UVA range lead to exosome release and consequent mitochondrial malfunction and elevated ROS in recipient cells. Photons, calcium, and neurochemicals are important in signal production while the exosome cargo, and cytokine mediated pathways especially TGFb determine response to the signal. By highlighting some key challenges and controversies, concerning the mechanisms and more importantly, the reason these effects exist, current ideas about the wider implications of non-targeted effects in radiation protection, evolution and biology in general will be discussed.

Biological effects of *in vitro* exposure to continuous and pulsed 1,6 GHz radiofrequency

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Keywords: RF-EMF, CW/PW, biological effects, RNA sequencing

The continuous increase of the use and diffusion of technologies making use of radiofrequency electromagnetic field (RF-EMF) is generating a continuous growing concern in both policy authorities and general public, about possible effects and risks on health and environment (1). Although several researchers have been performed to identify non-thermal effects by *in vitro* studies using various assays on different cells and tissues, the reported results are contradictory (2). This is probably due to high experimental variability, linked to the quality of the EMF exposure systems, the different type of cells, and the not optimized and robust experimental design (3). Moreover, the biological effects related to different modulation (continuous and pulsed waves) of the RF signal is a not yet sufficiently investigated topic (***4***).
In light of these observations, the purpose of the present research project is to investigate non-thermal biological effects in human dermal fibroblasts (HDF) exposed to 1.6 GHz using a multi-methodological approach. The HDF will be exposed to continuous (CW) and pulsed (PW) wave for 2 h at the specific absorption rate (SAR) of 0.4 W/kg. The different assays performed in this study will include: γ-H2AX, CREST-micronuclei, chromosome mis-segregation, mitotic spindle, cell cycle, ultrastructural and protein expression analysis. In addition, wide genome expression profile will be evaluated through the RNA sequencing approach by high-throughput NGS.
Preliminary results will be presented and discussed.
Acknowledgement: This work is performed in the framework of the European Defence Agency (EDA) project Nº. B 0987 IAP2 GP "Biological Effects of Radiofrequency Electromagnetic Fields (RFBIO)" funded by the Italian MoD.

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In Silico Comparisons of FLASH Proton Therapy versus Conventional Dose Rate Treatment Plans

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FLASH proton therapy (FLASH-PT) aims to deliver beams of ultra-high dose rates (≥ 40 Gy/s) to induce a normal tissue sparing (FLASH) effect whilst maintaining the anti-tumour effectiveness of conventional dose rates. Compared to conventional radiotherapy techniques, FLASH-PT differ in the beam delivery, dose rate, and fractionation schemes used. Hence, the logistics of the clinical implementation of FLASH-PT, including the treatment planning process, may need to be redefined. This study aims to compare the *in silico* results produced by a novel FLASH-PT treatment planning system to the results produced for conventional dose rate techniques such as 3DCRT, VMAT, and IMPT. In this study, nine patient cases of bone (3), brain (3), and lung (3) metastases were considered, all previously clinically treated with 3DCRT/VMAT. Treatment plans for FLASH-PT and IMPT were created using a research version of the MIROpt TPS, developed by Ion Beam Applications SA from the open source version of UCLouvain. Conformal FLASH proton beams are produced using monoenergetic spot scanned protons traversing through a conformal energy filter, range shifter, and an aperture. A FLASH dose rate constraint of ≥ 40 Gy/s was included in the plan optimisation. Comparisons of the conformal FLASH-PT plans to the 3DCRT/VMAT and optimised IMPT plans were made using dose volume histograms, boxplots, dose maps, and the Wilcoxon Rank Sum Test ($p < 0.05$). Conformal FLASH-PT treatment plans, satisfying the FLASH dose rate constraint, exhibited no significant differences when compared to the 3DCRT/VMAT and optimised IMPT plans. This study demonstrates that conformal FLASH-PT treatment plans, showing equivalence to 3DCRT/VMAT and optimised IMPT plans, can be produced. Future work involves the physical verification of the calculated versus delivered doses, to confirm the safety and accuracy needed for the clinical implementation of conformal FLASH-PT.

4-methylumbelliferone enhances the radiosensitivity of the radioresistant oral squamous cell carcinoma cells through hyaluronan synthesis 3 suppression

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Keywords: radioresistant cancer cells, hyaluronan, 4-MU, HAS3

Cancer cells acquired radioresistance after radiotherapy cause recurrence and metastasis. We previously found that 4-methylumbelliferone (4-MU), which is a hyaluronan (HA) synthesis inhibitor can enhance the radiosensitivity of the radioresistant cancer cells (RR cells). In this study, to elucidate the relationship between HA inhibition and radioresistance, we used the RR cell lines (HSC2-R cells and HSC3-R cells), which were established by long term fractionated X-ray irradiation to the oral squamous cell carcinoma (OSCC) cell lines (HSC2 cells and HSC3 cells).

In the RR cells, the mRNA expression of HA synthase (HAS) 3 was significantly higher than that of the parental cells, whereas 4-MU significantly suppressed its expression and HA concentration of the culture medium. The intracellular reactive oxygen species (ROS) level of the RR cells, which is an DNA damage inducer, was lower than that of the parental cells, and significantly enhanced by 4-MU compared with non-treatment control cells. The wound healing ability of the RR cells, which represents cell migration ability was significantly suppressed by 4-MU treatment compared with non-treatment control cells. The same effect was confirmed in the induction of epithelial mesenchymal transition by epithelial growth factor. To confirmed that the extracellular HA involved in the radioresistance and cell migration, it was depleted by using *streptomyces*-hyaluronidase (*St*-Hyal), which is a HA degradation enzyme. Although *St*-Hyal administration completely depleted HA in the culture medium, the cell survival and migration ability were not change. Based on these results, the radiosensitization and anti-migration effects of 4-MU may be caused by suppressing the oncogenic and DNA damage protection signaling via HAS3 expression rather than depletion of extracellular HA. Therefore, we investigated whether the HAS3 knockdown mediated by siRNA sensitize the RR cells. The cell survival of HAS3 knockdown cells treated with X-ray irradiation was significantly suppressed compared with their control cells treated with X-ray, suggesting that HAS3 can be a target for radiosensitization in RR cells.

In conclusion, our study suggested that HA synthesis inhibition, especially HAS3 inhibition caused by 4-MU treatment enhances the radiosensitization of RR cells.

DNA methylation alterations in fractionally irradiated rats and breast cancer patients receiving radiotherapy

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Introduction: Breast cancer Radiotherapy (RT) has significantly improved patient treatment outcomes, however, it also increased cardiovascular mortality due to radiation-induced cardiovascular disease (RICVD). DNA methylation is an epigenetic mechanism which can regulate gene expression that was found to be dysregulated in cardiovascular diseases. We therefore investigated RT-induced DNA methylation alterations in cardiac-relevant genes and the subsequent effects on gene expression. **Methods**: Female wistar rats received whole heart fractionated X-irradiation (0, 0.04, 0.3 and 1.2 Gy) for 23 daily fractions. Blood was collected at 1.5, 3, 7 and 12 months after irradiation. SureSelect MethylSeq was used to identify differentially methylated genes (DMG) at 1.5 and 7 months and expression of the top cardiac-relevant DMG was evaluated by RT-qPCR. In addition, methylation and expression levels of these selected DMGs was explored in blood of breast cancer patients receiving RT and sampled at diagnosis, after RT and 6 months after RT using Illumina EPIC beadchip array and RT-qPCR, respectively. **Results:** Overall, radiation induced global hypomethylation. SLMAP showed hypomethylation at 1.5 months which correlated with significantly increased gene regulation in rats. For the other selected genes, (ITPR2, E2F6 and PTPN2), the correlation between methylation status and gene expression was less clear. In breast cancer patients, ITPR2 and E2F6 exhibited differential methylation which was associated with differential expression. **Conclusion:** Local rat heart irradiation induces alterations in methylation and expression of SLMAP, ITPR2, E2F6 and PTPN2. ITPR2 and E2F6 were differentially expressed in irradiated rats and in breast cancer patients received radiotherapy, therefore present promising candidates as biomarkers for RICVD risk assessment and warrants further research in a bigger cohort.[i]

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ORAL PRESENTATION

Targeting DNA repair and oxidative stress response pathways to overcome radioresistance of glioblastoma cancer cell

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Ionizing radiation, Cancer stem cell, Oxidative stress, Nrf2, Parp1

Ionizing radiation (IR) kills cells primarily by generating reactive oxygen species (ROS) and cytotoxic double strand breaks (DSBs) in DNA. Cancer stem cells (CSC) have often higher expression of antioxidant and an effective DNA repair systems that protect them from the effect of IR and lead to relapse, consequently. The main objectives of our project are to establish CSC line with combined deficiencies in two pathways namely oxidative stress response and DNA repair, using inhibitors and/or knock down of the involved genes and to analyze the effect of combined inhibitions with IR (x-rays, protons and carbon ions) on the radioresistant of the cells.
U87 glioblastoma cancer stem cells were cultured as spheres in medium in present of Parp1, Nrf2 inhibitors. Counting of the sphere numbers and sizes showed that treatment of the cells with inhibitors decreases the sphere formation and the size of sphere. Our preliminary results showed that pre-treatment of cells 76 hours prior exposure to IR with Parp1i and Nrf2i has no effect on the number of formed spheres as compared to control without inhibitors.
However, when the inhibitors were present 76 hours before and 7 days after irradiation, the number of spheres decreased, with Nrf2 inhibitor after carbon ions irradiation and with the double inhibition (Parp1 and Nrf2) after X-ray exposure. Further, to compare the effect of radiation quality, we irradiate the cells (+/- inhibitors) with x-rays, protons and carbon ions. Sphere formation assay showed carbon ions irradiation is more effective in killing cancer stem cells, comparing to the protons and x-rays. Preliminary results indicate that treatment with the inhibitors modify the effect of x-ray more than carbon ions and the analysis of proton irradiated cells are ongoing.

Role of DAP3 in cell cycle regulation in human lung adenocarcinoma cell lines exposed to ionizing radiation

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Keywords: DAP3, G2 arrest, cdc2, checkpoint kinase 1

Mitochondria play important roles in cellular response to various stresses including ionizing radiation (IR). Previously, we reported that mitochondrial ribosome protein death-associated protein 3 (DAP3) regulates radioresistance of human lung adenocarcinoma cell lines A549 and H1299 [1]. However, the underlying mechanisms by which DAP3 regulates the radioresistance remain to be elucidated. Here, we investigated the role of DAP3 in cell cycle regulation after IR to obtain clues for the mechanism of DAP3-mediated radioresistance in A549 and H1299. We prepared DAP3-knockdown A549 and H1299 cells, and analyzed the effect of DAP3 knockdown on cell cycle regulation after IR. Notably, although DAP3 knockdown hardly affected cell cycle distribution of non-irradiated cells, it attenuated the increase in G2/M population by IR. Therefore, we next analyzed the protein expression of G2/M regulators such as cdc2-Tyr15 phosphorylation (pcdc2). The results showed that DAP3 knockdown suppressed IR-increased pcdc2 expression accompanied by G2/M arrest. Furthermore, the analysis of phosphorylated expression of checkpoint kinase 1 (pchk1)-Ser296 and pchk2-Thr68, which coordinate cell cycle progression, revealed that DAP3 knockdown decreased IR-induced pchk1 and pchk2 expression. Intriguingly, chk1, but not chk2, inhibitor could suppress IR-induced G2/M arrest.
Collectively, these results suggest that DAP3 regulates IR-induced G2/M arrest through chk1-cdc2 axis in human lung adenocarcinoma cells.

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POSTER SESSION 1

Radiology during pregnancy: risks, radiation protection in medical practice, and communication with the patient

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Keywords: radiation protection, pregnancy, radiology, communication

Purpose: Radiation protection practices differ between hospitals when it comes to radiology during pregnancy. To remedy this best practices were identified by a literature review, interviews with radiographers, and interviews and a survey among pregnant women.

Materials and Method: The literature review was used as input for both the interviews and the survey. The interviews were conducted with 52 radiographers in focus group sessions. Three main topics were selected: (1) dose reduction, (2) confirmation of pregnancy, and (3) risk communication. In addition, 150 recently pregnant women were involved: 10 were interviewed and 140 filled in a questionnaire.

Results: The literature review showed: (1) deterministic effects will in radiological practice never occur, because of the threshold dose of around 100 mGy; (2) stochastic effects have not been observed in recent studies, but may occur when the fetus is inside the X-ray beam. However, even for high dose procedures, the risk is very low.
The outcomes of the focus groups were: (1) no consensus about shielding when the fetus is outside the X-ray beam, (2) better justification by requesting physicians, (3) a need for a multi-lingual, informative website and (4) a need for a list of dose-reducing measures. (5) Both the requesting physician and the radiographer should inquire after pregnancy (because of the time between request and actual procedure).
The interviews with pregnant women showed that radiation risk information fell short. The survey indicated that 68% would like to have information about radiation risks beforehand. Most women (56%) chose a pictogram matrix as their preferred way to visualize the risk.

Conclusions: The scientific literature shows that risks of radiology during pregnancy are minimal, but anxiety among patients can be reduced by proper communication about radiation risks and uniform evidence-based procedures in all hospitals.

Study of potentially renal cancerous effect of uranium in genetically-engineered mouse models: UKCAN project

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Keywords: Uranium, kidney cancer, occupational exposure, ionizing radiation, instillation

Exposure of uranium compounds can occur in several situations including nuclear fuel processing, military activities, and natural exposure. Uranium (U) is a radio element with known radiological (α-emitting radionuclide) and chemical (as a heavy metal) toxicities, which accumulates preferentially in kidneys. Renal carcinogenic effect in U-exposed population is suspected and need additional epidemiological or experimental studies.

Since the process of initiation and propagation of cancer can be particularly long, we have chosen to use two genetically-engineered mouse models (GEMM) predisposed to its development to investigate the link between uranium exposure and the risk of renal cancer.

The project aims to:

- Assess the relevance of non-invasive imaging tool (ultrasonography, metabolic cages) and innovative biomarkers in urines to determine the incidence and grade of tumors
- Study of benign and malignant renal tumors in GEMM models as a function of time and dose of uranium exposure through histo- and immunohistology
- Determine the renal oncogenesis and carcinogenesis biological mechanisms and cancer hallmarks (initiation, promotion, proliferation) in GEMM by clinical biochemistry, molecular biology.
- Analyze the uranium content in targets organs and excreta's by ICP-MS

Our preliminary studies aim to correlate specifically histology to ultrasound echography in order to follow-up endogenous renal tumors and determine their characteristics (type, number, volume). The phenotypic characterization is done at regular time intervals up to the age of 1 year (bodyweight, diuresis, ultrasonography). The evolution of renal injuries and masses leading to tumors as small as 200 µm are detected and quantified. Histological analyses showed delimited areas of different tumor types in GEMM. Immunostaining for CAIX, CK7, PS6 or VIM protein allows the identification, localization, and quantification of tumors in renal tissue. Immunostainings are in progress to complete the phenotypic characterization of RCC.

The comparison of the increase, or not, of tumor effects in these respective models will provide experimental knowledge to analyze the possible link between uranium exposure and the development of renal cancer.

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Stromal Vascular Fraction mitigates radiation-induced gastro-intestinal syndrome

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Accidental or intentional radiation exposures have serious health consequences for exposed individuals and can affect a large number of people. Large volume irradiation at high irradiation doses induces multiple tissue lesions. The gastro-intestinal tract is particularly sensitive to irradiation and lethality. At dose more than 10 Gy results in diarrhea, dehydration, sepsis with mortality within 10 days post-exposure. Radiation-induced gastrointestinal syndrome (GIS) results from direct cytocidal effects on intestinal stem cells and crypt stroma impairing epithelial regeneration. Given the logistical hurdle and the urgency for treatment in large numbers of casualties, there is a tremendous need for effective therapeutic measures, even if implemented several days after radiation exposure. The stromal vascular fraction (SVF) derived from adipose tissue is an easily accessible source of cells with angiogenic, anti-inflammatory, immunomodulatory, and regenerative properties. We examined whether SVF restores the irradiated intestinal cells niche and mitigates the GIS.

Mice exposed to abdominal radiation (18Gy) received an intravenous injection of SVF ($2*10^6$ cells) on the day of irradiation.

When injected before 24hours post-irradiation, the SVF limited the weight loss and inhibited the intestinal permeability and mortality after abdominal irradiation[1]. Histological analyses of intestine showed that SVF stimulated the regeneration of the epithelium by promoting the restoration of the cell population in the intestinal stem cell compartment. The intestinal “organoid” model that mimics the *vivo* response confirmed that SVF treatment stimulated the intestinal stem cell compartment. SVF has an anti-inflammatory effect by repressing pro-inflammatory cytokines, increasing the presence of anti-inflammatory monocyte subtypes $CD11b^{+}Ly6c^{low}CX3CR1^{high}$ in the ileum.

The SVF by inducing regeneration of intestine could be a promising therapeutic approach for the treatment of GIS.

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Extracellular vesicle mediated bystander effects in ionising radiation induced leukaemia

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Extracellular vesicles (EVs) are a class of small biologically active lipid-membrane enclosed vesicles, comprised of microvesicles and exosomes. They are an important component of intercellular communications, capable of shuttling proteins, lipids, lncRNA and miRNA cargoes from cell to cell (1). Cancer cells are very active in EV production (2), and tumour-derived EVs carry a high proportion of oncogenic miRNAs and proteins (3,4,5,6), suggesting that they participate to the remodelling of their immediate environment, mainly promoting growth. Acute myeloid leukaemia (AML) is one of the most common secondary cancers post ionising radiation (IR) exposure. EVs can possibly propagate radiation effects, e.g. DNA damage via the bystander effect (7), and are implicated in radiation-induced AML (rAML).
The gene PU1/Sfpi1 exhibits a dose dependent deletion of a single allele post IR exposure in the mouse CBA mouse model with a proportion of mice developing rAML (8,9). To investigate the role of EVs in rAML, a tailored transgenic CBA mouse model was used, engineered with mCh and GFP fluorescent markers integrated next to Sfpi1 on chromosome 2 alleles, as such, loss of mCh or GFP signal is indicative of a preleukaemic clonal expansion (10). Mice were either mocked or 3 Gy X-irradiated at 10-12 weeks of age and blood samples were analysed by flow cytometry for a period of 12 months (or until death if before this period) for monitoring allele deletion. A distinct, radiation and pro-oncongenic miRNA signature was found in mice with pronounced levels of leukaemic clonal expansion. Dysregulated miRNA included mmu-miR-761 and mmu-miR-29b, among others. Further experiments on murine bone marrow derived primary cells (macrophages, mesenchymal stem cells, and haematopoetic stem cells) demonstrated a conserved miRNA profile post X-irradiation, such as an upregulated level of mmu-miR-582-5p, previously found upregulated in rAML (8), as well as other miRNA related to the regulation of lysine degradation. In parallel, the direct impact of EVs on bystander cells was also assayed, wherein the human myelomonocytic cell line CESS was irradiated, its EVs labelled and coincubated with human T-lymphocytes, which were subsequently sorted into EV-positive and EV-negative populations. The

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levels of DNA and chromosomal damage were measured by yH2AX and dicentrics respectively.
The role of EVs in radiation-induced carcinogenesis will be discussed.

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Radon progeny adsorption on facial masks

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Keywords: Radon progeny, filtration, radiation protection

The radioactive noble gas radon (^{222}Rn) is responsible for around half of annual radiation exposure from natural sources. Radon will decay into several short-living progeny (^{218}Po, ^{214}Pb, ^{214}Bi and ^{214}Po) which can be inhaled and will deposit their energy in the lung. Therefore, progenies are responsible for more than 95% of the total effective dose and together with radon are classified as carcinogenic for lung cancer.
Filtration of the progenies will reduce the dose to the lung and is typically done by HEPA-filter or electrostatic air cleaners. Due to the Covid19 pandemic, wearing of face masks as a safety measure is recommended by WHO. In our study we investigate the filtration properties of FFP2 mask and surgical mask (II R) for radon progeny.
Therefore, FFP2- and II R-mask were attached to a measurement device (EQF 3220, Sarad GmbH), which can distinguish between radon progeny in dependence of their size distribution, ranging from unattached (< 5 nm) and clustered (20-100 nm) to attached (> 100 nm) progeny. In parallel, it is measuring the radon activity-concentration during experiments. This setup is placed inside a radon chamber[1]. Usual measurement times were up to 5 hours, allowing radon to achieve radioactive equilibrium with its short living progeny after approximately 3 hours. Therefore, only data 180 minutes after start of radon exposure were analyzed. The measured progenies were normalized to the radon activity concentration. By comparing background measurements without filter and experiments with FFP2- and II R masks, the percentage of retained radon progenies was determined.
For unattached progeny the percentage of retained radon is almost the same for FFP2 (98.8 ± 0.6%) and II R mask (98.4 ± 0.7 %). For clustered progeny, there are minor differences (FFP2: 85.2 ± 18.1 %; II R: 79.5 ± 22.1 %). Therefore, we show that both masks are effective in filtering radon progeny and thus are capable of reducing the total effective dose to the lung.

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Radon solubility in human blood and components

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Keywords: Radon solubility, human blood, radiation protection

Radon as a natural occurring radioactive noble gas contributes significantly to annual radiation dose. Despite being carcinogenic, it is also used to treat inflammatory diseases like rheumatoid arthritis. Detailed knowledge of the radon distribution within the blood is needed to evaluate partition coefficients. However, data for human blood is scarce and therefore we tested human whole blood, plasma and erythrocytes. In order to understand the underlying mechanisms determining radon solubility bovine protein solutions of hemoglobin and albumin were tested. Additionally oleic acid and pentanol were used to test additivity of solubility as assumed by ICRP[1]. There it is stated that solubility in tissue can be calculated on the basis of its composition, for instance solubility in breast tissue is calculated as a combination of fatty and muscle tissue.

Samples were exposed to a defined radon activity concentration under controlled conditions for one hour. After sealing the samples radon tight, the radioactive equilibrium between radon and its γ-emitting progeny (^{214}Pb, ^{214}Bi) is achieved after a few hours. Extrapolating the activity of γ-emitting nuclei measured with γ-spectroscopy, the initial radon concentration was determined. Subsequently, using the samples mass and radon activity concentration during exposure the solubility is calculated.

Comparing the human samples, plasma showed highest solubility followed by erythrocytes and whole blood. Solubility in protein solutions was in the same range and only significantly decreased when hemoglobin was heat denatured before exposure. Measurements of oleic acid and pentanol seem to contradict the additivity of solubility assumed by ICRP. Based on this, a re-evaluation of solubility data should be envisaged. Our measurements indicate that a there are more complex mechanisms on the microscopic scale determining solubility than pure tissue composition.

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Interphase Fluorescence In Situ Hybridization (FISH) for interphase chromosomal aberration-based biological dosimetry

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Keywords: Radiation, Biodosimetry, Chromosomal aberration, Interphase nucleus, fluorescent in situ hybridization (FISH)

Metaphase spreads stained with Giemsa or painted with chromosome specific probes (FISH) have been in use since long for retrospective dose assessment (biological dosimetry). However, in cases of accidental exposure to ionizing radiation the culturing of lymphocytes to obtain metaphase chromosomes and analysis of chromosomal aberrations is time-consuming. Similarly, analyzing chromosomal damage in G0/G1 cells or non-dividing cells by premature chromosome condensation (PCC) is laborious. Following large scale radiological emergencies, the time required for analysis is more important than precision of dose estimate. Painting of whole chromosomes using chromosome-specific probes in interphase nuclei by the FISH technique will eliminate the time required for cell culture and allow a fast dose estimate, provided that a meaningful dose response can be obtained by scoring color changes between chromosomal domains visible in interphase nuclei.

In order to test the applicability of interphase FISH for quick biological dosimetry, whole blood from a healthy donor was irradiated with 8 Gy of gamma radiation. Irradiated whole blood was kept for 1 h at 37°C to allow DNA repair and thereafter processed for FISH with probes specific for chromosome 1 and 2. Damaged chromosomal fragments, distinguished as having an extra color domain, were observed in interphase nuclei of lymphocytes irradiated with 8 Gy. These fragments were efficiently detected and quantified by the FISH techniques utilizing both confocal microscopy and fluorescence microscopy. Furthermore, our investigation showed a clear dose response curve for exposure to 0, 1, 2, 4 and 8 Gy of gamma radiation. These results demonstrate interphase FISH as an interesting test for biodosimetry and for studying cytogenetic effects of radiation in non-dividing cells.

Shielding of the femur reduces the severity of the gastrointestinal subsyndrome of ARS and increases survival in mice

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Keywords: Acute radiation syndrome, Radioprotection

Acute radiation syndrome (ARS) appears in individuals that receive a large, acute radiation dose to a significant amount of the body. The condition is classified according to the affected organ system into the hematopoietic, gastrointestinal and neurovascular subsyndromes. Depending on the dose received, the mortality can reach 100% within days after exposure. Today, there is no preventative or curative treatments available for ARS, and treatment options generally consists of supportive care including blood transfusion and bone marrow transplant, administered according to the symptoms presented.

In this study, C57BL/6j mice received a 225 kV x-ray dose of 8.5 Gy at a dose rate of 59 Gy/h. For 50% of the mice, a lead shield was placed in front of the left hind leg to shield the femur and mimic bone marrow transplant, as demonstrated by Van Bekkum et al.[1] The rest received 8.5 Gy as a total body dose. We monitored pain scores according to a system developed by Nunamaker et al.[2], and weighed the mice twice per day in the critical period.

All unshielded mice reached humane end points within 10 days. Symptoms included high pain scores, depilation, diarrhea and weight loss. Necroscopies revealed empty and partially hemorrhaged stomachs and intestines, suggesting that death was caused by the gastrointestinal subsyndrome. This was confirmed by histological examination of the small intestine.

The mice that were partially shielded all survived until termination of the study (110 days). In the first 10 days, symptoms in this group included moderate pain scores, depilation and weight loss. After 10 days, the pain scores stabilized at a low level and all mice eventually gained weight above the baseline level.

Our results show that the subsyndromes of ARS are not easily separable, and that early treatment of the hematopoietic subsyndrome has potential to alleviate the gastrointestinal subsyndrome to a point that significantly increases the chance of survival in the affected individuals.

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Low doses of chronic ^{137}Cs contamination impairs olfactory capacity of mice with sexes differences

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Keywords: 137Cs, olfactory function, ionizing radiation, sexes

Resuspension of Cesium 137 (^{137}Cs) present around the contaminated Chernobyl Exclusion Zone is possible by diverse external elements. Wildfire occurring in April 2020[1] or military moving in February 2022 induce respectively a release of ^{137}Cs and possibly a stirring up dust. People living around this zone can therefore be exposed to low doses of ^{137}Cs by inhalation and contaminated food. Little is known about effects of ^{137}Cs inhalation, although, a study found a link between moderate doses of ionizing radiation and olfaction[2]. This study has shown that people exposed to external radiation presented impaired olfactory function. The aim of our study is to understand if low doses of ^{137}Cs contamination could also impairs this olfactory function.

ApoE$^{-/-}$ male and female mice received water ad libitum supplemented or not with 20, 100 or 500kBq/L of 137Cs for 6 months. We expect that ^{137}Cs present in the litter is inhaled by mice. In parallel, to evaluate their olfactory capacity, some mice received predator odor to test the induction of stress. The weight of mice was monitored, behavior test to explore olfactory activity and stress are realized before euthanasia and olfactory epithelium and bulb were collected.

Our results show that male mice exposed to ^{137}Cs spend less time exploring unknown odors than controls mice. They also spend less time doing olfactory exploration in general. On the other hand, this effect seems absent in female but they failed to stress in response to predator odors assuming an impairment of olfactory system. Moreover, only non exposed animals to ^{137}Cs lost weight du to stress induced by predator odor. Immunostaining of olfactory epithelium is ongoing to explore potential alterations which could explain the loss of olfactory function and the difference between males and females.

Thereby, we found that low doses of chronic 137Cs contamination could impair olfaction of mice in different levels between males and females.

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Effects of low and moderate doses of ionizing radiation on a aortic aneurysmal model of Angiotensin II infusion ApoE$^{-/-}$ mice

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Keywords : Aneurysm, abdominal aorta, microenvironment, radiation

Exposure of populations to ionizing radiation show a correlation between this exposure and the occurrence of cardiovascular disease (CVD)[1,2,3]. While systemic review of published data did not find a clear link of low doses radiation to circulatory diseases[4,5]. *In vivo* experimental studies suggested that chronic low dose rate ionizing radiation induced a protective effect on atherosclerosis in rodent studies with a decrease in inflammation in atheromatous plaques and plaques size[6,7]. The first cause of CVD morbidity and mortality is atherosclerosis and is followed by aortic aneurysm. In our study we evaluated the impact from low to moderate dose of ionizing radiation in aortic abdominal anevrysm. We used a model of male ApoE$^{-/-}$ mice at the age of 10-12 weeks. Those mice were implanted with subcutaneous osmotic pump (model 2004 Alzet) filled with a solution of angiotensin II (Angio II) to perfuse at 1000 ng/kg/min during 7 or 28 days[8]. Mice received X-ray total body irradiation with the same dose rate at different doses (50, 500, 1000, 5000 mGy and control) 3 days before or after the Angio II infusion. We observed in mice irradiated before aneurysm induction, a significant decrease in the incidence of pathology with the highest doses (1000 mGy and 5000 mGy) and a significant decrease in the systolic aortic diameter with 500, 1000 and 5000 mGy. Moreover, at 1000 mGy, we shown a decrease in the actin contained in the smooth muscle cells suggesting vascular wall remodeling. Whereas in mice irradiated after Angio II infusion, we didn't observed any tendency in pathological incidence or the dilatation mesurement. Our results suggest a dose and microenvironment dependant effect on a mice model of aortic anevrysm.

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←CONT'D

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Low doses of chronic ^{137}Cs contamination effects on atherosclerosis

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Keywords: 137Cs, atherosclerosis, low doses, ionizing radiation, sexes

Epidemiological studies have shown a relationship between high doses of ionizing radiation (IR) and cardiovascular diseases, however studies are uncertain for low doses[1]. Populations living in radiation-contaminated territories, such as Chernobyl and Fukushima, are chronically exposed to external and internal γ radiation due to the ^{137}Cs released in the environment. Thus, a central question in radiation protection research is to understand if low-doses exposure to IR play a role in progression of cardiovascular diseases. Previous animal studies have shown some protective effects of chronic low dose γ exposure on vascular system[2]. These data were obtained in male mice with chronic contamination to ^{137}Cs until 100kBq/L in drinking water for 6 months. However, chronic higher doses in male and female mice have never been studied.

In our study, we evaluated a potential threshold effect on cardiovascular system up to 100kBq/L in male and female mice. ApoE$^{-/-}$ male and female mice receiving water ad libitum supplemented or not with 20, 100 or 500kBq/L of ^{137}Cs for 6 months before euthanasia and collection of plasma, aorta and heart.

We measured differences in absorbed doses between males and females mice which is confirmed by a higher activity per gram of skeletal muscle in male for the 500kBq/L groups. Histological, immunohistochemical analyses of aortic plaque phenotype didn't show any differences in plaque size and lipid content in male mice contaminated with different concentrations of ^{137}Cs. However, the identification of immune cells recruited near the plaque could change with the increasing dose from 100 to 500kBq/L of ^{137}Cs and data for female are still expected.

Thereby, this work could help to identify the potential existence of a dose threshold, below that which harmful effects are not exhibited, and beneficial effects are potentially observed. Furthermore, these findings permit to explore differences between males and females.

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Radon exposures delay the development of psoriatic skin lesions in a mouse model

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Keywords: radon therapy, psoriasis, immune modulation

The chronic inflammatory skin disease psoriasis is on the indication list for radon therapy. Therapeutic success is reported but the mechanisms leading to observed clinical benefits are unknown. The pathology of psoriasis is caused by an autoimmune reaction inducing hyperproliferation of keratinocytes and infiltration of immune cells into the skin. The proinflammatory cytokine IL-17A is described as a key player in this mechanism.
To investigate the effects of radon exposures on a psoriatic phenotype, we used transgenic DC-IL-17A$^{ind/ind}$-mice that constitutively express IL-17A and therefore gradually develop psoriatic skin lesions (plaques); breeded in the group of Prof. Clausen, Gutenberg University Mainz [1].
Before plaque induction, mice received single (~539 kBq/m³, 1 h) or multiple (10x ~39 kBq/m³, 1 h) radon treatments as in patient therapy [2]. The occurrence of skin lesions was observed and scored from 0–6 (0=normal skin, 4=plaque induction, 6=spread lesion) for ~20 weeks after treatments. In a second setup, mice were sacrificed 3 days or 2 weeks after multiple radon exposures to collect tissue samples for molecular analyses.
After radon exposures, the development of psoriatic skin lesions was delayed for several weeks as compared to sham exposed mice, with more pronounced effects in multiple than in single exposed animals. Molecular analyses indicated transient immunosuppressive effects of radon in skin and lymph nodes. In particular, following multiple radon treatments, NGS data showed a downregulated gene expression of factors related to initiation phase of psoriasis in skin. Furthermore, distinct subtypes of dendritic cells were depleted in lymph nodes. Instead, the amounts of anti-inflammatory components like regulatory T cells were increased in these organs.
This study provides the first *in vivo* evidence for alleviative effects of radon on a chronic psoriatic disease model which so far has only been reported by patients where placebo-effects cannot be excluded.

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Capturing the complex biology of low-dose effects using multimodal data integration: analysis of pros and cons using a mouse model of colon cancer as a case example

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Keywords: low-dose radiation, systems biology, multimodal data integration, colon cancer

Biological mechanisms of low-dose radiation (LDR) health effects are very complex. Analytical methods using systems biology approaches to analyze complex multimodal and/or multiomics data have shown great potential for revealing networks of causally related biological changes spanning molecular, cellular, tissue and organism levels. However, these methods are facing multiple challenges pertinent to the structure and nature of the data. Examples are: 1) “fat data” or “high dimensionality” of data – a combination of thousands to millions of variables are obtained from a single sample (e.g. RNA-seq or DNA-seq) with a small number of replicates/animals per group; 2) two types of heterogeneity of data, one being biological heterogeneity (e.g. omics vs. physiological data) and the second being data scale heterogeneity (e.g. millions of variables in omics vs. few physiological variables); and lastly, 3) the origin of the data when different data modalities are coming not from the same individual animal/subject, which is a common feature of animal radiobiological experiments. We present here our systems biology approach to tackle some of these common challenges using data collected in a recently completed study wherein the effects of LDR on intestinal tumorigenesis were examined in the $APC^{min/+}$ mouse model of human colon cancer. The data comprised of RNA-seq transcriptomes, DNA-seq methylomes, blood cellularity and biochemistry, blood cytokine profiles, and tumor formation. In step one, each data modality was analyzed separately, followed by step two where challenges 1) and 2) above were addressed using the RGCCA/SGCCA[1] multiblock algorithms. Finally, integrative analysis was carried out to extract biologically relevant information, such as biological pathways. Our results suggest that the approach used can help decipher biologically relevant information for complex pathological conditions from a highly heterogenous data sets encompassing various levels of biological organization. One of the major limitations of the used algorithms is, however, the inability to address the type 3) challenge above, which informs planning of future LDR animal studies. Not only can this approach be applied to animal studies of the effects of LDR on other health conditions, e.g. on atherosclerosis, but also it can facilitate the development of adverse outcome pathways by identifying causally related key biological events.

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Omics molecular biomarkers of localized radiation injury in mice biofluids

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Keywords: non-invasive biomarkers, localized radiation injury, microRNA, metabolomics

A radiological accident, whether from industrial, medical, or malicious origin, may result in localized external exposure to high doses of ionizing radiations. Such exposure leads to the development of a local radiation injury (LRI) whose kinetics and severity depend on the absorbed dose, the duration of exposure and the volume of irradiated tissue. After an asymptomatic phase of variable latency, the LRI manifests as an erythema that may evolve from dry desquamation to deep ulceration and necrosis through unpredictable inflammatory waves. Early diagnosis and prognosis of victims of LRI is therefore crucial for the effectiveness of medical management and the reduction of deleterious effects.

To respond to the constraints of a radiological emergency, a fast and non-invasive diagnostic method is needed to facilitate identification and care of victims. This study aims to identify, through an omics approach carried out in biofluids, molecular biomarkers associated with LRI in a preclinical C57BL/6J mouse model of hind limb irradiation using different 10 MV X-ray doses that lead to injuries of different severity grades. We performed broad-spectrum profiling of microRNAs (miRNA) and metabolites in blood and urine, using quantitative real-time polymerase chain reaction and mass spectrometry, respectively.

Using a multivariate sparse partial least square discriminant analysis (PLS-DA), we identified panels of miRNAs and metabolites in both biofluids, that can differentiate groups of mice according to the radiation dose or the severity of the injury. Furthermore, an integrative analysis was conducted to establish multi-scale correlations between specific miRNAs/metabolites levels and various biological parameters, *i.e.* blood cell counts and circulating C-reactive protein levels (inflammation marker), as well as physiological/functional parameters including observational lesion score, cutaneous blood perfusion (laser Doppler), and skin barrier integrity (Tewameter®). The identified biomarker signatures were further confirmed in an independent validation mice cohort.

Our results demonstrate relevant plasma and urine molecular signatures associated with LRI in mice and support the use of miRNAs and metabolites in biofluids as suitable molecular biomarkers for the prognosis and diagnosis of LRI.

Design of a preclinical proton minibeam radiotherapy facility

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Keywords: proton minibeam radiotherapy, dose monitoring, beam quality assurance, cancer model

Spatial fractionated radiotherapy using protons, so-called proton minibeam radiotherapy (pMBRT) was developed for better sparing of normal tissue in the entrance channel of radiation. Preclinical in-vivo experiments conducted with pMBRT in mouse ear models or in rat brains support the prospects. However, the research on radiobiological mechanisms and the search for adequate application parameters delivering the most beneficial minibeam therapy is still in its infancy. Progressing towards clinical usage, pMBRT research should overcome technical and biomedical limitations of the current irradiation test stages and animal models.
This work discusses the design of a preclinical pMBRT facility located at an existing 68.5 MeV cyclotron. Two major parts, which have to be designed and constructed are the dose monitor and the range shifter. To be able to monitor high local dose rates a parallel plate ionization chamber was constructed. As electrodes 6 µm thick mylar foils coated with aluminum at a distance of 5 mm were used. Here, we present the final prototype construction and first reference measurements using an x-ray beam. The range shifter needs to be designed such that a beam diameter of ~200µm (□) is not exceeded. We show beam spreading simulations of different materials, which can be used as range shifter, such as PMMA, carbon and aluminum. Also we present the possibility of using a hybrid range shifter splitted in two parts, one in vacuum and one in air. This on the one hand supports keeping beam size small and on the other hand offers maximum flexibility. Additionally we introduce a possible quality assurance procedure, which has to be conducted to be able to apply the pMBRT in small animals with sufficient small beam size, positioning accuracy as well as lateral and axial dose profiles. For this purpose we show first pilot studies of a cancer model which will be used as a preliminary stage of the animal model.

Model study of ^{49}Ti(p,2pn)^{47}Sc and comparison with first production measurement within the LNL-REMIX experiment

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Keywords: ^{47}Sc cross section, cyclotron-based radiopharmaceuticals, theranostics

Radionuclide ^{47}Sc has gained interest in precision nuclear medicine thanks to its favourable decay properties, suitable for targeted radiotherapy and theranostic applications, also in combination with the β^+ emitters ^{43}Sc/^{44}Sc[1]. However, efficient production routes, appropriate for pre-clinical and clinical investigations, are hard to find and still represent an open issue. Herein we investigate the modeling of ^{47}Sc cyclotron production using proton beams on enriched titanium targets. This theoretical analysis takes also into account the very recent ^{49}Ti(p,2pn)^{47}Sc production measurements within the INFN-LNL REMIX (Research on Emerging Medical radIonuclides from X-section) project. The analysis consists in the modeling, using the nuclear reaction code TALYS[2], of the relevant ^{47}Sc cross section, and includes the production of its main contaminants, starting from the long-lived ^{46}Sc. Since a large variety of pre-equilibrium and level-density models can be selected within TALYS, we describe statistically the results by means of the interquartile band and the Min/Max excursion obtainable from all considered models[3]. Then we optimize the level density parameters (starting from the existing data for ^{46}Sc) to obtain a better agreement between measurements and theoretical cross-section[4]. The new, preliminary data for ^{47}Sc and contaminants, from the REMIX project, allow to refine the optimization to obtain more reliable cross sections and from there the evaluation of yields, radionuclidic purities and dosimetric impact of contaminants. Finally, from these results we will discuss the feasibility of production routes employing enriched titanium targets.

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[2] *S. Goriely, S. Hilaire, and A. J. Koning. Astron. Astrophys., 487:767, 2008.*

[3] *A. Colombi et al. Nucl. Technol., 208:4, 735-752, 2022. doi:10.1080/00295450.2021.1947122.*

[4] *F. Barbaro et al. Phys. Rev. C, 104:044619, 2021.*

Comparison of ^{nat}Gd(p,x), ^{155}Gd(p,n) and ^{159}Tb(p,5n) reaction modeling for ^{155}Tb production

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Keywords: ^{155}Tb, theranostics, nuclear reaction modeling

In the field of radiopharmaceutical production, terbium has gained the attention of the community since it is the only element in the periodic table with four isotopes (^{149}Tb, ^{152}Tb, ^{155}Tb and ^{161}Tb) that can be used for both imaging and therapy[1]. In particular ^{155}Tb is a Auger-electron emitter and has γ rays suitable for SPECT (Single Photon Emission Computed Tomography) imaging.
In this work we investigate and compare different nuclear reactions for the production of ^{155}Tb using medium-low energy proton beams on ^{nat}Gd, ^{155}Gd, and ^{159}Tb targets, contributing to the activities of the INFN-LNL REMIX (Research on Emerging Medical radIonuclides from the X-sections) project. The considered production routes are analyzed with the nuclear reaction code TALYS[2] performing initially a statistical analysis[3] to take into account the variability of the models for the level density and preequilibrium processes implemented in the code. In a subsequent step, we perform an optimization of the calculated cross sections considering specific combinations of the models and varying their parameters, in order to improve the agreement between the cross sections and the data[4]. This analysis is carried out for the reactions with ^{nat}Gd and ^{159}Tb since many experimental data are available for these production routes, and it is tentatively applied to the ^{155}Gd(p,n)^{155}Tb reaction, whose cross sections have not been measured yet. After an inspection of the complex decay schemes of all the Tb isotopes produced in the reactions, the extended Bateman's equations are solved to finally calculate the yields and purities for optimal irradiation conditions, in view of possible dosimetric studies and clinical applications.

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[2] *S. Goriely, S. Hilaire, and A. J. Koning. Astron. Astrophys., 487:767, 2008*
[3] *A. Colombi et al. Nucl. Technol., 208:4, 735–752, 2022, doi: 10.1080/00295450.2021.194712*
[4] *F. Barbaro et al. Phys. Rev. C, 104:044619, 2021.*

Imidazolyl Ethanamide Pentandioic Acid as a mitigator of Acute Radiation Syndrome in a Total Body Irradiation juvenile rat model

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Keywords: Acute Radiation Syndrome, Medical countermeasure, Juvenile, Total Body Irradiation

Introduction: Acute ionizing radiation exposure at doses of 2 to 6 Gy in humans results in Hematopoietic Acute Radiation Syndrome (H-ARS). The disease manifests through severe depletion of hematopoietic stem cells and reduced blood cell counts, leading to increased susceptibility to infections, hemorrhages, and premature death. Imidazolyl Ethanamide Pentandioic Acid (IEPA; Myelo001), an orally administrable small molecule drug with a good safety profile, mitigated myelosuppression in adult mice after sublethal and lethal (≤LD50/30) total body irradiation (TBI). The radiation effects after TBI exposure are reported to be age-dependent. The data presented here tested two doses of IEPA to determine the drug's survival and hematological efficacy in a special juvenile WAG/RijCmcr rat population. **Methods**: Juvenile male rats (5-6 weeks old, n=16-25/group) were irradiated at 6.25 Gy TBI (X-RAD 320 Precision, 320 kVp, 13 mA, 1.73 Gy/min). Rats received vehicle (sterile water) or IEPA (10 or 60 mg/kg p.o.) at 24h, 48h, and 72h post-TBI. Peg-G-CSF (s.c.) was used as a positive control, given at 0.55 mg/kg once 24h post-TBI. Endpoints included 30-day survival, body weight, peripheral blood counts on days 3, 7, 10, 15, and 21, with each rat bled twice using a staggered collection method during the study, and a terminal bleed of the survivors on day 30. Bone marrow was analyzed via fluorescence-activated cell sorting (FACS) and Colony Forming unit assay (CFU-G, CFU-M, CFU-GM). **Results**: TBI resulted in 84% survival (n=21/25) in IEPA 10 mg/kg-treated (IEPA10) rats as compared to 72% survival (n=18/25) in the vehicle-treated group and 92% survival (n=23/25) after Peg-G-CSF treatment. In contrast, IEPA 60 mg/kg (IEPA60) treatment showed a 56% survival (n=9/16). A small positive increase in body weight was observed in IEPA10 versus the vehicle group. In contrast to the vehicle group, the IEPA10-treated rats showed a slower decline, reduced nadir, and an accelerated recovery of neutrophils, monocytes, and red blood cells. In addition, IEPA10 led to improvement of bone marrow cells with significantly increased CFU-GM populations. **Conclusion**: Dose optimization of IEPA shows survival and hematological efficacy for the lower 10 mg/kg dose at LD30/30 in the juvenile rat TBI model. **Funding:** NIH-NIAID contract 75N93020C00005.

2D dosimetry for evaluation of *in vitro* clonogenic survival following exposure to spatially fractionated GRID radiation field

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Abstract

Background: Spatially fractionated (GRID) irradiation is an approach to deliver high local radiation doses in an 'on-off' pattern. The radiobiolgical effects from GRID needs to be better characterized and understood to promote clinical applications. The purpose of the current work was to develop a framework to evaluate *in vitro* effects of GRID.
Materials and methods: A549 lung cancer cells cultured *in vitro* in T25 cm^2 flasks were irradiated using 220 kV X-rays with an open field or through a tungsten GRID collimator with periodical 5 mm openings and 10 mm blockings. Delivered doses were 2, 5, and 10 Gy. A novel approach for image segmentation was used to locate the centroid of surviving colonies in scanned images of the cell flasks. Gafchromic film dosimetry (GFD) and FLUKA Monte Carlo simulations (MC) were employed to map the dose distribution in the flasks at each surviving colony centroid. Fitting the linear-quadratic (LQ) function to open field survival data, the expected survival level at a given dose level was obtained. The expected survival level was then mapped together with observed levels in the GRID-irradiated flasks.
Results: GFD and FLUKA MC gave similar dose distributions, with a mean peak-to-valley dose ratio of about 5. LQ-parameters for open field irradiation gave α=0.16±0.04 Gy^{-1} and β=0.001±0.004 Gy^{-2}. Using the image segmentation method, the surviving colony distribution in the cell flasks gave a pattern qualitatively resembling the GRID collimator outline. The mean absolute percentage deviation between predicted and observed survival in the (peak;valley) dose regions was (8;10) %, (4;41) %, and (3;138) % for 2, 5 and 10 Gy, respectively.
Conclusion: A framework for mapping of surviving colonies following GRID irradiation together with predicted survival levels from homogeneous irradiation was presented. For the given cell line, our findings indicate that GRID irradiation, especially at high peak doses, causes reduced survival compared to an open field configuration.

Italian dosimetry audit: service for radiotherapy centres and a new study for non-reference conditions

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Keywords: dosimetric audit, radiotherapy photon beams, non-reference dosimetry, TLD

The Italian National Institute of Ionizing Radiation Metrology (ENEA-INMRI) and the Italian Association of Medical Physics (AIFM) offer a certified audit service to radiotherapy (RT) centres for dosimetry in photon beams.
Audits are provided for photon beams in the range 6-18 MV including flattening filter free beams, CyberKnife and TomoTherapy.
Thermoluminescent dosimeters consisting of a set of TLD chips embedded in a PMMA waterproof holder are used. The dosimeter signal is the average value of 20 or 10 TLD chips depending on the beam uniformity at the measurement point. Dosimeters are calibrated at ENEA-INMRI in terms of absorbed dose to water in a reference ^{60}Co gamma beam. Correction factors accounting for energy dependence, signal reproducibility and response stability are applied to evaluate absorbed dose.
For the audit, measurements are performed in reference conditions according to the international dosimetry protocols [1-3]. For each beam, irradiation of two dosimeters with 2 Gy is required. Audit results are evaluated in terms of the normalized error E_n [4]: it is satisfactory if $|E_n| \leq 1.0$. Distribution of E_n scores for all irradiated dosimeters shows that 99.4% of values are in the range [-1.0,1.0]. As for the single unsatisfactory result, data from the form filled in by the RT centre allowed to identify an error in the dosimeter positioning.
In non-reference condition, a technical protocol for verification of dose delivery in VMAT treatments is being developed. VMAT delivery simulating head-neck treatments with Simultaneous Integrated Boost on two different target volumes while sparing the parotid glands and the spinal cord to the proper dose constrains

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have been planned. A uniform dose (within 2%) was required in the planning phase on a region with dimensions comparable to sensitive volume of the detector used to measure the delivered dose in a cylindrical water phantom. TLD measurement accuracy is evaluated by comparison to a reference ionization chamber. From preliminary results, it emerges that dosimeters with 10 TLD chips are suitable for dose measurements also in non-reference conditions.
The reference dosimetry audit was successfully performed for the 38 RT centres and 84 photon beams. Meanwhile, other measurements will be planned to evaluate different VMAT plan configuration.

[1]*IAEA TRS 398 - Absorbed Dose Determination in External Beam Radiotherapy: An International Code of Practice for Dosimetry based on Standards of Absorbed Dose to Water.*
[2]*IAEA TRS 483 - Dosimetry of Small Static Fields Used in External.*
[3]*AAPM TG-51 protocol for clinical reference dosimetry of high-energy photon and electron beams.*
[4]*ISO/IEC 17043:2010 Conformity assessment – General requirements for proficiency testing.*

Simulation of microgravity with random positioning machine induces promoting effects in Triple Negative Breast Cancer cells.

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Keywords: Microgravity; Cancer; Triple Negative Breast Cancer

Space environment is a harsh place to host human life and exposes the crew to many physical challenges. The absence of gravity (known as microgravity, □g) affects many sides of human biology and the human body requires to adopt several mechanisms of adaptation as a countermeasure to restrain the effects induced by gravity unloading [1]. The interest towards □g is not only speculative but it could help to predict potential long-term alterations and to develop countermeasure to manage healthy problems in space. Microgravity can be reproduced in vitro with the help of microgravity simulators and simulated microgravity (s-□g) is applied in many fields of medical research, including cancer biology [2,3]
In our study, we aimed to determine in vitro the effects that simulated microgravity is able to induce in a model of Triple Negative Breast Cancer (TNBC), an aggressive form of tumor. In fact, the role played by □g in cancer progression still remains an open debate [4]. The investigation on the effects induced by s-□g in neoplastic cells is not related to the need to cure cancer in space, instead, it's aimed to clarify if the absence of gravity is associated with the acquisition of □g-related phenotypes both in morphology and at molecular level which may help to highlight novel cancer biomarkers. For our analysis we used a random positioning machine (RPM) which is able to counterbalance gravity forces by rotating along the x, y and z axis. In particular, we investigated and compared the effects played by s-□g at two different timepoints (24 and 72 hours), analyzing cell viability by MTT test and the change of expression of some genes, driving proliferation, survival, cell death, cancer stemness, and metastasis, in the human TNBC MDA-MB-231 cell line by qRT-PCR.

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Our biological findings demonstrated that s-□g can sustain some key aspects of TNBC development and dissemination. To date, results about this field of investigation are controversial, in fact, both pro- and anti-tumoral roles of microgravity are reported in the literature when different cancer settings are analyzed (e.g: lymphomas, gliomas and pancreatic cancer) [5-7] Hence, our results may help to shed the light on the potential involvement of gravity unloading as a stressor whose role requires a deeper knowledge to understand its effects in tumor development and maintenance.

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Recommendations for optimizing radiation protection of patients and health workers: results of the European Project MEDIRAD

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Key words: radiation protection, patients, health professionals, ionizing radiation

MEDIRAD - Implications of Medical Low Dose Radiation Exposure is a European research project, funded by EURATOM under the Horizon 2020 programme which involved 35 institutions from 14 EU states with the aim of increasing the scientific basis and clinical practice of radiation protection (RP) in the medical field. In particular, the project aimed to improve the understanding and evaluation of the health effects of exposure to low doses of ionizing radiation resulting from diagnostic and therapeutic applications (off-target exposures), to optimize practices to reduce doses and to develop dose assessment tools that can be used in clinical practice and to develop of a series of science-based recommendations to ensure adequate and better RP for patients and staff.
Specifically, the work package 6 of the project foresaw:

- drafting of recommendations (RECOs) based on scientific results of the project; these RECOs are mainly addressed to institutional bodies and scientific associations to drive research on ionizing radiation towards an improvement of the RP of patients and health workers;
- wide stakeholder involvement at European and international level to discuss and share the RECOs;
- dissemination of MEDIRAD RECOs

The scientific basis for the RECOs stems from the research developed in the course of the MEDIRAD project. In order to achieve a sufficient degree of consensus, MEDIRAD engaged in a substantial dialogue with relevant stakeholders in Europe and

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internationally. The MEDIRAD Stakeholder Forum, which underpinned this dialogue, included representatives from 86 organisations who were invited to express their views on issues to be considered as priority and to comment on the draft formulation of MEDIRAD recommendations.
Among the four RECOs developed, the ISS contribution was mainly focused on RECO1, especially on the aspects related to the GDPR Directive [1] compliance. This RECO aims to facilitate the development of large scale multinational epidemiological studies by proposing for example guidelines to help European countries implement European regulatory requirements on ethics and encouraging harmonization of regulatory practice notably through the collection of experience gathered through the EURATOM research projects. A synthetic excursus of the RECOs together with a deep description of RECO 1 "GDPR and Medical Radiation Protection Research" will be presented.

The four RECOs are available at http://www.medirad-project.eu/recommendations.

[1] *Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation)*

Retrospective dosimetry using components of mobile phones

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Keywords: Retrospective dosimetry, accident dosimetry, luminescence methods, dosimetric properties

Retrospective dosimetry refers to dose assessment of individuals after a radiological incident or in situations when conventional personal dosimeters were not available or sufficient (ICRU, 2019, Bailiff et al., 2016). In daily life, a mobile phone is usually carried closely to a body and is a perfect candidate to fulfil the requirement as a fortuitous dosimeter. Consequently, different components of a mobile phone have been investigated by different research groups and the dosimetric properties were examined. There are systematic investigations available using different materials which are found on or inside a phone, such as glass extracted from the display or from its covering, Al_2O_3 ceramics extracted from the circuit board or silicate-based filler materials found in chip cards (i.e. SIM cards).
This paper gives a brief overview of the different luminescence materials available from a phone and highlights their dosimetric properties by analysing the strengths, weaknesses, potentials and challenges of the different luminescence methods.

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Bailiff I.K., Sholom S., McKeever S.W.S., Retrospective and emergency dosimetry in response to radiological incidents and nuclear mass-casualty events: A review. Radiation Measurements. Volume 94, November 2016, 83-139.

Validation and automation of a novel biological dosimetry method combining dicentric chromosome and micronucleus assays

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Keywords: biological dosimetry, dicentric chromosome assay, micronucleus assay, automatic scoring

In large-scale radiological and nuclear emergencies the early individual dose assessment is of primary importance to rapidly identify overexposed individuals requiring medical intervention. In this regard, we are validating a novel biological dosimetry method combining the dicentric chromosome assay (DCA) and the micronucleus test (MN) in a single protocol [1].

The method was optimized within the *BioPhyMeTRE* project "Novel biological and physical methods for triage in radiological and nuclear (R/N) emergencies" (https://biophymetre.com/) [2], modifying key steps (i.e. colcemid treatment and fixation) in order to obtain an adequate number of good quality metaphases and binucleated cells for triage procedure, both in control and in irradiated samples.

Validation of the combined biological protocol was performed by comparing it with the standard methods for DCA and MN in terms of numbers of dicentrics and micronuclei induced by ^{60}Co gamma rays at the following doses: 0.5, 1, 2, 3 Gy. The reproducibility of the method is under evaluation: dicentric and micronuclei yields in irradiated blood samples from healthy donors are being compared, taking into account inter-individual variability in response.

The production of dose-response curves for the combined protocol is ongoing, on a panel of 10 doses in the range of 0.25–5.0 Gy, according to IAEA recommendations.

Moreover, the automation of the scoring for the combined protocol has been performed by the Metafer 4 Scanning Platform (MetaSystems): for the analysis a single classifier called SIF (Search Information File) is used, which combines three different search/analysis algorithms by executing them in an ordered sequence (micronucleus analysis, metaphases search and automated metaphases capture).

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Experimental determination of concentration factors of Mn, Zn and I in the phytoplankton species Phaeodactylum Tricornutum

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Keywords: Environmental radioactivity, concentration factors, phytoplankton, diatom, manganese, zinc, iodine

During normal operation of nuclear facilities, small and controlled amounts of radioactive elements are released into the environment. The resulting dose to humans is estimated using ecosystem models, and one category of model parameters is concentration factors (CFs), which relate the concentration of an element in organism, under equilibrium conditions, to the concentration in the surrounding medium. Many nuclear facilities, in particular nuclear power plants, are located at the sea and hence the marine ecosystem is important. The primary producers in the marine ecosystem are phytoplankton, and hence their CFs are central for correct dose predictions.

In this work, we have identified elements with phytoplankton CFs that need to be better known, due to their relatively large impact on the dose assessment as well as scarcity and spread of literature data. These elements were Mn, Zn and I. Their phytoplankton CFs were experimentally determined in this work. Cultures of the diatom species *Phaeodactylum Tricornutum* were grown in saline and brackish seawater samples (from Skagerrak and the Baltic Sea, respectively) with additions of nutrients and radioactive isotopes of the concerned elements (Mn-54, Zn-65 and I-131). The CFs were determined by relating the activity concentration in the seawater to the activity in the phytoplankton.

Based on the present data, our conservative estimates (the highest of the values obtained for saline and brackish water respectively) of these CFs are (relative standard deviations in parenthesis) 7 200 L/kg (36%) for Mn, 9 100 L/kg for Zn (45%) and 33 L/kg (38%) for I, all phytoplankton masses referring to fresh weight. The passive adsorption of radionuclides on senescent cells was also quantified and found to be much lower than the active uptake.

Our results are in general similar to or lower than the lowest published values, which may be due to the particular phytoplankton species considered, phytoplankton being a diverse organism category.

Development of a real time reporting system for dose assessment of operators in abnormal events in nuclear medicine therapy: the SIREN project

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Keywords: radiation protection, health professionals, ionizing radiation

The SIREN Project (2020-2022) aims to develop procedures for reporting abnormal events in a Nuclear Medicine Therapy Unit by the implementation of an Internet of Things (IoT) dose monitoring system and a mobile application. The involvement of the operators in all phases, from the design of the IoT system and app up to their final test, is expected to contribute significantly to a more positive attitude of the staff towards incident reporting and proactive actions and in a general increase in incident prevention and preparedness. This seemed to be a necessary step to increase the perception of the usefulness of reporting or reduce the fear of the consequences.

Face-to-face interviews were carried out with 24 staff in different roles in the Unit (radiation protection experts, physicians, physicists, nuclear medicine technicians and nurses). The interviews with the staff were aimed at collecting a list of needs and expectations with respect to the reporting system, the attitude towards radiation protection devices as well as opinions on the abnormal events considered most frequent and at highest risk of accident. Based on this list, a mobile application has been designed and is under development.

For the dose monitoring system, the type, number, and location of dose detectors will be based on the dose levels estimated at different distances from the patient and at different times after radiopharmaceutical administration. These estimates were obtained from a literature review and simulations (obtained with ResRad Build) of the dose distribution in the inpatient room under abnormal event scenarios, such as patient's emesis or catheter leakage.

There was a good response from the staff with being involved in the design of the app and of the dosimetry system. This involvement is expected to increase the users' acceptance and the availability to use it.

Tomato hairy roots accumulating anthocyanins as a test bed for space food anti-oxidant properties

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Keywords: MicroTom, hairy root cultures, agrospace, biofortification, anthocyanins, gamma radiation

In the near future, NASA plans to launch the Artemis program with the aim to look further into human life in deep space. Ionizing radiation is considered the main hazard in spaceflights both to humans and plants, due to the generation of cell metabolic stress leading to free radical species. The issue of counteracting overproduction of free radicals is crucial for survival in space outposts and opens the way to the idea of the 'antioxidant space fresh food'. As a consequence, there is great interest in the development of plants producing natural antioxidant food that may be introduced into space crews' diet. Among flavonoids, anthocyanins are well-known as health-promoting and chronic-diseases-preventing molecules due to antioxidant, anti-inflammatory, anti-proliferative and anti-neurodegenerative functions.

Here, tomato hairy root cultures engineered to synthesize anthocyanins, normally not accumulated by commercially available tomato crops, were used as a test bed for the design of bio-fortified whole plants aimed at agrospace applications. This model allowed to profile the accumulation of 5 major anthocyanins in the engineered tomato cells. A detailed analysis on phenylpropanoids was carried out by LC-HRMS. The antioxidant properties of the engineered tomato cells were evaluated upon *ex-vivo* high dose radiation, as a potent pro-oxidant stimulus (i.e. 2 kGy, administered through the Calliope ^{60}Co gamma irradiation facility, at ENEA). Significantly higher free radicals scavenging activity and lower accumulation of reactive oxygen species were found in the engineered tomato model with respect to control, as demonstrated by Electron Spin Resonance Spectroscopy. Moreover, both UV-VIS spectra and photoluminescence analysis demonstrated that polyphenols content and folding of tomato soluble protein were not significantly affected by the pro-oxidant stimulus.

These results may have significance in the engineering of whole tomato plants that can benefit space agriculture.

A Monte Carlo simulation for estimation of percentage depth dose distribution using the PRAGUE detection system prototype

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Keywords: simulation, TOPAS, percentage depth dose distribution

The interaction of radiation with matter is an inherently stochastic phenomenon involving complicated analytical functions which are near-impossible and time-consuming to solve. At the same time, the needs of the nuclear and particle physics community for large-scale, accurate, and comprehensive simulations of particle detectors are on the rise. In the past decades, several Monte Carlo based simulation toolkits such as Geant4, PENELOPE, EPOTRAN, and TOPAS have been developed to meet these growing demands, with each suited to a specific scenario. TOPAS [1] is a simple, yet robust, toolkit based on Geant4 and offering all its versatility but adapted to advanced radiotherapy simulations. Considering its capabilities, it was used for analyzing a new detection system PRAGUE (Proton RanGe measurement Using silicon carbidE) - SiC detectors in stack configuration operated with conventional and ultra-high dose beams. Experimental setups were reproduced in the simulation space to obtain the theoretical dose profiles and compared with measured data. The results from simulation agree well with experimental data, with chi-square test analysis indicating consistency between the two at a significance level of 0.05.

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Long $^{222-220}$Rn time series at Campi Flegrei caldera (southern Italy)

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Keywords: radon, caldera, Campi Flegrei, α-spectrometry, time series.

A large study on long-term monitoring of radon time series data (10 years from 2011) at sites in the Campi Flegrei volcanic area (Naples, Italy) is presented. Measurements were performed with the RaMonA system which is based on measurement of the alpha particles of the daughters of radon (^{222}Rn) and thoron (^{220}Rn)[1]. The time series are drawn with powerful mathematical methods (hybrid methods) to capture and interpret radon trends and anomalies due to volcanic-seismic events[2]. The investigations on the gas composition of the monitoring area were carried out in relation to the characteristics of the time series studied. A particular novelty is that the radon, and here also the thoron, signal was studied in connection with the fumarolic tremors recorded in a site of the Campi Flegrei area. The results are also compared with the following indicators: the cumulative of background seismicity; the maximum vertical deformation acquired by GPS networks; the temperature-pressure of the hydrothermal system estimated based on gas geo-indicators[3]. The $^{222-220}$Rn trends and the results of the obtained anomalies confirm recent studies that have shown a new and long unrest phase in that area. The comparisons also suggest that the extension of the area affected by current Campi Flegrei crisis is larger than the area of seismicity and of intense hydrothermal activity from which the radon stations are 1–4 km away[3]. This unrest phase has greatly influenced public opinion in Italy in recent years. The results of the study represent an absolute novelty in the study of a such calderic area and mark a significant step forward in the use and interpretation of the radon, and mainly for the rarely studied thoron signal[4]. In fact, although thoron comes only from the most surface layer (half-life 55.6 s), so the characteristics of its time series are strictly connected to the shallow phenomena, this study provides good evidence that the gas is related to the carrier effect of CO_2 within the caldera. The hydrothermal alterations, induced by the increase in temperature and pressure of the caldera system, occur in the surface soils and significantly influence thoron's power of exhalation from the surface layer[4].

MORE→

←CONT'D

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The first map of the radon potential of the Campania region and the radiological characterization of natural building materials

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Keywords: radon indoor, building material, Campania, radon map, α+γ spectrometry, GIS.

For a better understanding of the ^{222}Rn risk associated with soils and building materials in the Campania region, extensive research has been done. The first potential map of the Radon distribution in the Campania region was created, using the kriging geostatistical interpolation method using the QGIS® software. The response variable is the activity concentration of indoor radon and the proxy variables used as predictors are: geology, faults, soil permeability, meteorological/climatic parameters, ^{238}U distribution, emanation coefficients and radon exhalation rate, ^{226}Ra in building materials and gamma dose rate in buildings. The obtained map highlighted the areas with a high Radon potential in correspondence with the main volcanic centers and the fault network of the region [1]. This map is a useful tool for identifying radon risk areas and assessing the level of radon risk for the population.
The characterization of the natural radioactivity content was also carried out in thirty-one natural construction materials representative of the Campania region, using gamma spectrometry to determine the activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K. Radiation hazard indices commonly used to assess human radiation exposure were calculated. The comparison of the results with the limit values established by Directive 2013/59/Euratom and by UNSCEAR 2000 demonstrates the high content of natural radioactivity and the risk of exposure to radiation from some samples of volcanic origin. The emitted fraction and exhalation rate of ^{222}Rn and ^{220}Rn (thoron) were also measured. The different materials show interesting differences in their radioactivity content and their ability to generate radon [2].

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Quantification of the hydrated electron by scavengers and Geant4-DNA simulation

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Keywords: Radiotherapy damage, Water radiolysis, Yield of hydrated electron, Experimental and simulated data

This study aims at better evaluating the indirect effects of water radiolysis on the ionizing radiation damage mechanisms during radiotherapy treatment. Under irradiation of a cell, ionizing radiations will generate radicals, which will yield damages to biomolecules, including proteins and DNA. Our body is mostly made up of water (~65 – 70%), proteins (~20%), lipids (~10%), with DNA accounting for only 0.1%. When an ionizing radiation enters a cell, it can either interact directly with a biomolecule (direct effect) or interact with water, resulting in the formation of reactive species by radiolysis of water. These reactive species will then react more or less quickly with the surrounding biomolecules, inducing damage by indirect effect. Given the proportion of water, indirect effects will play a very important role in the phenomena occurring under irradiation in the cell. The precise quantification of water radiolysis species is therefore essential for understanding the mechanisms of damage formation to biomolecules. Regardless of the ionizing radiation (accelerated ion, electron, X-ray or gamma), reactive species generated by radiolysis of water are about the same: hydroxyl radical $HO^{\bullet}$, hydrogen atom $H^{\bullet}$, hydrogen peroxide H_2O_2, and hydrated electron e_{aq}^-.

In this poster, I will present the production yield kinetics of hydrated electron e_{aq}^-, on time scales from nanosecond to microsecond, determined by scavenging. Experiments were realized with 6 MeV X-rays (ICANS – Strasbourg), 2 MeV protons (ACACIA – Strasbourg) and 1 MeV electrons (AERIAL – Strasbourg). Experimental data of reactive species have been compared to Monte Carlo simulation results produced with the Geant4-DNA software [1], an extension of Geant4 that was developed to simulate physical, chemical and biological effects of ionizing radiation on DNA.

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Coralyne radiosensitizes human lung A549 adenocarcinoma cells by upregulation of CDKN1A expression

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Keywords: coralyne; cell cycle progression; gene and protein expression; radiosensitivity

Introduction

Protoberberine alkaloids and their derivatives are used as antitumor agents in traditional medicine and have been investigated nowadays. Coralyne is a synthetic analogue of protoberberines alkaloids. Structure of coralyne is related to the isoquinoline four rings, but their arrangement in contrast to other protoberberines alkaloids is fully aromatic, which makes the structure flat. This alterations in their chemical structures significantly affect the biological properties of coralyne.
For this purpose in our study we analyse effects of coralyne and IR on cell cycle progression and expression of selected genes and proteins implicated in cell cycle regulation in human lung A549 adenocarcinoma cells. A549 cells were treated with 1–25 μM coralyne for 24 hours and then irradiated with 2 Gy of gamma-rays using a 6 MV Medical Linear Accelerator Artiste (Siemens). 24 h after irradiation cells were harvested and proceed for cell cycle analysis and genes and proteins expression.

Results and conclusion

The results showed that treatment of cells with different concentration of coralyne alone did not affect the cell cycle progression. Exposure to 2 Gy of ionizing radiation alone resulted in an enhanced accumulation of A549 cells in the G2/M phase. This effect was significantly reduced when cells were pretreated with coralyne in a dose 25 μM for 24 h before irradiation with 2 Gy, suggesting that coralyne can eliminate the radiation-induced G2/M arrest what effectively increases A549 cells radiosensitivity. We investigated the expression of three genes related to cell cycle progression in cells: cyclin B1-interacting protein 1 (CCNB1IP1), cyclin-dependent kinase inhibitor 1 (CDKN1A) andcyclin D-binding Myb-like transcription factor 1 (DMTF1). We have observed that the combined treatment of A549 cells with coralyne and IR strongly upregulated CDKN1A expression. The level of CDKN1A protein was significantly increased only in cells treated with 25 μM coralyne and 2 Gy of IR.

Adverse Outcome Pathways and Linkages to Genomic Signatures Relevant to Ionizing Radiation Injury

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Keywords: gene expression, radiation, risk assessment, AOPs, adverse outcome pathways

A large body of data on the effects of radiation on gene expression has been generated over the past three decades. The data has allowed for an understanding of events at the molecular-level and has shown a level of consistency in response despite the vast formats and experimental procedures being used across institutions. However, clarity on how the accumulated data is applied to regulatory decision-making is needed. An approach to bridge this gap is the adverse outcome pathway (AOP) framework. AOPs represent an illustrative framework, characterizing a stressor associated with a sequential set of causally linked key events (KEs) at different levels of biological organization, beginning with a molecular initiating event (MIE) and culminating in an adverse outcome (AO). Launched by the Organisation for Economic Cooperation and Development (OECD), AOPs enable the union of existing biological knowledge to essential KEs that initiate a path to disease, and are utilized in both chemical and ecological risk assessment. Here, we demonstrate the potential application of the AOP framework within the field of ionizing radiation illustrating a transcriptomic gene-informed radiation-related AOP to lung cancer (the AO). Radiation-induced gene alterations and pathways found within the AOP have been characterized, however, there is a need to apply the gene signatures and pathways within KEs identified and to elucidate causal gene linkages and pathway interactions. This approach not only enhances the AOP under development, but also provides a means for the data to be used to inform regulatory-decision making and highlights important research gaps. Accordingly, through systemic mining of the literature and publicly available databases using the software 'DistillerSR', we selected radiation-specific transcriptomic studies and data within these KEs at the molecular, cellular, organ, and population level. Gene signatures and pathways were then associated to each KE and an evidence map was constructed of consistent gene responses and areas that require directed research by Reactome and GeneMANIA. This approach allowed for the identification of previously described genes and pathways (e.g. DNA repair, inflammation) associated with the new identified associate events (AEs), KEs, and novel characterization of radiation-induced gene signatures enabling AOP enhancement.

Radiotherapy induced alteration in cytokine levels are mitigated by TSA in C57Bl/6 mice

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Keywords - Radiotherapy, Leukemia, Histone deacetylase inhibitors (HDACi)

Secondary malignancies caused by exposure of radiation during radiotherapy in the course of treatment of cancer, have become a clinically significant issue. Hematopoietic stem cells (HSCs) are fast-dividing cells that can become cancerous if exposed to whole-body radiation during cancer treatment. There is an increased chance of developing secondary cancers such as chronic myeloid leukemia (CML), Acute myeloid leukemia (AML) and Acute non lymphocytic leukemia(ANLL). Trichostatin A is a very potent histone deacetylase inhibitor (HDACi) at very low concentration. It has anticancer activity too. We hypothesized that administering TSA shortly after exposure to radiation will enhance the acetylation status of genes, lowering the risk of subsequent cancers by reducing apoptosis and pro- and anti-inflammatory responses. To detect various cytokine levels C57Bl/6 mice were used and TSA was administered 1 and 24h after 5 Gy of gamma radiation (Co^{60}) exposure and various cytokine levels were measured after 2 and 24h post irradiation. For apoptotic studies TUNEL assay was performed at 2^{nd}, 4^{th} and 8^{th} day after radiation exposure. Radiation-induced changes in the levels of IL-2, IL-5, TNF-α, GM-CSF and apoptosis were mitigated by TSA administration. Therefore, our study suggests that all of these cytokines increase the recovery of hematopoietic stem cells, reduce the amount of apoptotic cells in the bone marrow and hasten the recovery of a variety of blood parameters. The JAK-STAT pathway, as well as numerous anti and pro apoptotic proteins, are being studied to validate the mitigation of probabilities of radiation-induced leukemia by TSA.

Challenges and opportunities of ultra-high dose rate dosimetry for FLASH radiotherapy

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Keywords: FLASH radiotherapy, dosimetry, beam monitoring, high dose-rates

Preclinical studies have shown that FLASH radiotherapy (RT) may substantially improve normal tissue sparing while maintaining high tumor control probability compared to conventional dose-rate RT [1]. However, the clinical translation of FLASH RT requires challenges related to dosimetry and beam monitoring of ultra-high dose rate (UHDR) beams to be addressed. Active detectors currently in use suffer from saturation effects under UHDR regimes and passive detectors able to measure on site in a reasonable time are not available [2]. There is a significant interest in identifying the most reliable experimental approach for UHDR dosimetry. In this presentation, the main challenges coming from the peculiar beam parameters characterizing UHDR beams for FLASH RT will be discussed. These challenges vary considerably depending on the accelerator type. A detailed status of the current technology will be presented, with the aim of discussing the detector features and their performance characteristics and/or limitations in UHDR regimes [3]. Further developments for established detectors will be also reported together with novel solutions currently under investigation, based on new prototypes of solid-state detectors, scintillators, modified ionization chambers and portable calorimeters, with a view to predicting future directions in terms of dosimetric procedures. In particular, a description of the new approaches for dosimetry and beam monitoring, developed in the framework of the Italian INFN FRIDA (FLASH Radiotherapy with hIgh Dose rate particle beAms) collaboration will be provided, discussing recently achieved results.

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[3] *M. McManus M., SCIENTIFIC REPORTS, vol. 10, ISSN: 2045-2322 (2020).*
[2] *F. Romano et al., Medical Physics, in-press, doi: 1002/mp.15649*

Verification of the accuracy of delivered dose by the treatment planning system for high-dose-rate skin brachytherapy through thermoluminescence dosimetry

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Keywords: brachytherapy, thermoluminescence, dosimetry.

Brachytherapy (BT) is a radiotherapy technique that allows to deliver dose through a radioactive source placed inside or next to the tumor. Due to the characteristic dose fall-off beyond the tumor, BT leads to an higher sparing of the surrounding tissues compared to conventional external beam radiation therapy. Despite BT is a technique widely implemented in the clinical setting, the dosimetric issue represents an interesting research field. In fact, the commercially available Treatment Planning Systems used to carry out the treatment plan are provided with a dose calculation algorithm (AAPM TG-43) affected by some limitations and approximations: it assumes that the patient is totally made of water neglecting tissue heterogeneities and intersource dose attenuation, leading to uncertainties affecting dose delivery. The aim of the presented work is to investigate the dosimetric accuracy of the TPS (Oncentra Brachy by Elekta) for high-dose-rate skin BT performing thermoluminescence dosimetry by the use of dosimeters LiF:Mg,Ti (TLD-100) provided by Harshaw Chemical Company. The work was carried out at the Istituto Nazionale Tumori IRCCS Fondazione G. Pascale (Naples) and at "LaRa" Radioactive Laboratory (Certified ISO 9001) of the Physics Department Ettore Pancini, University Federico II, were the TLD analyzing system is installed. The radioactive source used for treatments is Iridum-192 (Ir-192). The TLDs used were first characterized through the measurement of the individual sensibility factor with a 6 MV photon beam. Then, to use them as dosimeters, the calibration factor (CF) that converts the thermoluminescent signal into dose value was determined. The method implemented consists in an indirect calibration: the CF for the photon energy of Ir-192 was obtained by linear interpolation between the CF corresponding to 250 kV X-rays, Cs-137 and Co-60. Superficial and anthropomorphic phantoms have been used to simulate the treatment and verify the delivered dose. Dose comparisons have been performed by simultaneously measurements obtained with gafchromic EBT3 films. The preliminary results will be presented.

Experimental and Monte Carlo simulation study of Linear Energy Transfer with heavy ions beams (4He, 16O and 12C) for future applications in ion-beam therapy

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Keywords: LET, RBE, Monte Carlo, Microdosimetry, Geant4

In the present hadrontherapy scenario, there is a growing interest in exploring the capabilities of different ion species other than protons and carbons. The possibility of using different ions paves the way for new radiotherapy approaches, such as the multi-ions treatment, where radiation could vary according to the target volume, shape, depth and histologic characteristics of the tumour. In this work, the study and understanding of biological-relevant quantities were extended for the case of the 4He ion, 12C and 16O. Monte Carlo based algorithms in the dose and track-averaged LET (Linear Energy Transfer) calculations were validated also for the case of a mixed field characterized by the presence of secondary ions from both target and projectile fragmentation[1].
The simulated dose and track averaged LETs were compared with the corresponding dose and frequency mean values of the lineal energy, yD and yF, derived from experimental microdosimetric spectra. Three microdosimetric experimental campaigns were carried out at the Laboratori Nazionali del Sud of Istituto Nazionale di Fisica Nucleare (INFN-LNS, Catania, I) using the silicon microdosimeter MicroPlus probe. The MicroPlus probe is an array of 3D right parallelepiped shape sensitive volumes (diodes) with area 30 um X 30 um, fabricated using silicon on insulator wafers with an active layer of 10 um thickness. The LET distribution was evaluated in both irradiation condition: with a pristine Bragg Beak and modulated peak (obtained by using a proper ridge filter). The beam energy was 62 MeV/n in all the investigated cases. The obtained results verified with the statistical analysis based on the chi-squared goodness of fit test, the total averaged LET quantities obtained with the Monte Carlo calculations, including all the contributions of secondary ions, find a remarkable agreement with the microdosimetric experimental data, within their respective uncertainties.

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The cancer suppression mechanisms of elephants: An in vitro study to shed light on Peto's paradox

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Keywords: TP53, Peto's Paradox, Radiation Therapy, Evolution, Elephants, Cancer

The risk for cancer between mammalian species is not related to body size and species life span. This lack of correlation is known in evolutionary biology as Peto's Paradox. In elephants, the largest land mammals, the answer to this paradox might lie in the redundancy of the tumour suppressor gene *TP53* gene. The presence of 20 *TP53* copies and the more recently discovered *LIF6* pseudogene might suggest that the cancer defence of elephants is mediated by an enhanced apoptotic response to DNA damage, removing potentially cancerous cells at an early stage. However, the redundancy in tumour suppressor genes cannot resolve Peto's paradox completely in elephants, since they should have developed a trade-off between the aggressive elimination of damaged cells and senescence, resulting in depletion of their stem cell pool. Here, the first results of a joint project are presented, highlighting what implications Peto's paradox could have for radiation protection and radiation therapy strategies.
Blood samples were collected from elephants by experienced wildlife veterinarians in the Zoo of Naples (Italy) and private game reserves with free-roaming elephants in South Africa. After transport to the laboratories, the elephant blood samples were irradiated and apoptosis (Annexin V-FITC/PI) and DNA repair response (γ-H2AX foci) was compared to human samples. In addition, comparative next generation sequencing was performed on human and elephant blood samples, to investigate which specific pathways are up- or downregulated after radiation exposure.

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The results of the apoptosis assay illustrate that elephant cells go into apoptosis at much higher rates than human cells, even after exposure to doses as low as 0.125 Gy photons. While no statistically significant difference could be observed in the number of DNA DSBs at 1 hour post-irradiation, the 24 hours result confirm that elephant lymphocytes have lower numbers of residual DNA DSBs compared to human lymphocytes.
The results confirm the working mechanisms of the tumour suppressor gene and striking differences in DNA repair capacity between human and elephant cells. It is envisaged that this project could rapidly advance the development of new strategies for the prevention of radiation-induced cancers or the sensitization of cancer cells to radiotherapy.

Dose-response effects of ^{177}Lu-octreotide and ^{177}Lu-octreotate in neuroblastoma-bearing mice

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Keywords: Neuroblastoma, radionuclide therapy, somatostatin analogues, xenografted mice

Background: Patients with aggressive neuroblastoma (NB) have a 5-year survival rate of only 40-50%, indicating the need for novel and improved treatment options. Since NBs overexpress somatostatin receptors, radiolabelled somatostatin analogues have a potential treatment option. In our previous biodistribution studies with ^{177}Lu-octreotate, mice xenografted with human NB cell lines were shown to have high uptake in tumor tissue compared to risk organs. The aim of this work was to compare the biodistribution of ^{177}Lu-octreotide with ^{177}Lu-octreotate and to study their therapeutic effects in CLB-BAR xenografted mice.

Methods: Female nude BALB/c mice (5-6 weeks old) were s.c. injected on their flank with CLB-BAR cells. Biodistribution of 1.5 or 15 MBq ^{177}Lu-octreotide was investigated at 1h, 24h and 168h. For the therapeutic study animals were divided into groups and administered with 15, 30 or 60 MBq ^{177}Lu-octreotide or ^{177}Lu-octreotate. Other mice received fractionated administration with 1x15, 2x7.5, and 3x5 MBq ^{177}Lu-octreotate.Treatment effects were studied by tumour volume measurements. Results from both studies were compared with those from sham treated mice (i.v. saline injections). The tumour volume was measured with a caliper twice a week and the mice were killed when the tumour mass exceeded 10 % of the body weight.

Results: Comparable biodistribution profiles were observed after administration with ^{177}Lu-octreotide or ^{177}Lu-octreotate. Treatment with ^{177}Lu-octreotide displayed a clear dose-response relation and a clear anti-tumour effect was observed. No clear dose-response relationship was observed after treatment with ^{177}Lu-octreotate. Although the mice that received 60 MBq showed a better response, the tumour volume was not significantly lower than other groups. Furthermore, fractionation with 3x5 MBq ^{177}Lu-octreotate displayed a more profound effect on the tumour volume.

Discussion and conclusion: The mice that received ^{177}Lu-octreotide showed a clearer dose-response relationship with a stronger anti-tumour effect in comparison with ^{177}Lu-octreotate. Pronounced anti-tumour effects following fractionated administration merits our thoughts regarding saturation effects and further studies will investigate this theory.

Isotope labelled ferritin – an alternative drug delivery system

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Keywords: ferritin, radiolabeling, molecular imaging, radioconjugates

Cancers are the second most common cause of death worldwide. Each year, they cause the death of nearly 10 million people. The high diversity of neoplastic diseases and the existence of metastases, characteristic for malignant tumours, makes finding a universal diagnostic and therapeutic method a difficult and so far unresolved problem. Current research is focused on the development of methods enabling detection of smaller metastases and diagnosis of cancers at early stages of growth, as well as more effective and safer therapies. This is expected to reduce mortality and improve patient welfare.

The aim of our studies is to assess the applicability of ferritins as an alternative delivery system of isotopes for tumour diagnosis. Ferritins are proteins with a cage-like structure and they are responsible mainly for the storage and transport of iron in the organism. Our experiments with ferritin molecules labelled with the copper-64 isotope indicate highest concentration of the compound in the tumour tissue 12-24 h after injection. Therefore, we decided to perform experiments with other radionuclides selected based on their half-life and characteristics of the emitted ionizing radiation, i.e.: iodine-131 and lutetium-177. We have carried out an optimization procedure of ferritin labelling experiments and gathered extensive data on biodistribution of the radioconjugates.

The obtained results indicate the possible use of radioisotope labelled ferritins for diagnosis and treatment of tumours, especially when combined with molecular imaging techniques such as PET or SPECT.

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From design to in vitro evaluation of potential radioprotectors

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Keywords: apoptosis inhibition, gamma irradiation, in vitro, radioprotective agents

Purpose: The risk of radiation exposure, which is increasing due to recent events, highlights the need for new radioprotectants. The aim of this work was to design, synthesize and determine the toxicity and radioprotective effects of a series of 1-(2-hydroxyethyl)piperazine derivatives *in vitro*. The substances were *in silico* modeled and in-house synthesized.
Materials and methods: First, a docking study toward Bcl-2 anti-apoptotic protein was performed and the most suitable structures were designed and synthesized. 10 human cell lines, 9 cancerous and 1 non-cancerous were used for *in* vitro evaluation of toxicity of concentrations 10 μM and 100 μM by the WST-1 proliferation assay. MTT test was used to assess half inhibitory concentration (IC_{50}) and maximum tolerated concentration (MTC) in A-549 lung cancer cell line. The radioprotective effect was tested by flow cytometry with Annexin V/propidium iodide staining on the MOLT-4 T-lymphoblastic leukemia cell line, which was treated by 100μM concentration of tested compounds 1 hour before irradiation by gamma source ^{60}Co.
Results: Tested compounds showed MTC ranging from 0.002 to 6.25 mM and IC_{50} from 0,04 to > 25 mM, nevertheless the least toxic substance was considerably unstable. Several compounds significantly increased survival fraction (SF) ranging from 51 to 65 % in the MOLT-4 cell line after 1 Gy irradiation compared to non-treated irradiated control (SF=40 %) and non-treated non-irradiated control (SF=92 %). An overview of the impact on viability will be given.
Conclusion: The results suggest that some of the 1-(2-hydroxyethyl)piperazine

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derivatives have promising radioprotective potential. Currently, the structure of the best substance is being improved to more pronounced radioprotectivity and lower toxicity. In parallel, the putative mechanism of action is investigated and indications are that it might involve inhibition of the intrinsic apoptotic pathway.

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Monitoring of the long-term trends in parameters of electromagnetic radiation in urban environment of Warszawa

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Keywords: electromagnetic exposure, radiocommunication networks, exposure evaluation, non-ionizing exposure

The highest density of radio communication antennas and electromagnetic radiation (EMR) with the most complex frequency spectrum may be found in the urban environment. The aim was to identify and assess the trends in long-term changes of parameters of EMR emitted by urban radiocommunication systems in Warszawa (capital city of Poland). The frequency spectrum of EMR was measured and analyzed in order to determine the components of exposure in particular locations, and monitoring of the time-variability of the level of EMR exposure (using exposimeters – multi frequency-narrow-band electric field data recorders – collecting characteristics of exposure to EMR harmonized with the frequencies of typical urban radiocommunication systems: mobile phones (downlink signal transmission from the base station to the terminal: LTE 800, GSM 900, DCS/LTE 1800, UMTS/LTE 2100, LTE 2600) and radio and television transmitters (FM, TV VHF/UHF), local network of communication between devices and access to the Internet (Wi-Fi 2.4/5 GHz).
It has been shown that the level of exposure in a given place depends mainly on the impact from the nearest EMR transmitters – mobile phone base station in the typical urban location. A comparative analysis of the results of EMR over years did not show any significant changes in the total level of exposure in Warszawa during the last 20 years. However, changes in the composition of dominating frequency components have been documented - related to the migration of radiocommunication services into new technologies and frequency bands (such as migration of analog TV into the digital service, or introduction of new mobile phone systems – 3G, 4G, LTE). Currently, the observed dominating exposure is associated with the use of mobile internet access (LTE) and to a lesser extent from voice calls (GSM). The results of the EMR monitoring in urban environment correlate with reports regarding changes in the structure of telecommunication services usage.

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POSTER SESSION 2

Exploring human plasma proteome in context of radiation biomarkers

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Keywords: ionising radiation; biomarker; TBI; plasma; proteomic

PURPOSE: The increasing risk of acute large-scale radiological/nuclear exposures of population underlines the necessity of developing rapid and high-throughput biodosimetric tools for estimation of received dose and initial triage. As ionizing radiation triggers complex response on genome and proteome level, both were already reported as suitable indicators of radiation-induced damage in vitro or in animal models. Our goal is to identify and quantify radiation-responsive plasma proteins in TBI patients using mass spectrometry.
MATERIAL AND METHODS: Peripheral blood was taken before and 24 hours after TBI (2 x 2.0 Gy). Plasma samples of leukaemic patients (n=24) were immuno-depleted, reduced, alkylated, and digested. Healthy donors (n=15) of corresponding sex and age were sampled in parallel to reduce bias caused by oncological condition and temporal effects. Both “label-free” and iTRAQ relative quantification approaches were applied using RP-nanoLC-ESI-MS/MS system with Q-Exactive mass spectrometer (Thermo). Proteins were identified using Proteome Discoverer v.2.2 platform (Thermo). Subsequent analysis was carried out using Metascape freeware (http://metascape.org).
DISCUSSION: We acquired a list of plasmatic proteins with statistically significant up-regulation (ratio ≥ 1.2) or down-regulation (ratio ≤ 0.83) 24 hours after irradiation ($p \leq 0.01$). We ruled out proteins deregulated in non-irradiated patients when compared to healthy donors as possibly associated with the disease and those changed in healthy donors within 24 hours as the timely unstable ones. Finally, we obtained a list of candidate proteins and assessed their biological function concerning radiation.
CONCLUSION: Finally, we obtained a list of candidate biomarkers and an overview of identified proteins will be presented. We assessed their radiobiological relevance and selected the top candidates for verification. As this is an ongoing project, the validation is still in the process. The human plasma proteome of oncological TBI patients represents an uncovered area, thus the obtained data might have future implications to biological dosimetry.

Role of protein glycosylation in radiation-induced immune cell recruitment

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Keywords: Radiation therapy; Immune cell recruitment; Vascular endothelium; Protein glycosylation

Radiation therapy damages tumors and surrounding normal tissue, probably in part through the recruitment of immune cells. Endothelial high-mannose N-glycans are, in particular, involved in monocyte-endothelium interactions. Trimmed by the class I a-mannosidases, these structures are quite rare in normal conditions. Here, we show that the expression of the endothelial a-mannosidase MAN1C1 protein decreases after irradiation. We modeled two crucial steps in monocyte recruitment after irradiation by developing *in vitro* real-time imaging models. Inhibition of MAN1C1 expression by siRNA gene silencing increases the abundance of high-mannose N-glycans and improves the adhesion rate of monocytes on endothelial cells in flow conditions. In contrast, inhibition of MAN1C1 expression decreases radiation-induced transendothelial migration of monocytes. Consistently, overexpression of MAN1C1 in endothelial cells using lentiviral vectors decreases monocyte adhesion and enhances transendothelial migration. Hence, we propose a role for endothelial MAN1C1 in the recruitment of monocytes through endothelial high-mannose N-glycan interactions, particularly in the adhesion step to the endothelium.

Role of KRAS mutation on NSCLC resistance to x-rays, protons and carbon ions

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Keywords: NSCLC, KRAS mutation, X-rays, carbon ions and protons irradiations

Unresectable locally advanced non-small cell lung cancer (NSCLC) is a frequent cancer with a prognosis that remains poor despite several treatments for radiotherapy, chemotherapy and immunotherapy. Mechanisms of resistance to treatments have been highlighted such as KRAS mutations. A new therapy type tyrosine kinase inhibitor targeting the KRAS G12C mutation has enabled efficacy in metastatic NSCLC and could have an interest in the treatment by radiotherapy locally advanced forms. However, the radiosensitizing effect of such molecules targeting KRAS remains unknown. KRAS resistance mechanisms could be related to hypoxia and the proliferation of tumor stem cells and irradiation by carbon ions and protons compared to photon irradiation might also have future interest in the treatment of these patients.
In this study, we propose to use *in vitro* models of KRAS G12C mutated cell lines (vs. non-mutated). These cells are treated with a combination of KRAS G12C inhibitors and irradiations (X-rays, carbon ions and protons). Following a complete characterization of the cell lines and drug toxicity tests, the clonogenic survival of the cells is then analysed.
Our preliminary results show a differential response of the cells, depending on the cell lines, irradiation doses and quality and drug combinations.

3D Tumor Models for Radiobiological Applications

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Keywords: 3D cell cultures, 3D bioprinting, alternative tumor models, tissues radio-response.

Cancer is intrinsically complex, comprising both heterogeneous cellular composition and extracellular matrix. *In vitro* cancer research models have been widely used in the past to model and study cancer. Although two-dimensional (2D) cell culture models have been the traditional hallmark of cancer research, they have many limitations, such as the disturbance of interactions between cellular and extracellular environments and changes in cell morphology, polarity, division mechanism, differentiation and cell motion. This implies that 2D tumor models are ineffective to accurately recapitulate complex aspects of tumor cells growth, as well as their drug and radiation responses.

Over the past decade there has been significant uptake of three-dimensional (3D) *in vitro* models by cancer researchers, highlighting a complementary model for studies of radiation effects on tumors, especially in conjunction with chemotherapy. In this work we present two alternative 3D *in vitro* models obtained by the use of: 1) 3D bioprinting techniques and 2) VITVO cell culture bioreactors containing a fiber-based matrix, to be used for radiobiological purposes. Preliminary results show clearly different spatial cell organization in the 3D compared to 2D environment, leading to different responses of medulloblastoma cells to radiations, also in terms of cell survival. Our findings can shed new light on understanding the features of the 3D cell model and its application in basic research into clinical radiotherapy and medicine. Furthermore, positioning itself halfway between 2D cell culture and animal models, 3D systems could affect modeling of radiation-tissue interactions, thus opening up new possibilities in the study of radiation response mechanisms of healthy and tumor tissues.

Gold nanorods and radioisotopes: future diagnostic and therapeutic applications in nuclear medicine. Preliminary in vitro radiobiological tests.

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Increasing the dose within the tumour mass and simultaneously reducing the dose to healthy tissues is still a major challenge in radiotherapy, although several strategies have been proposed. At present, nanotechnologies play an important role in biomedicine, and different types of nanoparticles have received significant attention in this field, such as gold nanorods (AuNRs) [1].
Low energy electrons (Auger electrons) are produced by the interaction of photons with the gold in the NRs. These electrons are similar to those emitted by the decay of ^{99m}Tc, a radioactive nuclide widely used for diagnostic purposes in nuclear medicine. At the cellular level, these short-range charged particles lead to a dense deposition of ionising energy associated with increased radiobiological efficiency. Auger and internal conversion (IC) electron emitters appropriately targeted to the DNA of tumour cells may therefore represent an interesting new radiotherapy system: ^{99m}Tc could indeed be used as a theragnostic radiopharmaceutical once loaded on AuNRs and delivered to the tumour site.
This work aims to be a proof of concept to evaluate in vitro the toxicity of AuNRs in T98G cells and their efficacy in inducing radio-induced damage at cellular and/or molecular level following photons irradiation, thus mimicking the labeled ^{99m}Tc used in clinical settings.
Preliminary data will be presented on the chemical characterization of AuNRs (with aspect ratio 3.2 and Surface Plasmon Resonance bands at 520 and 680 nm) and the loading of ^{99m}Tc on their surface. Spectroscopic characterization, such as Uv-vis, Fourier transform infrared spectroscopy (FTIR), and X-ray photoelectron spectroscopy (XPS), will be performed to investigate the drug-AuNRs interaction. Finally, preliminary radiobiological data on cell killing and/or chromosomal damage will be shown.

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Biomedical Applications of Radioactive ion Beams: First results of the BARB project at GSI

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Keywords: Radioactive Ion beams, particle therapy, online range verification, Image guidance

Heavy ion particle therapy is a rapidly growing and potentially the most effective and precise radiotherapy technique. However, range uncertainties jeopardize the benefits of the sharp Bragg peak and force to use wide margins extending in the normal tissue.
The use of radioactive ion beams (RIBs) for simultaneous treatment and online range verification using positron emission tomography (PET) could help to overcome this limitation, showing an increased signal/noise ratio, a reduced biological washout (thanks to the shorter measurement time required to collect the signal) and alignment of the activity peak with the Bragg peak compared to PET imaging of fragments produced by primary stable ion beams[1].
In this context, the BARB (Biomedical Applications of Radioactive ion Beams) project was initiated at GSI with the goal to assess the technical feasibility and investigate possible advantages of RIBs in preclinical studies[2,3].
During the first year of experiments within this project, radioactive Carbon and Oxygen beams (^{10,11}C and ^{15}O) were produced by isotopic separation with the fragment separator (FRS) and transported to the medical vault of GSI. Thanks to the upgrade of the SIS-18 in the FAIR in Darmstadt, it is possible to achieve RIB intensities sufficient to treat a small animal tumor.
Beam implantation in plastic phantoms was visualized by two independent imaging setups: a dual-panel PET scanner from the University Medical Center Groningen and a subset of a high-resolution small animal in-beam PET detector in development at the Ludwig-Maximilians Universität in Munich. Range and depth dose distributions measurements have been performed with a water column setup. These first experimental results will be presented.
Work supported by ERC Advanced Grant BARB (2020, Marco Durante) and ERC Consolidator Grant SIRMIO (2016, Katia Parodi).

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Combination of nutrient deprivation and X-ray exposure induce synergic effect

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Keywords: Colorectal cancer, Radiotherapy, Resistance to therapy, Synergic effects

Conventional treatments for cancer patients are based on two main approaches: radiotherapy and chemotherapy. Radiotherapy makes use of ionizing radiation, inducing damage to the DNA of target tissues, and it is often used as an adjuvant to surgical resections. Chemotherapy employs drugs that block specific targets involved in proliferative activity to inhibit the cell cycle progression. The combined administration of such treatments has proven to be more effective than treatments with individual therapies. More generally, oncological research has recently seen a very rapid progress towards the so-called personalized medicine approaches, which allows patients allocation and stratification into treatment schemes based on the differential analysis and subtyping of tumors, including consideration of tumour specific resistance to treatments and interactions with the immune system.

In the context of investigating radiation resistance, such as that observed *in vitro* in the Caco-2 cell line (a cell line derived from colorectal adenocarcinoma) (1,2), we wanted to test a possible synergistic effect between ionizing radiation (0.5-5.0 Gy from a conventional radiotherapy X-ray accelerator) and shortage or absence of metabolites induced by Asparaginase, a bacterial amidohydrolase used for many decades in the treatment of Leukemia (3). Preliminary data from our Radiation Biophysics and Radiobiology Lab obtained with different radiobiological techniques, including cell clonogenic inactivation assay and cell-cycle characterization, show that the combination of X-ray exposure and deprivation of specific nutrients (asparagine and glutamine) seem to exacerbate the cell stress response, mostly at low doses, and that the two stressors have a synergistic action.

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The impact of chromatin architecture, its geometry and topology, on radiation induced damaging and following DNA repair processes

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Keywords: chromatin architecture, DNA damaging, DNA repair, super-resolution localization microscopy, Ripley´s distance frequency statistics, persistence homology

The three-dimensional architecture of genomes on the micro-, meso- and nano-scale acts in combination with epigenetic modifications as an important player of gene regulation and, consequently, fundamental biological processes such as DNA damage response and repair. So far only little is known about the impact of chromatin architecture and its geometry and topology on DNA double strand break (DSB) induction and repair pathway selection and progression at individual damage sites. How does a **cell nucleus as system as a whole**, process DSBs and re-organize the chromatin towards functionally intact repair units? And how, on the other hand, are repair centres oriented towards specific repair mechanisms formed at individual DSB sites? We present investigations of spatial and topological parameters of chromatin domains and DNA repair foci during a time period of repair to glimpse key aspects related to these questions. Nano-probing of chromatin damage sites and the recruited DNA repair proteins in combination with super-resolution Single Molecule Localization Microscopy (SMLM) are powerful methods for geometric and topological analyses of meso- and nano-structures in single cells and at single DSB sites and, thus, to study mechanisms of their formation and repair pathway regulation. We used variable technological tools based on image-free SMLM, nano-scaled molecule distribution analyses, appropriate metrics following Ripley´s distance frequencies and cluster formation analyses, as well as topological quantifications employing persistence homology. Comparing the topology of repair foci suggests general similarities in repair cluster formation, indicating a non-random, molecule topology at given time points during repair. The data reveal a specific architecture of DNA damage foci for a given chromatin domain and cell type.

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Characteristics of chromatin architecture around complex damage sites, repair focus nano-architecture or spatial arrangements of repair proteins may contribute to control repair process. Our studies contribute to the understanding of whole system cellular radiation response.

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2 Hausmann M, Falk M, Neitzel C, Hofmann A, Biswas A, Gier T, Falkova I, Heermann DW, Hildenbrand G (2021) Elucidation of the clustered nano-architecture of radiation-induced DNA damage sites and surrounding chromatin in cancer cells: A Single Molecule Localization Microscopy approach. Int. J. Mol. Sci. 22: 3636 doi.org/10.3390/ijms22073636

The influence of the breast cancer cells sensitization by gold nanoparticles on the cell cycle distribution and the survivability rate in the case of Boron-Neutron Capture Therapy

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Keywords: Boron-Neutron Capture Therapy, gold nanoparticles, breast cancer cells, clonogenic assay, cell cycle distribution

Boron- Neutron Capture Therapy (BNCT) is a type of radiotherapy which is based on the ability of non-radioactive boron-10 isotope to capture thermal neutrons. In the experiment, triple-negative types (MDA-MB-231, MDA-MB-468) and luminal type (MCF-7) of breast cancer cells were used. The main aim of the research was to analyze the influence of the breast cancer cells sensitization by gold nanoparticles on their cell cycle distribution and their survivability rate.
MDA-MB-231, MDA-MB-468 and MCF-7 were exposed on average to 2,5 Gy of neutron radiation from MARIA research reactor located in National Center for Nuclear Research in Świerk. Before irradiating cells, solutions of boron-10 compound (BPA) and gold nanoparticles were added and nextly incubated. Cell cycle distribution was measured by flow cytometry method within 24h. In the case of the clonogenic assay, cells were incubated for 14 days and later colonies were counted.
Preliminary results show that there is difference in the cell cycle distribution and the survivability rate between cells after using BNCT treatment method with sensitization by gold nanoparticles and without usage of gold nanoparticles. The obtained results will be presented during the conference.

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Biological dosimetry for microbeam radiation therapy (MRT)

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Microbeam Radiation Therapy (MRT) is an innovative preclinical concept in radiotherapy that collimates X-ray radiation in micrometer-wide, planar beams. Previous research has shown that MRT substantially spares normal tissue, while being equally effective in tumor ablation. In order to validate doses measured with radiochromic film dosimetry, biological dosimetry using the cytokineses blocked micronuclei (CBMN) assay was applied on a cellular level.

CBMN assay was performed using CHO cells after homogeneous irradiation to establish a dose response curve, and afterwards with either sham or microbeam. At least 1000 binucleated cells were analyzed with Metafer (Metasystems, Germany). Microbeam radiation was performed at the XenX irradiation device (XStrahl, UK), equipped with a special microbeam collimator. Planar microbeams with a peak-width of 50 µm and a center-to-center distance of 400 µm were used.

The dose-response curve was fitted with the linear equation (y=0.44x + 0.13). Irradiations with a physically calculated peak dose of 2 Gy and a valley dose of 0.05 Gy resulted in 2.05 +/- 0.12 Gy and 0.02 +/- 0.05 Gy respectively using biological dosimetry. However, after irradiations with a calculated peak dose of 82 Gy and a valley dose of 2 Gy, the micronuclei which were counted in the valleys equaled to 2.29 +/- 0.02 Gy.

This is the first study determining precisely the absorbed doses in the peak and valley regions of MRT by film and biological dosimetry. Moreover, the slightly higher measured doses than the physically planned doses in the valleys indicate effects on a cellular level, which could be due to bystander effects and/or enhanced cell migration.

The effect of low- and high-dose rate brachytherapy on the innate and adaptive immune system of prostate cancer patients

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Keywords: prostate cancer, brachytherapy, immune phenotyping

Introduction: Radiotherapy, as one of the most important prostate cancer treatment modalities can modify systemic immune responses but little is known on how long immune dysfunction persists in cancer survivors. Our aim was to perform a detailed analysis of the systemic immune status of prostate cancer patients treated with various radiotherapy protocols.
Materials and methods: Blood samples were collected from 21 patients treated with low-dose rate (LDR) and 18 patients treated with high-dose rate (HDR) brachytherapy before and at 5 time points after therapy. Cellular and soluble changes were analysed in peripheral blood.
Results: In LDR patients before therapy both the adaptive (decreased CD4+ effector T cells) and the innate immune system (decreased NK cell pools) were changed. While the innate immune response recovered as the tumour was cured, a mild long-term deficit in the adaptive immune response persisted even 3 years later. Similar, but stronger changes were detected in HDR patients. Before therapy the level of B cells, memory T cells decreased, senescent T cells level and degranulating NK cell levels strongly increased compared to control group, and these alterations persisted up to 36 months. Levels of PDGF-AA, ENA-78 and RANTES increased in both patient groups before therapy, however they did not normalize in HDR patients potentially indicating an increased predisposition for the development of long-lasting side effects such as radiation-induced late fibrosis.
Conclusion: Significant differences were noted in systemic immune parameters of both prostate cancer patient groups highlighting the importance of radiotherapy in modulating systemic immune responses.
Funding: This work was supported by a Hungarian research grant of the National Research, Development, and Innovation Office (NKFI-124879) and by the H2020 Framework Program (grant agreement number 899995, EURAMED rocc-n-roll).

Fibroblast-based radiosensitivity assessment for primary immunodeficiency patients

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Keywords: Micronucleus assay, γ-H2AX foci assay, radiosensitivity, fibroblasts, primary immunodeficiencies

Background & aim
Several human syndromes with a genetic defect in one of the DNA double strand break (DSB) recognition and repair proteins have been described. These patients are defined by their immunological defects, cancer susceptibility, neurological abnormalities and sensitivity to ionizing radiation (IR). Malfunction of the patient's immune system may be the most overt clinical feature and initially present with a primary immunodeficiency disease (PID). As DNA damaging agents are often applied in diagnostic and therapeutic procedures, identification of radiosensitive individuals in this patient population is essential to optimize their clinical care. As an alternative for the well-established lymphocyte-based assays, which are not feasible for certain PID patients, reliable fibroblast-based radiosensitivity (RS) analysis is proposed in this study.

Materials & methods
Fibroblasts derived from PID patients with a confirmed or suspected DNA repair defect (mutations in ATM, Artemis, XLF, LIGIV, NBS1, RAG1/2) were irradiated with X-rays (0,5 and 1 Gy) in the G0 phase of the cell cycle. RS was assessed with the γ-H2AX foci
architecture of DNA damage foci for a given chromatin domain and cell type.

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test and an optimized cytokinesis-block micronucleus (MN) assay. Using fibroblasts from healthy individuals, the cut-off value for RS was determined.

Results & conclusion
Patients with an Artemis and RAG1/2 mutation could respectively be identified as radiosensitive and not radiosensitive with both the γ-H2AX foci and G0 MN assay. The MN test was not feasible for XLF and LIG4 mutated fibroblasts, but their high γ-H2AX foci levels post-irradiation clearly indicated a radiosensitive phenotype. For these patients, the observed in vitro RS correlated with the expected clinical response to IR. However, ATM defective fibroblasts, known for their extreme RS, show high MN yields following irradiation, while these cells were not considered radiosensitive with the γ-H2AX foci assay. Also for NBS1 mutated cells, residual foci levels did not reach higher than the RS cut-off value. Although limitations in the use of a single RS assay were observed, the combination of the fibroblast MN and γ-H2AX foci test clearly improved the RS assessment for PID patients.

Variation in intra- and intercellular response to radiation-induced DNA damage

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Keywords: 53BP1, micronuclei, intra- and intercellular sensitivity, DNA damage response

Cellular response to ionizing radiation-induced DNA damage may vary depending on genetic and environmental factors. The interesting question is how far environmental factors influence the radiation response of cells in culture. To investigate whether there is a difference between intra- and intercellular variation in sensitivity to radiation-induced DNA damage we compared frequencies of 53BP1 foci in nuclei of binucleated cells and in nuclei of neighboring cells. Frequencies of foci in micronuclei and mononucleated cells were also assessed, and nucleus areas were examined to see if the density of foci per unit nucleus/micronucleus area is different in nuclei in mononucleates vs binucleates and in micronuclei.

Experiment was carried out with U2OS cells which were stably transfected with a plasmid coding for 53BP1-GFP. Cells were seeded on glass coverslips, placed in Petri dishes, and exposed to 2 Gy of gamma radiation. Cytochalasin B was added immediately after irradiation. After 24 h the cells were irradiated again with 2 Gy of gamma radiation. Kinetics of foci formation was analyzed after 30, 60, 120 and 180 minutes of repair time. Foci were analyzed using a confocal microscope and scored manually on images.

The analysis has not been completed at the time of abstract submission. The preliminary results show that the intercellular variability in focus frequency is significantly higher than the intracellular variability suggesting that environmental factors have an impact on the response of cells in cell culture. A difference in focus frequency was also observed between nuclei of mononucleates and binucleates.

Margin reduction in particle therapy: potential benefits of using radioactive ion beams

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Keywords: particle therapy, carbon ions, image guidance, treatment planning, NTCP

One of the limitations of carbon ion therapy (CIT) is the range uncertainty, which is usually mitigated by adding wider margins to the CTV to ensure its dose coverage. While PET imaging is the beam range verification method with most clinical applications in CIT, for ^{12}C ion beams its applicability remains limited by the low signal-to-noise ratio and the shift between the dose and activity peaks. Instead, direct application of β^+-emitting radioactive beams (RIBs), such as ^{11}C, for treatment could increase the signal magnitude and improve its correlation with the dose distribution, allowing to reduce the tumor margins and, consequently, the dose to surrounding healthy tissues. The experimental campaign of the BARB project[1], ongoing at GSI, aims at the full physical and radiobiological characterization of RIBs for therapy, with the final goal of treating the tumor in a small animal.
This study aims to estimate the potential clinical benefits of tumor margin reduction

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granted by RIB treatments. We have developed a FLUKA beam model for radioactive ^{11}C ions to be used within the TRiP98 treatment planning system and established the workflow to predict the resulting activity maps. We demonstrate that in ^{11}C plans, the activity fall-off strictly follows the dose fall-off and could be theoretically used to trace the sub-millimeter dose distribution shifts, allowing to practically eliminate the range margins. Following that, we compared the robustly optimized ^{11}C and ^{12}C treatment plans for head-and-neck and liver tumors. Reduced margins in ^{11}C plans lead to a substantial decrease of dose to the bystander critical structures. The results of the NTCP evaluation, additionally performed to estimate the toxicity for critical organs in the target proximity, suggest a better toxicity profile compared to ^{12}C beams in selected patients.

Work supported by ERC Advanced Grant 2020 n. 883425 (BARB, Biomedical Applications of Radioactive ion Beams) to Marco Durante.

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Planar Proton Minibeam Irradiation Elicits Spatially Confined DNA Damage in a Human Epidermis Model

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Keywords: proton minibeam radiotherapy, DNA damage, human epidermis, 53BP1, gH2AX

High doses of ionizing radiation in radiotherapy can elicit undesirable side effects to the skin. Proton minibeam radiotherapy (pMBRT) may circumvent such limitations due to tissue-sparing effects observed at the macro scale. Here, we mapped DNA damage dynamics in a 3D tissue context at the sub-cellular level*.
Epidermis models were irradiated with planar proton minibeams of 66 µm, 408 µm and 920 µm widths and inter-beam-distances of 2.5 mm at an average dose of 2 Gy using the scanning-ion-microscope SNAKE in Garching, Germany. γ-H2AX + 53BP1 and cleaved-caspase-3 immunostaining revealed dsDNA (double-stranded DNA) damage and cell death, respectively, in time courses from 0.5 to 72 h after irradiation.
Focused 66 µm pMBRT induced sharply localized severe DNA damage (pan-γ-H2AX) in cells at the dose peaks, while damage in the dose valleys was similar to sham control. pMBRT with 408 µm and 920 µm minibeams induced DSB (double-strand break) foci in all cells. At 72 h after irradiation, DNA damage had reached sham levels, indicating successful DNA repair. Increased frequencies of active-caspase-3 and pan-γ-H2AX-positive cells revealed incipient cell death at late time points.
The spatially confined distribution of DNA damage appears to underlie the tissue-sparing effect after focused pMBRT. Thus, pMBRT may be the method of choice in radiotherapy to reduce side effects to the skin.

*H. Scherthan et al., Planar Proton Minibeam Irradiation Elicits Spatially Confined DNA Damage in a Human Epidermis Model, Cancers 2022, 14(6), 1545; https://doi.org/10.3390/cancers14061545

Mechanism of chemopotentiating effects of low-dose fractionated radiation in head and neck cancer cell lines

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Keywords: Low-dose fractionated radiation, carboplatin, paclitaxel, head and neck cancer cells.

In our previous study (1), we found the unique enhancing effects of low-dose fractionated radiation (LDFR) on cisplatin in two cervical cancer cell lines SiHa and CaSki (both cell lines with Human Papilloma Virus (HPV) presence and low-dose hyper-radiosensitivity (HRS) phenomenon absence). The aim of the present study is to identify molecular mechanisms underlying the chemopotentiating effects of LDFR, especially the role of radiation-induced ATM nucleoshuttling (RIANS), in four human head and neck cancer cell lines (with different status of HRS and with or without HPV infection). The presence of HRS phenomenon in the cell lines is estimated by flow cytometry-based clonogenic survival assay. The potentiating effects of LDFR 4 x 0.5 Gy *versus* a single dose of 2 Gy on carboplatin and paclitaxel are compared using clonogenic survival, pATM and gammaH2AX foci assays. For combined experiments, the cells are treated with cytostatic drug and irradiated 24 hours later with 6 MV X-ray beam. To date, HRS status was assessed for two cell lines. FaDu cells appeared to be HRS-positive (with extremely pronounced HRS region) and SCC-25 cells HRS-negative. In case of FaDu cells, preliminary results showed that LDFR 4 x 0.5 Gy enhanced the effects of carboplatin and paclitaxel (assessed by clonogenic survival assay) at the same level as a single dose of 2 Gy that suggests no sparing effect of fractionation when low radiation doses (0,5 Gy) are combined with these cytostatics. Research is ongoing and results for four cell lines will be presented at the conference.

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Investigating transcriptional and translational responses to low-dose ionising radiation: towards an integrated low dose response model

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Keywords: low-dose radiation, transcription, translation, DNA repair

The cellular response to ionising radiation, either naturally occurring or otherwise, involves an intricate and coordinated chain of events. These events encompass activation of DNA damage response and DNA repair pathways via post-translational modification, regulation of transcription and translation, defining the health relevant outcomes such as apoptosis, genomic instability or proliferation. The precise nature and interactions within the cellular response to ionising radiation largely depend on the damage incurred. Although the biological response and effects of high doses of ionising radiation are well documented, less is known about responses to low-dose exposure. Given the potential implications for human health, we wished to investigate the regulation and coordination of transcriptional and translational events and how they relate to DNA double-strand break repair after exposure to low-dose ionising radiation using biologically relevant *in vitro* models, namely human-derived fibroblast cell lines. Following a combination of molecular and bioinformatic lines of interrogation including RNA-seq, mRNA stability analysis, ribopuromycylation and γH2AX foci analysis, we present observed changes as a function of time in coordinated transcriptional, post-transcriptional, translational and DNA double-strand break repair pathway responses to low doses (20 mGy and 100 mGy) of γ-rays. Taken together, these data help to decipher early molecular responses to low-dose radiation within the context of delayed health related outcomes, such as genomic instability, and represent the first steps to building an integrated low-dose response model.

The role of hypoxia and radiation in the stemness of murine osteosarcoma cells

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Keywords: tumor hypoxia, radiation, Cancer Stem Cells, Circulating Tumor Cells.

Despite the improvements in cancer treatment over the past decades, tumor recurrence and metastases are still the main concern for the therapy's success[1]. Tumors are composed of a heterogeneous population of cells. Besides those cells, Cancer Stem Cells (CSCs) are the most aggressive and resistant subpopulation[2]. Circulating Cancer Stem Cells are particularly resistant to radiation and present specific cell surface markers needed to resist in the bloodstream[3]. An increased metastasis formation ability characterizes these cells. These cells are challenging to identify, and are present in a few number in the bloodstream. The possibility of culturing them in vitro and characterizing them would significantly increase our knowledge about the mechanisms responsible for metastasis formation. An essential role in forming these cells is due to the tumor microenvironment and, in particular, hypoxia. In this study, we select cells with CTCs (Circulating Tumor Cells)-like phenotypes for further characterization using stressors such as hypoxia and radiation. We cultivate the cells in hypoxia for one week (acute hypoxia) and two weeks (chronic hypoxia), while irradiation is performed with a target dose of 4 Gy of X-rays.
Our results evidence a subpopulation of cells with an increased sphere formation capacity and migration under hypoxia and radiation. Further, WB analysis shows an increase in CD133 expression, a cancer stem cell marker, after treatment. This study will shed light on the mechanisms responsible for the CTCs formation.

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Short- and long-term effects on AHH-1 after low doses and dose rates of gamma radiation

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Keywords: low dose, low dose rate, dose and dose rate effectiveness factor (DDREF)

The relationship between radiation dose, dose rate and cancer risk is uncertain. Accordingly, the justification for a dose and dose rate effectiveness factor (DDREF) to adjust cancer risk estimates at low doses and low dose rates (LDLDR) remains controversial. This study aims at investigating short- and long-term radiation-induced biological effects of low doses at different low dose rates as compared to a single acute dose rate of ^{137}Cs gamma radiation on AHH-1 lymphoblasts.

Cells exposed to 0, 25, 50 or 100 mGy delivered either chronically at 1.6, 8 or 12 mGy/h or acutely at 0.35 Gy/min are monitored over 30 days for molecular, cellular and cytogenetic endpoints. Short-term effects to be analysed include cell viability at 1-, 3-, and 6-days post-exposure by the MTT assay, and cell reproductive death by the colony formation assay. Additionally, cell growth is continuously followed up, and cell pellets, collected on selected days, will become available for transcriptional and post-transcriptional analyses to investigate immediate and long-term changes at these levels by RNA sequencing and/or quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blot, respectively. Finally, stable chromosomal aberration analyses will be performed by fluorescence in situ hybridization (FISH) 30 days after exposure to examine the potential detrimental effects of low doses and dose rates on AHH-1 cells. Analyses of the first two experiments are ongoing. Preliminary data do not show an impaired cell growth of irradiated cells as compared to control up to day 19, but there is a trend in the first available replicate for a reduced cell viability of exposed cells relative to control at 3- and 7- days post-exposure.

Our study will contribute to defining the shape of the dose response curve after low doses delivered at different dose rates, providing insight on whether the experimental data supports a DDREF for the analysed endpoints and conditions.

Chemopotentiating effects of low-dose fractionated radiation in normal fibroblasts from patients with head and neck cancer treated with LDFR combined with induction chemotherapy

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Keywords: Low-dose fractionated radiation, carboplatin, paclitaxel, fibroblasts, head and neck cancer patients

The phenomenon of low-dose hyper-radiosensitivity (HRS) is an effect in which cells die from excessive sensitivity to low doses (< 0.5 Gy) of ionizing radiation but become more resistant (induced radioresistance, IRR) to larger doses. One possibility to benefit in the clinic from the HRS effect is by using low-dose fractionated radiation (LDFR) as an enhancer of systemic chemotherapy. The fact that our National Research Institute of Oncology, as the first in Poland, has started a phase II clinical trial using LDFR (0.5 Gy fractions) combined with induction chemotherapy (ChT) in patients with locally advanced squamous cell carcinoma of head and neck (SCCHN) gave us a unique opportunity to recognize potential predictors and mechanism underlying the clinical response to such treatment. The aim of the study is to compare *in vitro* the effects (assessed by clonogenic, pATM and γH2AX foci assays) of LDFR (4 x 0.5 Gy) *versus* a single dose of 2 Gy on carboplatin and paclitaxel in normal fibroblasts derived from patients with SCCHN enrolled in the clinical trial (LDFR+ChT) and to answer the question whether the chemopotentiating effects of LDFR apply to normal cells and depend on HRS status. To date, skin fibroblasts from 10 SCCHN patients have been obtained. Preliminary results on fibroblasts of two patients showed that carboplatin- and paclitaxel-potentiating effects were two-fold greater with LDFR 4 x 0.5 Gy than those with a single dose of 2 Gy. Research is ongoing and all results will be presented at the conference.

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Mechanistic insights from high resolution DNA damage analysis to understand mixed high and low LET radiation exposure and its therapeutic potential

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Keywords: DNA damage, high LET radiation, low LET radiation, high-resolution microscopy

Cells exposed to both scattered low linear energy transfer (LET) and densely ionising high LET radiation delivered at the same time react more strongly than expected based on additivity. Here we studied the relationship between DNA double strand break (DSB) location inside the nucleus and chromatin structure. High-resolution transmission electron microscopy (TEM) was used to assay MDA-MB-231 cells 30 min after 5 Gy of gamma and/or alpha irradiation. DSB marker γH2AX immunolabelling was detected using nanosized gold beads. Additionally, cellular response to single (1 x 1.5 Gy) versus fractionated dose delivery (5 x 0.3 Gy) to low and/or high LET radiation was assessed in two cancer cell lines (MDA-MB-231 and U2OS). The highest total number of gold beads as well as foci were found in cells irradiated with alpha radiation just prior to gamma radiation (called mixed beam, 50% dose of each), followed by alpha, then gamma radiation. DSB induced by mixed beams tended to be surrounded by open chromatin (lighter regions in TEM), yet foci containing the highest number of beads, i.e. larger foci representing complex damage, remained in the heterochromatic areas. The γH2AX focus area was also greater in mixed beam-treated cells when analysed by immunofluorescence. The strongest reduction in cell viability and colony formation was demonstrated in MDA-MB-231 and U2OS cells after mixed beams, in comparison to each radiation quality delivered separately, after fractionated exposure. This may partially be explained by a delay in recompaction of chromatin in MDA-MB-231 cells, where the heterochromatin marker H3K9me3 was low after fractionated mixed beam as well as alpha radiation exposure. In conclusion, we provide information on induction and location of damage with higher complexity when cells are irradiated with a mixed field. The stronger cell kill induced by fractionated exposure to mixed beams in two cancer cell lines indicates a therapeutic potential of combined high and low LET irradiation.

Study of molecular pathways involved in second cancer induction after radiotherapy

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Keywords: radiotherapy, second cancer, childhood cancer survivors, PRDM1

Around 400 000 children and adolescents develop cancer each year worldwide, where Hodgkin lymphoma (HL) and non-Hodgkin lymphoma represent around 10% of all cancer cases [1,2]. The treatment regimen of HL consists of chemotherapy and radiotherapy. With this, 10 years overall survival is achieved in over 90% of patients. However, one severe complication is represented by the late toxic effects of the therapy, where radiotherapy is considered as the major contributing factor. There is an increasing occurrence of therapy-related second cancers (SCs), where breast cancer is the most common type among HL childhood survivors[3–7]. Even though the late consequences of radiation exposure are known, predisposing factors and adverse molecular pathways involved in development of SCs remain unclear.

Recent advances in genomics bring new approaches for finding biomarkers of susceptibility to SC. Genome-wide association studies of childhood HL survivors revealed the single nucleotide polymorphism located on chromosome 6q21 (gene *PRDM1)* as significantly associated with elevated risk of SC development. The presence of this risk allele resulted in lower basal and radiation-induced expression of PRDM1[8]. The aim of this study is to investigate the role of the gene *PRDM1* in the radiation response of healthy epithelial breast cells. We will present preliminary data from knockdown of *PRDM1* in MCF10A cells, where we see small, but consistent differences in response at the level of cell cycle arrest and apoptosis genes, cell viability and PRDM1 itself.

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Migration behavior of U87 glioblastoma cells after irradiation with varying LET

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Keywords: glioblastoma, high-LET, migration, cancer therapy

Glioblastoma multiforme is the most common malignant brain tumor with a very poor prognosis. High infiltration rates, uncontrolled cell growth, and the strong ability to develop therapy resistance are components of the aggressive nature of this type of cancer. Despite multimodal treatment, the tumor often recurs in the vicinity of 1-2 cm to the primary tumor.
In this study, the migration behavior of U87 glioblastoma after low- and high-LET irradiation is analyzed to figure out whether the migration is enhanced or also influenced in other ways by radiation exposure.
For the migration assay, the cells were seeded in Ibidi Culture-Inserts 2 Well to generate a cell-free gap of about 500 µm between two cell populations. Targeted irradiation was performed with 55 MeV carbon ions and 20 MeV protons at the ion microprobe SNAKE (superconducting nanoprobe for nuclear physics experiments) located at the 14 MV tandem accelerator in Garching (GER). The closure of the gap after irradiation was observed by phase-contrast microscopy (10x, PH1) under live-cell conditions.
There are differences in the migration behavior of cells regarding their velocity and directness visible. Cells irradiated with high-LET carbons tend to be faster, but have lost all orientation, therefore the gap is closing the slowest. Irradiation with low-LET protons cause also a loss of orientation but has nearly no effect on the velocity of the cells. When only one cell population on one side of the gap was irradiated, the migration behavior changes again. Here, the cells are better oriented regardless of the type of irradiation. However, the one-sided proton irradiation leads to a deceleration of the cells.
Our results show that with different irradiation conditions the migration behavior changes. Especially, the behavior changes when only a part of the cells is irradiated. These findings indicate that the close presence of non-irradiated cells has a strong effect on the migration behavior of the whole population.

Normal tissue effects in a murine model system from radiotherapy of the head and neck

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Keywords: mice, fractionated radiotherapy, early and late effects, head and neck

It is well known that radiotherapy of head and neck (H&N) cancer causes both early and late effects that may lead to unplanned interruptions in treatment and a reduced quality of life[1]. Previous animal studies have used treatment regimens and fields that are not clinically compatible. The aim of this study was to establish a preclinical model with relevant endpoints, optimized radiation field and delivery setup to study radiation-induced normal tissue responses in H&N cancer.
C57BL/6J mice were irradiated with X-rays to total doses ranging from 30 to 85 Gy in 10 fractions over 5 days. The radiation field in the H&N area covered the skin of the neck including lip, oral cavity and salivary glands and was controlled by an X-ray imaging system. The maximal follow-up time was 100 days post irradiation (p.i.). Early radiation-induced effects were monitored by macroscopic examinations of the oral cavity. Structural changes were examined through histopathological analysis of the lip, oral mucosa tissues and salivary glands. Blood and saliva sampling was performed at baseline and p.i.
X-ray irradiation with total doses above 30 Gy induced dose-dependent radiation dermatitis of the lower lip, while oral mucositis was observed for doses above 75 Gy. The peak of acute effects was observed around 15-20 days p.i for all doses, while the severity level and time of first appearance demonstrated a dose dependency. Histopathological examinations showed radiation damages in the tongue, lip and parotid glands. Significant reduction of saliva volume was observed in mice exposed to 75 Gy.

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The optimal dose for the current model was found to be 75 Gy, while 85 Gy was not tolerable for this mouse strain.
A preclinical model to study radiation-induced normal tissue changes in the H&N area was established. The model allows investigating several clinically relevant tissue responses simultaneously, with the opportunity to study systemic effects through e.g. biochemical analysis of body fluids.

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Exosome secretion and cellular response of DU145 and PC3 after exposure to alpha radiation

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Keywords: exosomes, high LET radiation, alpha particles, prostate cancer

The lung and prostate cancers are the most common men's cancers, which are treated in clinical practice with different methods, such as: surgery, radiotherapy, chemotherapy, immunotherapy.[1] However, new methods of cancer treatment are still being investigated therefore the interest in exosomes studies is increasing in the last few years.

Exosomes are spherical membrane nanovesicles with a diameter of 30 to 150 nm. Exosomes are the only extracellular vesicles formed by exocytosis.[2] They are released by most eukaryotic cells, both healthy and cancerous. Due to the fact that exosomes are present in human body fluids such as urine, blood or saliva, they can be used for noninvasive diagnostic tests. Exosomes play an important role in tumor immune response, metastasis, angiogenesis, and survival.[3] The studies on exosomes isolated from cells exposed to photon radiation used commonly in conventional radiotherapy show the influence of this radiation quality on exosome release and its features. There is no research done on densely ionizing particles such as protons and alpha radiation,

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thus we have evaluated the cellular response of human prostate cancer cells exposed to 0, 2, and 6 Gy of alpha radiation emitted from the Am-241 source. The irradiated PC3 and DU145 cell lines characterized by different radiosensitivity were studied with apoptosis, lactate dehydrogenase (LDH), and interleukin-6 (IL-6) assays. Additionally, the corresponding concentration and size of isolated exosomes were measured with nanoparticle tracking analysis (NTA).
We found that exposure to ionizing radiation resulted in gross changes in viability and cell damage. There were increased amounts of apoptotic or necrotic cells as a function of doses. We demonstrated that irradiated PC3 cells secrete higher quantities of exosomes compared to DU145 cells. We also discussed the diameter of isolated exosomes and no statistically significant differences were found between control and irradiated cells.

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Hadrontherapy and PARP inhibitor effects on Chondrosarcoma JJ012 Cancer Stem Cells

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Keywords: Hadrontherapy, PARP inhibitor, Chondrosarcoma, Cancer Stem Cells

Chondrosarcoma is a primary tumor of bone and cartilage. It is known for its resistance to conventional treatments such as conventional radiotherapy, as well as its high metastatic potential for high-grade tumors. A possibility that could explain this resistance, is the presence of cancer stem cells (CSCs described as having increased DNA repair systems. In order to overcome this problem, certain therapeutic strategies are being explored, such as hadrontherapy (proton and carbon ions therapy) and radiosensitizing molecules such as PARP inhibitors whose effects have been previously described in the context of chondrosarcoma.
In this study, we observed the effect of different irradiation qualities (X-rays, protons and carbon ions) coupled with a PARP inhibitor: olaparib, on a chondrosarcoma cell line presenting a high stem cell potential: the JJ012 cell line. The impact of combination of different irradiation qualities coupled with olaparib treatment, was analyzed by clonogenic assays. The effect of treatments on the cancer stem cells proportion was studied using an ALDH activity test by flow cytometry, sphere formation assay and RT-qPCR.
Preliminary results indicate that carbon ions are more effective than protons, which are themselves more effective than X-rays on JJ012 chondrosarcoma cells. It also appears that olaparib increases the radiosensitivity of cells to different treatments (X-rays, protons, C-ions). This radiosensitivity seems higher with X-rays and protons than with carbon ions treatment. According to the first results on CSCs, it seems that carbon ions are a good alternative against this subpopulation, while the effects of olaparib has not been proven yet.

Vares et al., « A Multimodal Treatment of Carbon Ions Irradiation, MiRNA-34 and MTOR Inhibitor Specifically Control High-Grade Chondrosarcoma Cancer Stem Cells ».
Césaire et al., « Sensitization of Chondrosarcoma Cells with PARP Inhibitor and High-LET Radiation ».

CeCILE - intelligent detection, tracking and cell cycle evaluation of eukaryotic cells on phase-contrast live-cell videos

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Keywords: deep-learning, cell-cycle evaluation, live-cell phase-contrast-microscopy, MCMC-tracking

The reaction of tissue to irradiation is based on the radiation effects to single cells. The most notable reactions of cells to irradiation are cell cycle arrest, proliferation and cell death. Here, we present a new approach to investigate these biological endpoints in one experiment: The use of long-term live-cell phase-contrast videos analyzed by a deep-learning-based algorithm called CeCILE (Cell Classification and In-vitro Lifecycle Evaluation). With this method, we can observe and analyze the behavior and the health conditions of single cells over several days after treatment. After irradiation up to six sample dishes containing the cells can be mounted in parallel on a microscope inside a top-stage incubator and are imaged for up to 5 days. The created videos can then be analyzed by CeCILE, which is based on a faster RCNN-based algorithm for object detection and is trained on a hand-labelled dataset of microscopic videos. In these videos all cells were assigned to one of four classes, which defines the cells' state in the cell cycle. Then, a tracking algorithm assigns an individual ID to every cell in the video. The tracking is conducted employing hidden Markov models to simulate the cell movements and states and an embedded recursive Bayesian filter to predict their behaviors via inference. It additionally provides an improved tracking with stable cell identification in complex scenes as well as the possibility to predict future states and fill previous gaps in a trajectory caused by detection failures.
In conclusion, we are able to investigate the behavior of irradiated cells in one simple experiment. The first version of of CeCILE was able to achieve similar results compared to state-of-the-art assays. The upgrade with the Markov-model-based tracking allows to go further by inspecting the behavior of every single cell by evaluating the cell-cycle, the lineages and the circumstances of cell death.

Rudigkeit S. et.al: "CeCILE - An Artificial Intelligence Based Cell-Detection for the Evaluation of Radiation Effects in Eucaryotic Cells" Frontiers in Oncology, Vol. 11 (2021) p. 2327, doi:10.3389/fonc.2021.688333

Improved cytotoxic effects of ionizing radiation in radioresistant tumor models using drug delivery nano-systems.

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Keywords: radioresistant tumors, drug delivery, nano-systems, ionizing radiation

Objectives: Translational cancer radiotherapy main goal is to deliver high doses at the tumor site, in order to inhibit the growth of resistant cancer cells, and simultaneous reduction of adverse effects in surrounding healthy tissues. For this purpose, targeted nanoparticle therapies have been proposed as a solution. Here, we propose a method based on iron oxide nanoparticles (IONP) for the intracellular delivery of doxorubicin in order to enhance the cytotoxic effects of ionizing radiation.
Materials and methods: Iron oxide nanoparticles functionalized with polyethylene glycol were synthesized in order to be used as drug delivery systems for doxorubicin. The physico-chemical characterization of the nano-systems was done using relevant methods. The biological effects of the IONP were assessed on both 2D and 3D cell cultures of human cervical adenocarcinoma cells, squamous cell carcinoma and normal keratinocytes. IONP uptake and retention was assessed through optical microscopy, while clonogenic survival was used to measure the radio-sensitization effect of the nanoparticles at different doses X-Rays (150 kV) in both 2D and 3D cell models. Data was presented as mean ± SEM.
Results: Efficient internalization of IONP occurred in cancer cells, with the nanoparticles accumulating in the perinuclear area. In 2D cell cultures, $IONP_{DOX}$ enhanced the cytotoxicity of 150 kV X-rays in 2D HeLa cell cultures with a $DMF_{SF=0.1}$= 1.29 ± 0.02. Efficient penetration of the IONP was obtained after 48h of exposure in 3D spheroids and exposure to 150 kV lead to a $DMF_{SF0.1}$=1.07 ± 0.07 for HeLa cells.
Conclusions: The IONP are good candidates for the controlled delivery of DOX to enhance the cytotoxic effects of ionizing radiation.
Acknowledgement: This work was supported by the Austrian Agency for International Cooperation in Education and Research OeAD grant No. ICM-2018-10056, by Romanian Ministry of Education and Research grants no. 543 PED/2020 and Norway-Romania Grants: RO-NO-2019-0510, CTR 41/2020.

The DNA damage response of peripheral blood lymphocytes exposed to X-rays with different beam qualities and quantities.

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Keywords: Lymphocytes, DNA Damage, cell death, X-ray beam quality

Introduction: In radiology, low X-ray energies (<120 keV) are used to obtain an optimal image while in radiotherapy, higher X-ray energies (MeV) are being used to eradicate tumor tissue. Within the energy range of 0.1- 6 MeV, the radiobiological effectiveness (RBE) has been stated as equal to 1. However, the energy deposition of X-rays shows differences in function of their energy spectrum, which might lead to changes in biological responses. Therefore, in this study we compare the DNA damage response (DDR) in peripheral blood lymphocytes (PBLs) exposed to different X-ray beam qualities and quantities.

Methods: The DDR was evaluated by the γ-H2AX foci assay, the cytokinesis-block micronucleus assay and a SYTOX-based cell death assay in peripheral blood lymphocytes exposed to X-rays. Cells were irradiated in T25 flasks with a 220 kV X-ray research cabinet (SARRP, *X-Strahl*) or a 6 MV X-ray Linear accelerator (*Elekta Synergy*). Three main physical parameters were investigated: beam quality (V), mean photon energy (eV) and dose-rate (Gy/min). The addition of Cu filtration influenced the mean photon energy at the SARRP while dose-rates were varied by adjusting tube current for 220 kV X-rays (0,33-3 Gy/min) and by adjusting water-phantom depth in the 6 MV set-up (3-6 Gy/min).

Results: The RBE of 220 kV X-rays compared to 6 MV X-rays was higher than 1. When an identical beam quality was filtered to increase the mean photon energy, the RBE decreased. Within the tested dose rate ranges no specific effects were observed.

Conclusion: The DDR is influenced by the beam quality and mean photon energies. This study demonstrates that it is crucial to consider and report these physical parameters in radiobiological experiments.

Validation of FACS-based analysis of γH2AX foci formation and decay in human peripheral blood lymphocytes and U2OS cells.

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Keywords: γH2AX foci, peripheral blood lymphocytes, U2OS, DNA damage response

Introduction

The formation and decay of γH2AX foci is measured to assess the response of cells to ionizing radiation. Foci can be measured microscopically or with the help of flow cytometry (FACS). The aim of the study was to compare the kinetics of foci formation and decay using both methods in U2OS cells and peripheral blood lymphocytes (PBL). Foci in U2OS cells were analysed after 2 Gy of gamma radiation at 0, 15, 30, 60, 120, 180 min and 24 h of repair time. In PBL foci were analysed after 2 Gy and 60, 120, 180 min and 24 h of repair time.

Results and conclusion

The results demonstrate that the kinetics of foci formation and decay in U2OS cells and PBL counted manually are more expressive and dynamic than results obtained by FACS. Fluorescence intensities measured by FACS are more spread-out than foci frequencies measured microscopically, resulting in smaller signal differences between selected repair times than in the manual method. This is probably due to the differences in the nature of measured signals: cell fluorescence intensity vs focus frequency. The advantage of FACS analysis is that the measured signal level is less dependent on the precision of sampling time post irradiation. This is an asset when the γH2AX focus assay is used to measure differences in individual response to radiation.

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Cancer-related changes in cells exposed to alpha radiation in combination with nicotine

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Keywords: alpha radiation, nicotine, DNA damage, radon, cigarette smoke

Exposure to radon often takes place in combination with chemical stressors such as cigarette smoke and epidemiological studies show that radon and cigarette smoke interact in inducing lung cancer. The mechanisms of this interaction are not understood. A component of cigarettes is nicotine, the intake of which improves physical and mental ability due to the release of catecholamines into the bloodstream, being the main reason for smoking and chewing tobacco. The interesting question is if nicotine modulates the DNA damaging potential of alpha particles emitted by radon.

Bronchial epithelial BEAS-2B cells were pretreated with 2 μM nicotine and given 1 or 2 Gy of alpha particles. γH2AX formation and decay were analyzed to measure the direct effects on DNA damage response. Alpha exposure lead to a biphasic response with peaks at 1 h and 6 h, whereas nicotine alone induced no foci. The combined exposure produced a delayed, flattened response, where more foci remained unrepaired after 24 hours indicating delayed DNA repair. Also, fewer foci were detected at the time of the alpha peak (1h) in the combined treatment group. Preliminary data of comet assay showed a reduction in DNA tail moment at 1 h post combined exposure. During a 13-day time period post-exposure both the cell viability kinetics and viable cell counts showed a faster recovery in irradiated cells with nicotine treatment. These results are consistent with results from colony formation assay and suggest that nicotine interacts with alpha radiation at the DNA damage and cellular level.

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Processed nanodiamonds as radiosensitizers for innovative applications in radiotherapy treatments

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Keywords: radiotherapy, nanodiamonds, radiosensitizers, in vitro measurements

Brain cancers are among the most difficult tumours to treat and remain a leading cause of death in Europe. Current treatments based on surgical removal followed by chemotherapy and radiotherapy (RT) can have devastating neurological consequences. An innovative approach to improve RT efficacy is to use radiosensitizers to increase the radiation effectiveness on tumours, sparing the healthy surrounding tissue and preventing the long-term cognitive side effects by reducing the dose. In particular, nanoparticles provide unique chemical and physical properties and following irradiation can intensify the production of secondary electrons and free radicals, like reactive oxygen species (ROS), which can cause indirect damage to the cells and in turn enhance RT effects (1). In this contest, nanodiamonds (NDs) are attracting increasing interest due to their appealing properties, including inertness, fluorescence, biocompatibility and the possibility of surface functionalization (2). In the hydrogenated form (H-NDs), obtained by adding hydrogen moieties as surface terminations, it has been proved that they exhibit a negative electron affinity together with a positive charge in aqueous solutions, which ensures their high reactivity, locally enhancing the damage caused by radiation and the dose deposit in their surrounding area (3). In this work, NDs were processed using thermal treatments carried out in a controlled atmosphere and their surface was modified to achieve the desired specific characteristics to optimize

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their radiosensitizing properties (4). Differently surface-terminated NDs undergone on both physical and chemical analysis and were employed in different concentration to study their possible application in RT treatments. *In vitro* Raman/photoluminescence microscopy was performed to assess NDs cellular uptake and localization in human medulloblastoma cell cultures and NDs toxicity and their effect following X-ray irradiation was evaluated by cell vitality assays.

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Analysis of γH2AX distribution at nanoscale on glioblastoma cell line

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Keywords: irradiation, γH2AX, confocal microscopy, dSTORM

DNA double-strand breaks (DSBs) produced by ionizing radiation (IR) induce the complex process of DNA repair signalised by histon phosphorylation (γH2AX)[1]. Quantitative analysis of γH2AX foci is a standard method in radiobiology; however, applying direct stochastic optical-reconstruction microscopy (dSTORM) enables deeper insight at nanoscale into the DNA repair process[2]. Our aim was to reveal the dynamics of γH2AX formation after X-ray irradiation at different dose levels in a time-dependent manner, using dSTORM over diffraction limited 3D confocal microscopy.
Glioblastoma cells (U251) were irradiated with 250 KeV X-ray at 0, 2, 5 Gy dose levels and were fixed at 30 min, 24 and 72 hours after irradiation for γH2AX immunofluorescense staining. Increased γH2AX foci, and cluster density was detected at both dose levels 30 minutes after irradiation, but both returned to the control level at 24 hours. At the same time, the highest volume of foci and clusters was measured at 24 hours. dSTORM-based analysis of γH2AX revealed that micron-sized foci are composed of distinct, smaller parts with a diameter of few tens of nanometers (nanoclusters). We analysed the epitope/cluster, area/cluster ratios, cluster densities, and spatial nanofoci distribution with dSTORM superresolution microscopy. The density of these nanoclusters and the number of epitopes showed correlation with the delivered dose in the first post-irradiation time point and with the dynamics of the loss of γH2AX nanofoci.
dSTORM superresolution microscopy provided higher accuracy over 3D confocal microscopy in unrevealing the molecular structure and organisation of radiation induced γH2AX foci and in the study of molecular rearrangements during the repair process. The superresolution imaging opens a novel perspective for radiation biology research.

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Cytogenetic effects of low and high LET radiation on TK6 cells

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Keywords: Genotoxicity, Ionizing Radiation, Hadrontherapy, CBMN assay, DNA Damage

Radiotherapy is the most widely used solid cancer treatment. It is based on the exposure of tumors to Ionizing Radiation (IR), by using photons or particles radiation such as carbon ions or protons. IR interacts with the molecules in a cell, which may cause DNA damage and oxidative stress and may lead to cellular damage and cell death. Here we focus on the effect of different Linear Energy Transfer (LET) on cells at the level of DNA damage formation. The increase in LET results in an increase in the ionization density along the particle track. This promotes an increase in the amount and complexity of DNA damages. Our aim is to compare the effect of ionizing radiation with different LETs on cell survival and DNA damage formation.
We irradiated wild-type TK6 cells with x-rays, protons and two different LETs of carbon ions. Thereafter, the survival and the formation of micronuclei (CBMN assay), an indicator of genotoxic lesions, are being investigated.
The analysis is ongoing and the results obtained until know show that carbon ions radiation has stronger DNA damaging and cell killing effects than x-ray. The results also indicate that irradiation of cells by LET 28 keV/**µm** c-ions produces more MN than LET 73 keV/**µm** C-ions which might be due to the fact the 28 keV/**µm** carbon ions induce more close and complex DNA damages due to release of energy in shorter distance than 73 keV/**µm.** Another explanation is that 73 keV/**µm** carbon ions has higher killing effects than 28 keV/**µm** thus the exposed cells are eliminated from being prepared for MN formation assay. The experiments on proton beam irradiation are planned and the results will be presented and discussed in my poster.

Activation of PPAR□□by fenofibrate attenuates the effect of local heart high dose irradiation on the mouse cardiac proteome

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Epidemiological studies demonstrated an elevated cardiovascular disease (CVD) risk associated with high local doses of ionizing radiation to the heart, as reported in patients undergoing thoracic radiotherapy for malignant diseases such as breast cancer, Hodgkin's disease, or childhood cancers.
Radiation-induced CVD is characterized by the metabolic remodelling in the heart mainly due to the inactivation of the transcription factor peroxisome proliferator-activated receptor alpha (PPAR□□□ thereby inhibiting lipid metabolic enzymes. The goal of the present study was to investigate the potential protective effect of fenofibrate, a known agonist of PPAR□□□on radiation-induced cardiac toxicity. To this end, we compared for the first time, the cardiac proteome of fenofibrate- and placebo-treated mice 20 weeks after local heart irradiation (16 Gy) using label-free proteomics. The observations were further validated using immunoblotting, enzyme activity assays, and ELISA [1].
The analysis showed that fenofibrate restored the signalling pathways negatively affected by irradiation such as lipid metabolism, mitochondrial respiratory chain, extracellular matrix hemostasis, redox response, endothelial NO signalling and inflammatory status. These findings emphasize the role of PPAR□□related metabolic pathway in cardiac injury after irradiation and suggest a molecular target for potential diagnosis and prognosis of radiation-induced CVD risk to select individuals for optimal prevention and therapeutic interventions.

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A new biophysical model for treatment-plan biological optimization in hadron therapy

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Heavy-ion cancer therapy requires modelling of ion-beam biological effects in tumors and normal tissues. The Relative Biological Effectiveness (RBE) of heavy ions is a complex quantity, characterized by significant variations along the beam path and by the dependence on several factors including radiation quality, dose, and considered cell type and endpoint. Therefore, it requires a precise modelling, especially for the pencil-beam scanning technique. Two radiobiological models, LEM I (Local Effect Model) and MKM (Microdosimetric Kinetic Model), are currently in use for heavy ions in clinical facilities, although other models are available including BIANCA (BIophysical ANalysis of Cell death and chromosome Aberrations), which has shown good agreement with in vitro and in vivo carbon-ion experimental data [1, 2].

In this work we present the first application of BIANCA to carbon-ion treatment planning scenarios. Following an interface with the FLUKA Particle Therapy Tool [3], BIANCA was applied to re-calculate the RBE-weighted dose distribution for three patient cases (chordoma, head-and-neck and prostate) previously irradiated at CNAO (Centro Nazionale di Adroterapia Oncologica) in Pavia, where the radiobiological optimization was based on LEM I. The predictions obtained by BIANCA were based either on chordoma cell survival (RBE_{surv}), considered as representative of the effectiveness in the tumor, and on dicentric aberrations in peripheral blood lymphocytes (RBE_{ab}), which are indicators of normal tissue damage, including secondary tumors. In the target and in the entrance channel the values predicted by BIANCA were lower than those obtained by LEM I, whereas the two models provided very similar results in the Organs At Risk.

The observed differences between RBE_{surv} and RBE_{ab} suggest that, in normal tissues, the information on cell survival should be integrated by information more closely related to the induction of late damage, such as chromosome aberrations. This peculiar ability of the BIANCA model may represent an improvement for treatment plan optimization in ion-beam therapy.

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Investigation of DNA damage repair dynamics of NBS1 foci in U2OS cells exposed to mixed beams

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Keywords: alpha particles, gamma radiation, mixed beams, NBS1 foci, live-cell imaging

Different types of ionizing radiation (IR) interact differently with the cellular genome. Densely interacting particles with high linear energy transfer (LET) form concentrated double-strand breaks (DSBs) along particle tracks. Sparsely IR with low LET induces dispersed simple DSBs that are easier to repair [1]. It is of interest whether the sequential order of high and low LET IR affects the DNA damage response and DSB repair focus formation.

To investigate the DNA damage response after high and low LET IR and mixed beams (MB) thereof, we irradiated U2OS cells expressing a GFP-tagged version of the DSB sensor protein NBS1 with alpha particles, gamma radiation, and an alternating consecutive combination of both. NBS1 foci were recorded in live cells using an inverted fluorescence microscope during 5 h after irradiation. Time-lapse movies were analyzed for different parameters and the temporal dynamics of NBS1 foci occurrence and decay.

The results of our analyses align with the prediction that high LET IR causes fewer microscopic DSB foci in a small area of the cell nucleus as compared to low LET IR. In the case of MB, the sequence of the IR types applied showed significant differences. The cells irradiated first with alpha particles showed a greater number of foci that repaired more slowly. When low LET gamma radiation was applied first, the decrease in the large number of NBS1 foci over time was rapid. In both cases, there was a significant increase in the foci size after the 2nd hour of observation, especially in the case of irradiation with alpha first.

The current results suggest that the presence of high LET IR damage delays the sensing and repair of successive low LET-induced DSB damage. It can also be considered that high LET IR leads to more excessive chromatin damage which may include oxidative damage. An increase in the size of the foci may indicate the amassing of damaged DNA segments that are difficult to repair or that are congregated to facilitate repair [2].

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A comparison of Geant4-DNA and TRAX-Chem toolkits to evaluate the effect of oxygen depletion in FLASH irradiation

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Keywords: FLASH, Geant4-DNA, TRAX-Chem, oxygen effect.

Monte Carlo simulations of electron and proton irradiations in an oxygenated water with different oxygen concentrations were carried out using GEANT4 and TRAX-Chem toolkits by different groups. In this paper, the accuracy and efficiency of these codes are compared in order to evaluate the effect of oxygen in FLASH (ultra-high dose rate) irradiation.

The recent studies on animal models indicated that FLASH radiation treatment, remarkably increases the radio resistance of normal tissues, while the tumor control remains similar to conventional treatment. Also, observations show that oxygen plays a significant role in this kind of irradiation, but the mechanism of the oxygenation effect in FLASH irradiation is not completely clarified.

Simulated oxygen consumption, interaction of water radicals induced by particle radiation in the pre-chemical and chemical stages, radiolytic reactive oxygen species production and the calculated temporal yield (G-values in the chemical evolution time) were compared for electron and proton irradiations at various energies.

A general close agreement was observed in all cases analyzed by these codes separately. It was concluded that both toolkits are suited for this class of simulation, although further improvements are needed in both cases.

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New insights into metal nanoparticle-mediated effects on chromatin organization at the micro- and nano-scale: Relevance for tumor cell radiosensitization

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Keywords: metal nanoparticles (MP); nanoparticle-enhanced cancer radiotherapy; DNA damage and repair; ionizing radiation-induced (repair) foci (IRIF); chromatin micro and nano-architecture; single-molecule localization microscopy (SMLM)

Despite all improvements in radiotherapy, many tumors resist to this treatment and require high doses causing inacceptable damage to healthy tissues. Noble metal nanoparticles (MP) with high atomic number (high *Z*) have been shown to preferentially accumulate in proliferating tumor tissues and amplify radiation dose on the microscale. Hence, MN can serve as radiosensitizers, enhancing both radiotherapy efficiency and specificity. Simultaneously, MN allow tumor imaging (theranostics). Despite these advantages, biological processes provoked by MN in cells prior to and after irradiation (IR) remain obscure. We combined 3D confocal microscopy, single molecule localization microscopy (SMLM), flow cytometry and other biophysical/biological methods in combination with advanced software approaches to shed a new light, at the multiscale, on contradictory results presented in the literature on IR-mediated DNA double strand break (DSB) induction and repair in presence of MN. While we observed more DSBs in tumor cells irradiated in presence of MN (MN+), compared to MN(-) controls, this phenomenon reflected increased proportions of G2 cells in MN(+) cell populations rather than MN-mediated augmentation of direct DSB induction by IR.

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This is supported by micro- and nano-morphology of γH2AX/53BP1 foci, which slightly differed for MN(+) and MN(-) cells. At the nanoscale, Ripley´s distance frequency analysis of SMLM signal coordinate matrices revealed relaxation of heterochromatin (H3K9me3) clusters upon IR, which was more prominent in MN(+) cells. The expansion of radiosensitive G2 cells correlated with slightly decreased post-IR (PI) survival of MN(+) cells. Interestingly, low MN concentrations accelerated DSB repair but increased presence of unrepaired γH2AX/53BP1 foci at 24 h PI. MN thus exert multiple but mostly indirect effects on chromatin. Cytoplasmic effects, e.g., lysosome damage, cannot be excluded although they are not sufficiently supported by our preliminary results.

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Deciphering the role of the proteasome to explain the high biological efficacy of carbon ion irradiation

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Keywords: carbon ion irradiation, photons, oxidized protein, proteasome

Carbon ion irradiation (C-ions) present a high biological efficacy and an excellent ballistic precision compared with photons. Indeed, a body of arguments supports their superiority related to the spatial distribution of Reactive Oxygen Species (ROS) at the nanometric scale, condensed in their tracks whereas diffused in the cells exposed to photons[1]. The ROS induced by both radiation lead to misfolded and oxidized proteins addressed to the ubiquitin-proteasome system or the autophagy pathway to maintain cellular homeostasis. Understanding the fate of oxidized proteins and the role played by the proteasome could help decipher the molecular advantages of carbon ions.
The activity of the proteasomal catalytic 20S subunit, the levels of oxidized proteins through Oxiblot®, and their proteasomal addressing through the K48 ubiquitin expression, were studied in response to C-ion and photon irradiation for two Head and Neck Squamous Cell Carcinomas (HNSCC) cell lines and their radioresistant subpopulation of Cancer Stem Cells (CSCs). The activation of the autophagy was investigated by flow-cytometry (Muse®).
First, we showed a decrease in the 20S proteasomal activity after C-ion exposure compared with photons in HNSCC cell lines and their CSCs sub-populations. We also observed lower proteasomal addressing of the abnormal proteins traduced by a decreased K48 ubiquitin expression. However, the accumulation of oxidized proteins differs with enhanced oxidation after C-ions in parental cell lines, not observed in CSCs. These results, supported by preliminary data recently obtained at the French Heavy Ions Accelerator (GANIL), suggest a different fate of the abnormal proteins in CSCs after C-ion exposure, potentially addressed to the autophagy pathway. Besides, the

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condensation of ROS along the particle tracks, as evidenced by Monte-Carlo simulations, may lead to heavily oxidized protein difficult to repair and, therefore, toxic for the cells.
Altogether, these data confirm the central role of the proteasome in the biological specificities of C-ions, especially on CSCs, and support the relevance of the proteasome as a therapeutic target.

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Evaluation of innovative radiotherapy treatments in zebrafish

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Keywords: radiation therapy, curcumin, zebrafish, embryogenesis

In the era of personalized therapy, radiotherapy (RT) could be used in combination with radiation modifying agents both to improve the therapeutic index and to personalize cancer treatment plans [1]. In this scenario zebrafish embryo represents a potential model in the radiobiology field, considering that embryogenesis is the most radiosensitive stage in the vertebrate life cycle and that the aqueous environment in which embryos develop favours homogeneity in the radiation dose distribution [2].
In order to suggest innovative RT treatments, we describe the characterization of the biological effects induced in zebrafish embryos exposed to X-ray beams, alone or in combination with curcumin, already known for anti-oxidant and anti-tumor properties [3].
Distinct batches of 24 hours post fecondation (hpf) embryos were exposed to 0-15 Gy X-rays. For the combined treatment, embryos were pre-treated for 18 hours with 0-10 μM curcumin, and subsequently irradiated using the above mentioned dose range. Sister batches of 6 hpf embryos were either used as untreated controls or subjected to single treatment with curcumin following the same experimental setting used in the combined treatment. Treated and control embryos were carefully examined by daily steromicroscope observation until 120 hpf, to estimate the mortality rate as well as developmental delay and alterations. In addition, behavioural analysis was performed to assess alteration in swimming capacity or delay in response to induced physical stimuli, as well as any possible variation in the heart rate values at 48 and 72 hpf.
We found that the X-ray single exposure in a dose-dependent manner, as well as the treatment with curcumin alone at concentrations greater than 5 μM, inflicted gross malformations (including pericardial and yolk-sac edema, skeleton defects, cardiac dysfunctions and variation in the pigmentation degree), behavioural defects and lethality. In striking contrast, the occurrence of these phenotypic alterations was markedly reduced, at different extents, in embryos exposed to the combined treatment, strongly suggesting that the adverse effects induced by RT were mitigated in these embryos by 0-5 μM curcumin pre-treatment.

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Additional experiments are planned to accomplish the characterization at a molecular level of the observed effects.

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Radioresistant primary breast cancer cells and hormone receptor status: looking for their main link.

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Keywords: Radioresistance; Breast cancer; Gene Expression Profile.

In breast cancer (BC) care, radiotherapy (RT) is considered an efficient treatment, both for controlling localized tumors or as a therapeutic option in case of inoperable or recurrent tumors. However, the choice of a unique treatment plan for all BC patients may not be the best option. As known, BC is a heterogeneous disease at both clinical and molecular levels, with distinct subtypes also linked to the hormone receptor (HR) status (i.e. estrogen – ER, progesterone – PR) [1-2]. Thus, radiobiological research is needed to understand molecular differences that affect the radiosensitivity of different BC subtypes to obtain more successful treatments plans. For this purpose, the aim of this study was to analyze gene expression profiles (GEPs) induced by high ionizing radiation (IR) doses (9 and 23 Gy) in primary radioresistant BC cells. Firstly, we selected, collected (72h and 1-week post-RT), and expanded the IR radioresistant cell fractions of two primary BC cells isolated from surgically removed breast tumors, with opposite HR expression status: BCpc7 (ER+/PR+) and BCpcEMT (ER-/PR-): the negative expression of HRS is often described in radioresistant BC and associated with a bad prognosis. Secondly, differential gene expression analyses revealed that a conspicuous number of genes had significantly altered expression levels in 72h and 1-week post-RT radioresistant fractions compared to the early irradiated cells (24h) used as reference. Pathway analyses revealed that GEPs of the two radioresistant cell fractions, were strictly involved in the regulation of the cell cycle/cell death balance, hypoxia, inflammation as well as in viability, and in cell communication. Further investigation is now in progress to identify pathway signatures linked to HR status.

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We trust in the idea that genes may function as biomarkers of disease providing the rationale for the development of molecularly based signatures to predict the RT responses in cancers, including BC [3-4].

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RNA-Sequencing Analysis of Glioblastoma xenograft mice models proton boron capture therapy treated: a portrait of its molecular response.

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Keywords: Proton Boron Capture Therapy, Glioblastoma, Radioresistance, RNA-Sequencing Analysis

Today, the technological development of radiation therapy (RT) has led to more performing and innovative technologies which can deliver, with high precision, increasing doses of ionizing radiation (IR) saving the organ at risk. This property of the proton beam is due to its typical curve of energy deposition through the matter (Bragg peak), which represent a better conformational option with respect to conventional photon beams [1]. Additionally, in Proton Boron Capture Therapy (PBCT), the reaction between protons and boron particles enhance the Relative Biological Effectiveness (RBE) of protons, representing a chance for radio-resistant and hypoxic malignancies for which there is no effective therapy [2-3].
Here, we investigate the molecular responses induced by PBCT in Glioblastoma (GBM) U87 xenograft mice models, which belongs to the category of the foremost radio-resistant and hypoxic cancers, by using the RNA-Sequencing approach, to study the biological processes activated by PT with or without boron administration. Comparative differential gene expression analyses revealed that a conspicuous number of genes had significantly altered expression levels compared to the reference mock-irradiated cells. Pathway analyses revealed that differentially expressed genes (DEGs) compared to the

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control, were strictly involved in the regulation of actin cytoskeleton, axon guidance, and in the binding and communication with other cells through adherent, gap, and Focal junctions. Moreover, we reported the deregulation of the cell cycle /cell death balance, probably driven by Notch signalling, known to be a key regulator of neuronal cell growth and homeostasis in particular down-regulation of cells survival, growth, and viability and, on the other hand, an up-regulation of cell death (apoptosis, necrosis, and others), were described in PBCT GBM cells. Taking together these data, we encourage clarifying the biological response and therapy efficacy induced by PBCT to enhance cancer therapies' success rate, especially for the foremost radio-resistant and hypoxic cancers [4-5]. In addition, we trust that these gene expression data could be useful to select potential new biomarkers against which specific targeted therapies could be directed, in order to suggest a combined treatment approach with Proton Therapy.

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Tumor Microenvironment Alteration Induced By Radiotherapy Modifies Glioblastoma Cells Proliferation And Mitochondrial Fitness

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Keywords: Tumor microenvironment, Glioblastoma, Radiotherapy, Microglia, Immune escape, Mitochondria, Metabolism.

Tumor microenvironment (TME) consists in a complex interplay of cells and soluble factors holding a critical role in neoplastic development. Glioblastoma (GBM), a WHO grade IV glioma, is a malignant primary brain tumor for which combination of surgery, chemotherapy and radiotherapy is the first-line approach despite severe adverse effects. Significant pathophysiological changes have been found in GBM TME, such as oxidative stress, neuroinflammation and glia activation. This has been reported to occur spontaneously and upon severe therapeutic regimens, resulting in dismal prognosis and recurrences. Microglia, is among the most important players in favouring GBM growth and proliferation, representing target cells of immune escape mechanisms. Our study aims at analysing radiation-induced effects in modulating intercellular communication and how the plethora of molecules secreted in TME determines protective mechanisms in naïve GBM cells through mitochondrial activation and metabolic rearrangement. We first evaluated irradiated microglia and microglia-to-GBM cells interactions mediated by both paracrine and autocrine signalling. Conditioned media (CM) from human irradiated microglia were collected and GBM cell lines (i.e. U-87 MG and U-251 MG) were exposed to either mock irradiated or 2 Gy/15 Gy irradiated microglia-derived CM. We observed not significant changes in apoptosis, promotion of proliferation and colony formation and mitochondrial fitness modulation. In particular, we found that mitochondrial metabolism was affected by direct irradiation and such an effect was not observed in GBM cultures exposed to 2 Gy or 15 Gy irradiated microglia-derived CM.

Our results suggest that irradiation direct damages on either GBM cells or microglia are not transferred to naïve cells and that off-target radiotherapy modulates microglia to support GBM proliferation and metabolism.

Freezing and thawing cells to radio-sensitize tumour cells

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Keywords: cell freezing/thawing, DNA damage, Ionizing Radiation-Induced Foci (IRIFs), tumor cell radiosensitization, tumor cryoablation

In this work, we shed new light on the highly debated issue of chromatin fragmentation in cryopreserved cells. We describe replicating cell-specific DNA damage and higher-order chromatin alterations after freezing and thawing. We identified DNA structural changes associated with the freeze-thaw process and correlated them with the viability of frozen and thawed cells. And simultaneously evaluated DNA defects and the higher-order chromatin structure of frozen and thawed cells with and without cryoprotectant treatment. We found that in replicating (S phase) cells, DNA was preferentially damaged by replication fork collapse, potentially leading to DNA double strand breaks (DSBs), which represent an important source of both genome instability and defects in epigenome maintenance.
This induction of DNA defects by the freeze-thaw process was not prevented by any cryoprotectant studied. Both in replicating and non-replicating cells, freezing and thawing altered the chromatin structure in a cryoprotectant-dependent manner.
Freezing and thawing effects are tested to radio-sensitize the tumor cells.

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Metabolic regulation of the radiation response in cancer cells: opportunities for biological radiotherapy individualization

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Energy metabolism, Antioxidant defense, Radiation response

Radiotherapy plays a key role in the management of cancer patients. Technical and physical innovations have helped to enhance accuracy of radiotherapy dose delivery. Furthermore, multimodal combinations with molecularly tailored drugs or immunotherapy yield promising survival benefits in selected patient subgroups. Yet high loco-regional failure-rates and frequent development of metastases still limit patient outcome in relevant cancer subtypes. We will present our most recent findings and concepts on the role of cancer metabolism in the radiation response and radioresistance.
We used a systematic collection of metabolic and radiobiological data from irradiated cancer cell lines including syngeneic cell pairs for mathematical modeling of metabolic adaptative processes, as well as genetic and pharmacologic approaches to gain more insight into the interplay between metabolic adaptation to radiotherapy-induced cell stress, clinically relevant oncogenic drivers, the cellular radiation response, and cancer cell radiosensitivity. Our data demonstrate that in addition to DNA damage and oxidative stress exposure to ionizing radiation induces a severe energy stress. Mutant KRAS or the activation associated protein kinase B mutant protein AKT-E17K enhanced the capacity of cancer cells to adapt their metabolism to cope with these radiotherapy-induced stresses with impact on energy metabolism, antioxidant defense, and repair of DNA damage damage. These metabolic adaptive processes were associated with specific metabolic dependencies allowing for a targeted radiosensitization [1, 2].
Understanding metabolic phenotypes of radioresistance and associated metabolic bottlenecks in a cancer cell-specific context offers largely unexploited avenues for a

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future biological individualization and optimization of clinical radiotherapy, e.g., by targeting critical metabolic nodes of oncogene-dependent metabolic reprogramming.

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Tumour cell damage after proton irradiation in the presence of boron compounds

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The killing efficiency of proton beams in the presence of boron increases as was previously observed in *in vitro* cultivated cancer cells. The number of tested cell lines was low and the mechanism of the increased cell killing in the presence of boron is not understood. The insight and understanding of what happened to and within cells doped by boron compounds after protons irradiation could open new perspectives for streamlining proton therapy of aggressive tumours. Here we tested the glioblastoma U-251 MG and U-87 MG cell lines which were adherently cultivated. Adherent U-251 MG and U-87 MG cells were doped by boron compounds with different isotopic ratios 24 hours before irradiation. Cell samples were irradiated by a 190.6 MeV proton beam in plateau and Bragg peak positions. Control boron compound free cell samples were also included in the experiments. To evaluate the effect of proton therapy in the presence/absence of boron compounds the clonogenic cell survival test was used as a basal method. To examine the intercellular damage in relation to cell death or decreasing cellular proliferation, we analysed the number of DNA double-strand breaks marked by the expression of gammaH2AX and the number of lysosomes in which an increasing amount of number is related to damage to organelles.

The use of the ATR inhibitor VE-821 as a potential tool for radiosensitivity estimation of cancer cell lines.

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Keywords: DDR inhibitors; G2-M checkpoint; G2 chromosomal radiosensitvity assay; VE-821; chromatid breaks

While current technology in radiation therapy permits precise delivery of radiation dose to the tumor, with a decreased risk of side effects to the healthy surrounding tissue, still the mechanisms underlying DNA damage response (DDR) that lead to tumor resistance, are not yet clearly understood. After irradiation, signaling pathways are activated, enabling cell cycle arrest for DNA repair via the DDR-related kinases and their downstream targets. ATM, ATR, and Chk1 kinases play an important role in DDR activation and enhance resistance to radiotherapy. In our previous study, the use of ATM, ATR and Chk1 inhibitors have been studied as potential tools for radiosensitivity estimation. More specifically, these inhibitors, including the ATR inhibitor VE-821, have been used as alternatives to caffeine in order to modify the classical G2-chromosomal radiosensitivity assay [1], and this modified assay was performed in exponentially growing RPE and 82-6 hTERT human cells lines, proposing the efficacy of the above inhibitors, especially of VE-821, in radiosensitization [2].

For this reason, in this study, the classical G2 assay, using caffeine, as well as the modified G2-assay, using VE-821, have been performed in several cancer cell lines, such as epidermoid carcinoma (A431), lung cancer (A549) and prostate adenocarcinoma (PC3). Cells were irradiated during the G2/M-phase under the presence or absence of caffeine (for the classical G2-assay) and VE-821 (for the modified G2-assay). The induced chromatid breaks were recorded and used in order to evaluate the radiosensitivity of these cancer cell lines and their potency for radiosensitization. The comparison between the classical and the modified G2 assay shows the potential use of VE-821 for the evaluation of cancer cell radiosensitivity and validates this enhanced modified G2 assay. In addition, the results strongly support the concept that ATM and ATR inhibitors, can possibly act as attractive anticancer agents in radiation oncology.

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Radiolysis of concentrated myoglobin by accelerated ions

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Keywords: Radiolysis, proteins, Accelerated ions, Molecular effects

In order to better describe the biological effects of ionizing radiation, understanding the mechanisms of radiolysis at the molecular scale is a key step. Proteins are by far the most abundant biomolecules in the cell, yet very few studies describe their radiolysis by accelerated ions. The aim of our team is therefore to develop a systematic study of these effects on protein biomolecules, from amino acids to whole proteins, and with various ions, energies and dose-rates.

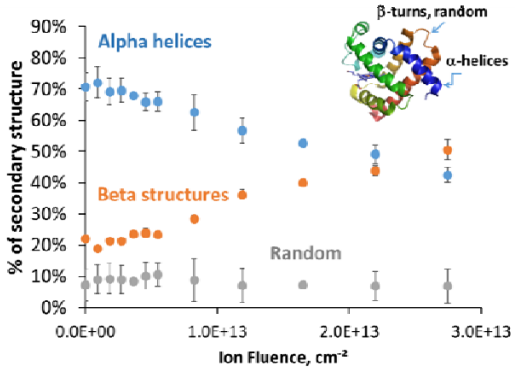

Figure 1 Evolution of the secondary structure of myoglobin under irradiation by 2 MeV protons, as a function of cumulative ion fluence. Error bars were determined with a duplicate of irradiation experiments.

ns of a few MeV energies, in highly concentrated native gels, 20 % w/w, similar to protein content in the cell. The impact of ions on its secondary structure was followed by mean of infrared spectroscopy, showing reproducible and organized change in its conformation, from alpha helices to mostly beta-structures (cf. Figure 1). UV-Visible spectra were also recorded under irradiation, and the combination of the data allowed identifying the formation of a significant quantity of carbon monoxide under irradiation.

The results obtained with low–energy protons will be presented and compared to helium and carbon ions, and the possible source for carbon monoxide will be discussed.

Dose-rate effect towards Radiolysis of Water and small Protein Biomolecules

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Keywords: Radiolysis, dose-rate, hydroxyl radical, biomolecules

Studying fundamental chemical mechanisms of the effects of ionizing radiations on biomolecules is crucial to have a better understanding of their radiobiological effects. These ionizing radiations can be used in a therapeutical context in radiotherapy to treat cancers by damaging tumoral tissues. FLASH radiotherapy, using very high dose rates (>40Gy/s) could have a preserving effect towards healthy tissues, prompting a lot of interest recently [1]. At this moment, molecular mechanisms of the FLASH effect are still far from being completely understood.

Dose-rate effects at the chemical stage, on water radiolysis and radiolysis of biomolecules, could be an essential element of the FLASH effect observed on biological systems.

Water radiolysis produces several reactive species, among which hydroxyl radical is the most potent one towards biomolecules. We have quantified and reconstructed the kinetics of formation of hydroxyl radical $HO^{\bullet}$, using several scavenging probes. Experiments were conducted at several dose-rates, from 0.1 to more than 2000 Gy/s, under irradiation by 1MeV electrons. The data obtained show a significant dose-rate effect on the yields of $HO^{\bullet}$.

Radiolysis of amino acids and of a small peptide, aspartame, was also studied in solution, the very same conditions. Even taking into account the dose-rate effect on $HO^{\bullet}$, a clear effect could be observed on the yields of radiolysis products of both biomolecules. Similar results were obtained with phenylalanine and aspartame, showing that the radiolysis mechanisms remain similar when the amino acid is included in a peptide.

These results could be very interesting to better understand FLASH effect.

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The lack of p21 sensitizes colon cancer cells to radiation-induced apoptosis.

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Radiation therapy is the most-effective cytotoxic therapy available to treat localized solid cancers. With the introduction of charged particle radiotherapy (proton therapy), the area of irradiated healthy tissue surrounding the tumor was further decreased. The aim of this study is to investigate the role of the p53 pathway in response to both X-rays and proton therapy treatments. p53 is a transcription factor with a key role in the stress-depended regulation of DNA repair, cell cycle arrest, and apoptosis. As a model, we used 3 isogenic derivatives of the colon cancer-derived cells HCT116: parental, $TP53^{-/-}$, and $CDKN1A^{-/-}$ (coding for p21, the main p53 target involved in cell cycle arrest). We analyzed cellular responses to irradiation, focusing on DNA damage, p53 targets activation, apoptosis induction, and 3D culture disaggregation.
As expected, X-rays and proton irradiation caused DNA damage an hour after treatment in all cellular systems, detected by the formation of γ-H2AX foci. Interestingly, despite their different genetic background, all three cell lines retained a similar ability to repair the DNA damage. Surprisingly, the p53 and p21 null cells showed a higher apoptotic rate, indicating that the two cell lines could be more radiosensitive than the parental cells. Moreover, to better mimic the shrinkage effect of radiation therapy on solid cancers, 3D spheroids were also used. HCT116 parental, $p53^{-/-}$, and $p21^{-/-}$ cells spontaneously formed spheroids in ultra-low attachment plates. Notably, while parental spheroids showed a reduction in diameter 10 days after X-rays and proton irradiation but still maintained a proper 3D organization, the $p53^{-/-}$ and notably $p21^{-/-}$ spheroids completely disaggregated. Furthermore, the viability of the $p21^{-/-}$ spheroids drastically dropped in response to X-rays and proton irradiation, and the analysis of PARP cleavage and activation of Caspase 3 highlighted an increase in apoptosis, particularly in p21 null cells.
Taken collectively, these data suggest that the absence of p53-dependent responses through p21 enhances the sensitivity to irradiation. This study revealed a dichotomy in p21 role: in addition to its canonical tumor-suppressive role, it seems to hold a radioprotective function in these cancer cells that, when depleted for p21, are considerably more prone to apoptosis. These findings could set the stage for future studies based on therapies targeting p21 in combination with charged-particles radiotherapy.

Raman spectroscopy of plasma for prediction of radiotherapy response in patients with HPV-positive head and neck cancer

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Keywords: Raman spectroscopy, radiotherapy, head and neck cancer, plasma

Globally, head and neck cancer (HNC) is the sixth most common cancer. Developed countries have seen an increase in the number of HNC cases, and this can be explained by HPV being a primary etiological source of HNC. Radiation therapy is a common treatment for HNC, and HNC patients can experience severe late radiation toxicity such as dry mouth and dysphagia that can significantly impact their quality of life. HPV-positive HNC patients are usually younger than HPV-negative HNC patients and with a better prognosis, HPV-positive HNC patients will have to deal with the long-term effects of radiation for longer. It is not known why some patients develop toxicity, and currently, it is difficult to predict before radiotherapy which patients will experience these long-term and sometimes irreversible late toxicities.
Optical spectroscopic methods, such as Raman spectroscopy, can provide a rapid, label-free, and non-destructive measurement of the biochemical content of cells and biofluids. This project aims to identify Raman spectral biomarkers from blood plasma to predict the development of radiation-induced toxicity prior to treatment commencement.
Baseline plasma samples (n=40) were collected from HPV-positive HNC patients who were enrolled in the De-ESCALATE clinical trial. Raman spectra from plasma of patients who experienced late toxicity grade 0-2 and grade 3+ were analysed using principal component analysis (PCA) for the exploration and visualisation of trends in the data, then partial least squares discriminant analysis (PLS-DA) was used for the development of classification models.
Identification of predictive spectral biomarkers would allow the stratification of HNC patients according to the risk of developing radiation toxicity and could guide the selection of treatment modalities to reduce this risk in high-risk patients or allow dose escalation in low-risk patients to improve tumour control.

Radiation therapy affects cellular internalization of hyaluronic acid coated-nanoparticles into breast cancer

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Keywords: radiation therapy, hyaluronic acid, nanoparticles internalization, breast cancer

Breast cancer is the most common malignancy accounting for 29.2% of all cancers in women[1] and it has been one of the first models treated with radiotherapy (RT) by stages I (tumor size up to 2 cm and no affected lymph nodes) to stage III (spread of the tumor to the lymph knots or tissue near the breast) to reduce the risk of recurrence after surgery. However, increasingly, the treatment plan requires the addition of chemotherapy drugs that often cause resistance. Nanotechnology proposes the use of nanoparticles (NPs) as novel gateways to enhance the therapeutic efficacy of anticancer agents at the target site of action due to their tumor-targeting abilities, which can limit the undesired systemic effects of chemotherapy agents and also reduce drug resistance[2,3]. In particular, hyaluronic acid (HA) coated NPs (HA-NPs) represent a very promising candidate for ligand-targeted therapy, considering the high expression of surface receptor CD44, that specifically binds with HA, in breast cancer cells[4]. This work aims at investigating the effects of ionizing radiation on cell's ability to internalize HA-NPs. Specifically, fluorescent (rhodamine B) HA- coated poly lactic-*co*-glycolic acid (PLGA) NPs (HA-PLGA-NPs) were formulated and placed in contact for 5h with healthy breast cell line (MCF10A) and its triple-negative cancerous counterpart (MDA-MB-231) irradiated with two doses of X-rays: 2 and 10 Gy. To quantify the cellular internalization capability of HA-PLGA-NPs, the samples were observed with a confocal microscope and the fluorescent signals was analyzed using ImageJ Fiji software. The

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results demonstrate an amplifying effect of the internalization process of HA-coated NPs by irradiated tumor cells, representing the background to continue the internalization study related to the response of cells to irradiation to finally establish the most suitable time to administration of NPs - basic therapy after radiotherapy.

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Nanodosimetry based radiobiological outcomes assessment

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Keywords: nanodosimetry, ionisation cluster size distribution, biological response, V79

Despite the enormous advances that have been made in medical technology and techniques using ionising radiation, the challenges associated with these rapid developments are still relevant. The biological outcomes assessment is subject to uncertainty due to the use of averaged, macroscopic physical quantities. That is why we notice the importance of the choice of physical values best describing the quality and quantity of radiation used. Since the initiation of radiation-induced damage is dominated by interactions occurring in the DNA or within its environs, the distribution of such interactions is crucial to properly assess the biological effects.

The first results of the attempt to connect fundamental nanodosimetric concepts with radiobiological parameters characterising the survival of a well-established cell line irradiated with an ion beam will be presented. To obtain a mathematical formula, we have analysed radiobiological data for V79 cells derived from the PIDE database[1] and correlated the parameters describing biological endpoints with nanodosimetric characteristics of the radiation used in these experiments. The nanodosimetric quantities were determined retrospectively based on available information about irradiation conditions using Monte Carlo simulation for the JetCounter device with the Geant4-DNA physics option 4. Since larger clusters are more efficient with delivering lethal damage, a simple sum of probabilities associated with cluster size from 2 to infinity, namely F2 parameter, cannot be a good predictor of radiobiological outcome, although it is often considered a valuable candidate[2,3]. Instead, we use a different quantity that takes into account both the cluster-size probabilities and the probability of damage due to ionisation in a minimal possible way. For now, the proposed model based on this quantity does not include any intermediate steps but provides good data consistency.

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Mathematical modelling of low dose hyper-radiosensitivity and induced radioresistance

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The surviving fraction of cells decreases exponentially with the increase of the absorbed dose at high doses[1]. At low doses, however, experiments show that surviving fraction differ from this due to the effects of hyper-radiosensitivity and induced radioresistance in many different cell lines[2]. The result is a function that starts steeper and after a local minimum starts to increase to a local maximum as the dose increases before following the exponential decrease at higher doses.
The aim of this study was to test if the hypothesis of minimum mutation load[3] can describe both hyper-radiosensitivity and induced radioresistance at low doses. In this case the principle means that the most damaged cells in a vicinity use apoptosis to reduce the mutation rate in the tissue. For this purpose, a python code was created to simulate the surviving fraction on different doses, with the following presumptions:

- the number of cells is in equilibrium in the tissue with random placement in a given radius circle,
- the radiation induced mutations follow Poisson distribution,
- and the cells are able to communicate the condition of their DNA with signals whose concentration distribution follow a normal distribution centered on the given cell.

The cells whose DNA damage are above a threshold (compared to the average damage of the vicinity) go into apoptosis and the neighboring cells divide to uphold the equilibrium. Thus, the overall rate of mutagenic damage decreases in the tissue. In order to test the model, results were compared to a large volume of experimental data[4]
The initial parameters were calculated from the experimental data and the results show that this model gives comparable result to the commonly used phenomenological induced repair model, which can be further improved with additional fit of the parameters. While the exact procedure of communication between the cells is not known, this model gives a possible general explanation to the hypersensitivity in the tissue due to any kind of mutagenic effect not just ionizing radiation.

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Hyper-fractionation of ^{177}Lu-octreotate increases the uptake in human GOT1 tumours in a mouse model

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Keywords: Biodistribution, GEP-NET, DOTATATE, somatostatin, radiopharmaceutical, radionuclide therapy, PRRT, Lutathera

Background: Radionuclide therapy using ^{177}Lu-octreotate in patients with spread neuroendocrine tumours show promising results, but could be optimised. A previous study showed slower tumour regrowth and prolonged overall survival for mice that received hyper-fractionated versus single injection with the same total dose of ^{177}Lu-octreotate, but the reasons for these differences are not known. **Aim:** The purpose of this study was to assess differences in biodistribution in tumour and normal tissues after single administration and hyper-fractionated treatment regime. **Methods:** Fifteen female BALB/c nude mice were treated with a total of 120 MBq, injected as 1x120 or 3x40 MBq, of ^{177}Lu-octreotate (n=3/group). The mice were euthanized after 1, 3 or 7 days after treatment start and tumour and normal tissues were excised, weighed and measured for ^{177}Lu activity to determine activity concentration. From the activity concentration, tumour-to-normal-tissue activity concentration values (T/N) was determined. **Results:** Overall, the activity concentrations in the various tissues were higher after hyper-fractionation, compared to single injection. The mean ^{177}Lu activity concentration in tumours were 4 times higher 7 days after treatment start, for the group that received hyper-fractionated treatment compared to single administration. T/N values increased by approximately a factor of 2-3 at day 7 for normal tissues, including kidneys and bone marrow, after hyper-fractionation compared to single administration. **Conclusion:** Hyper-fractionation results in higher uptake of ^{177}Lu-octreotate in tumours and higher T/N values, and could be beneficial in the treatment of tumours with overexpressed somatostatin receptors.

Comparative studies of intestinal tumorigenesis in the *Apc*$^{Min/+}$ mouse following acute or chronic gamma irradiation.

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Keywords: Apc$^{Min/+}$ mouse, intestinal cancer, gamma irradiation

Estimation of cancer risks following ionising radiation exposure are mostly based on epidemiological studies of the Japanese atomic bomb survivors who received acute high-dose exposures to external radiation (gamma rays and neutrons). Animal studies have been used to provide additional information on the effects of different dose rates on tumorigenesis, however, most of these studies have used acute high-dose rate radiation and there is less information about the effects from chronic exposure to low dose-rate radiation.

This study examines the quantitative effects of exposure to chronic low dose-rate gamma radiation compared with acute high dose-rate gamma irradiation in the *Apc*$^{Min/+}$ mouse model. F1 C57BL/6 x CBA/Ca *Apc*$^{Min/+}$ and *Apc*$^{+/+}$ mice were bred at UKHSA and shipped to Norway for exposure to chronic low-dose gamma radiation (2.1 mGy h^{-1}, total doses 0, 1.7 and 3 Gy) in the FIGARO facility at the Norwegian University of Life Sciences, Aas, or exposed to high-dose rate gamma irradiation (0.3 Gy min^{-1}, total doses 0, 1.5 and 3 Gy) at the Medical Research Council Co-60 gamma irradiation facility (Harwell Campus, Didcot, Oxfordshire, UK). Following the completion of the irradiations the mice were returned to UKHSA and killed 200 days after the end of their exposure period for tumour evaluation.

Intestinal tumour numbers in the different exposure groups will be presented and

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compared. The results will contribute to knowledge of low-dose rate radiation intestinal tumorigenesis and therefore inform judgements on Dose-Rate Effectiveness Factor values for low dose-rate vs. high dose-rate gamma exposure.

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Antiproliferative and radiosensitizing effects of the G4-ligand RHPS4 and its analogue 190 in breast cancer cells

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Keywords: G-quadruplex ligand, RHPS4, telomere, Ionizing radiation, radiosensitization

Strategies to overcome tumor radioresistance are urgently needed to increase radiotherapy effectiveness, reducing the overall treatment time and spare as much as possible normal tissues. In this regard, telomere targeting has been recently proposed as a radiosensitizing strategy. Molecules known as G4-ligands interact with telomeres stabilizing secondary structures known as G-quadruplex (G4s), inducing telomere uncapping and dysfunction, increased chromosomal instability, decreased cell proliferation and radiosensitization. RHPS4 is one of the most effective and studied telomeric G4-ligand, and our laboratory provided evidence of its *in vitro* and *in vivo* radiosensitizing effect on glioblastoma cells suggesting the potential of telomeric G4-ligands for therapeutic applications in radiotherapy.
Unfortunately, RHPS4 route to clinical testing was halted by off-target cardiotoxicity and analogues were developed to overcome this problem. In the present work, we investigated the capability of RHPS4 and one of its most promising analogs named 190 to impair proliferation and to induce telomere dysfunction and radiosensitization in breast cancer cells (MCF7, MCF7^{Y537S}, HCC1937, MDA-MB-231) and in a non-tumorigenic mammary epithelial cell line (MCF10A) as normal control.
Here we provided the evidence of strong antiproliferative action of RHPS4 and 190 compounds, as well as the ability to induce genomic and telomeric replicative stress, which is turned into genomic and telomeric damage.
However, despite the RHPS4 and 190 capability to increase IR effects in glioma cells (used as reference) we did not observed radiosensitization in breast cancer cell lines that displayed higher resistance to telomeric DNA damage induced by the ligand.

Proton therapy synergistic effect with *Betula etnensis* Rafin. (Betulaceae) extract in breast cancer cells

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Keywords: proton therapy; breast cancer; oxidative stress; betulinic acid

Breast cancer is the most common type of malignant disease in woman worldwide and currently it represents the second leading cause of death due to cancer in women. Radiotherapy, in addition to chemotherapy, is currently the primary method for cancer treatment, based on damage of malignant cells by ionizing radiation. Proton therapy (PT) provides a substantial physical advantage compared to conventional RT by using X or Gamma radiation rays, even if its biological advantages still remain understudied. Many natural compounds investigated for their potential usefulness as cancer chemopreventive agents might suppress carcinogenesis mainly during the initiation phase because of their radical scavenger activity (1).

Natural products, by enhancing selectivity of chemo- and radiotherapy, would allow to lower their doses, reducing side effects and risk of second cancers. It's reported that many Betula species are used in folk medicine to treat skin diseases, infections, inflammations, rheumatism and urinary disorders (3). Nearly, all species contain flavonoids, tannins, saponins, sterols and pentacyclic triterpenoids, such as betulin, betulinic acid and ursolic acid which have shown multiple antioxidant, antitumor and antiviral activities (4). In particular, betulinic acid is considered a promising anticancer agent (5). Since there is an increasing interest in the *in vivo* protective effects of natural compounds contained in plants against oxidative damage involved in cancer, in this study we investigated the effects of *B. etnensis* Rafin bark alcoholic extract on the viability of MCF7 cells and also on cell death after treatment with ionizing radiation (2-4-6-9 Gys), proton beams at 62 MeV (7). In addition, in order to elucidate mechanisms of action of this extract, several markers of oxidative stress were also evaluated.

Results obtained showed that alcoholic extract of *B. etnensis* Rafin significantly reduced cell viability of MCF7, inducing apoptotic and/or necrotic cell death in a concentration-depending manner. Moreover, clonogenic assay demonstrated that extract of *B. etnensis* Rafin was able to potentiate the antiproliferative effect of ionizing radiation. These results confirmed that extract *B. etnensis* Rafin. could be helpful both as preventive and as adjuvant in antineoplastic radiotherapy.

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Effects of ^{4}He irradiation on cell cycle progression and induction of apoptosis in radioresistant cancer cell lines

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Keywords: cancer cells, helium ions, cell cycle, apoptosis

Despite more sophisticated delivery technology, modern radiotherapy is faced with many challenges.[1] Clinical results have shown that, apart from well-known advantageous effects of hadrons, less conventional beams might provide additional benefits. [2] Due to their favorable physical and biophysical characteristics, helium ions (^{4}He) arose as promising therapeutic modality.[2,3] They provide minimal lateral scattering compared to protons, and reduced fragmentation tail compared to carbon ions.[2] Since innovation in radiation therapy requires understanding of the complex biological effects of ions, more experimental data about the effects of ^{4}He ions on cancerous cells is needed.[4] To get deeper insight into their biological effects, ^{4}He ions are used on two types of human cancer cell lines of different origin, i.e. MCF-7 breast adenocarcinoma and HTB140 melanoma cells. Previous experiments with gamma rays as well as with protons and carbon ions, have demonstrated high level of radioresistance of these cells.[5] Changes in cell cycle as well as the induction of apoptosis are used as endpoints to evaluate biological response of selected cell lines to ^{4}He irradiations. Irradiation dose was adjusted to the cell line and previously determined level of radiosensitivity.[6] Cell cycle distribution and induction of apoptosis were monitored in equal time intervals, up to 72 h after irradiations. According to the results, for MCF-7 cell line significant changes in terms of reduction in the number of S-phase cells was pronounced at 24 and 48 h time points, while in HTB140 cells changes in cell cycle were induced somewhat later and were still detectable 72 h after irradiations. Analysis of apoptosis has shown that ^{4}He ions make MCF-7 cells more prone to apoptotic cell death than HTB140 cells. This

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was confirmed by both changes in the number of SubG1 cells and changes in Bax/Bcl-2 ratio. The obtained results point to resistance to apoptosis as a possible mechanism underlying high level of radioresistance of HTB140 cells. Further experiments will be conducted to determine which modes of cell death contribute to biological response of this cell line to irradiation with ^{4}He ions.

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Direct and indirect effects of proton and carbon ion irradiations on breast adenocarcinoma cells

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Keywords: protons, carbon ions, direct and indirect effects, DMSO

Radiation-induced DNA damage is considered as the most important cellular damage as it could lead to the loss of clonogenic capacity and cell death.[1,2] Cell inactivation caused by irradiation results both from direct as well as from indirect actions mediated by free radicals.[3] To discriminate the direct from indirect radiation actions is important because it provides essential information about the mechanisms by which radiation ultimately affects cells.[4] Free radical scavengers, such as DMSO, can be used to reduce effects of indirectly induced DNA damages without affecting the direct effects of irradiation.[4,5] The aim of this study is to estimate the contribution of each of these effects on breast adenocarcinoma cells irradiated with three types of irradiations, i.e. γ-rays, protons and carbon ions. The MCF-7 cells were pre-treated with DMSO and then irradiated with ^{60}Co γ-rays, protons or carbon ions with doses ranging from 1-5 Gy. Cells were exposed to 62 MeV/u protons and carbon ions. Radiation position for protons was in the middle of the spread-out Bragg peak, while irradiations with carbon ions were carried out within slightly broadened Bragg peak to obtain LET with the highest biological effectiveness. Degree of protection (DP) was calculated for each dose and plotted as a function of the DMSO concentration. The contribution of indirect action in cell killing was obtained from the maximum DP provided by DMSO.[4,5,6] According to the results, the DP of DMSO increased in all irradiated cells, in a concentration dependent manner. After γ-irradiations, the estimated direct effects were around 35%, while the 65% of total irradiation effects could be attributed to indirect actions. In cells irradiated with protons and carbon ions, higher contribution of direct effects was observed, being around 44% and 48%, respectively. Although contribution of direct effects was higher in

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proton and carbon ion irradiated cells, a substantial fraction of indirect actions was found, being around 56% for proton and 52% for carbon ions. This points to important role of free radical actions in high LET irradiation induced cell death. The calculated relative biological effectiveness at 10% survival (RBE_{D10} values) also show that in high LET radiations, direct actions had a stronger impact on cellular growth inhibition than indirect effects.

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Breast Cancer Cell Microtubules: Target for Ionizing Radiation Effect

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Keywords: Microtubules, Breast Cancer Cells, Proton Irradiation

Microtubules (MTs) are one of the three components of cell cytoskeleton composed by 13 protofilament laterally tied together and assembled around a hollow core, defined as a rod or strand, with a diameter about 25nm. MTs have some similarities with DNA, the preferred macromolecule target of ionizing radiation damage, but also many differences. Among the common points we can mention the dimensions and involvement of acetylation and methylation process. On the other hand, microtubules are uniformly distributed over the cell volume and are characterised by completely different repair/reorganisation mechanisms than DNA. A Previous work has shown effects from ionizing radiation on MT of non-tumor breast cells[1]. In this work MTs rearrangement and distribution of breast cancer cells after irradiation with proton in the dose range between 2 and 9 Gy, will be presented.

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