NORTH ATLANTIC TREATY ORGANIZATION

SCIENCE AND TECHNOLOGY ORGANIZATION

AC/323(HFM-291)TP/1067

www.sto.nato.int

STO TECHNICAL REPORT **TR-HFM-291**

Ionizing Radiation Bioeffects and Countermeasures

(Effets biologiques du rayonnement ionisant et contre-mesures)

HFM-291 Research Task Group activity – final report.

Published May 2022

NORTH ATLANTIC TREATY ORGANIZATION

SCIENCE AND TECHNOLOGY ORGANIZATION

AC/323(HFM-291)TP/1067

www.sto.nato.int

STO TECHNICAL REPORT **TR-HFM-291**

Ionizing Radiation Bioeffects and Countermeasures

(Effets biologiques du rayonnement ionisant et contre-mesures)

HFM-291 Research Task Group activity – final report.

Senior Task Report Editor: Michael Abend

The NATO Science and Technology Organization

Science & Technology (S&T) in the NATO context is defined as the selective and rigorous generation and application of state-of-the-art, validated knowledge for defence and security purposes. S&T activities embrace scientific research, technology development, transition, application and field-testing, experimentation and a range of related scientific activities that include systems engineering, operational research and analysis, synthesis, integration and validation of knowledge derived through the scientific method.

In NATO, S&T is addressed using different business models, namely a collaborative business model where NATO provides a forum where NATO Nations and partner Nations elect to use their national resources to define, conduct and promote cooperative research and information exchange, and secondly an in-house delivery business model where S&T activities are conducted in a NATO dedicated executive body, having its own personnel, capabilities and infrastructure.

The mission of the NATO Science & Technology Organization (STO) is to help position the Nations' and NATO's S&T investments as a strategic enabler of the knowledge and technology advantage for the defence and security posture of NATO Nations and partner Nations, by conducting and promoting S&T activities that augment and leverage the capabilities and programmes of the Alliance, of the NATO Nations and the partner Nations, in support of NATO's objectives, and contributing to NATO's ability to enable and influence security and defence related capability development and threat mitigation in NATO Nations and partner Nations, in accordance with NATO policies.

The total spectrum of this collaborative effort is addressed by six Technical Panels who manage a wide range of scientific research activities, a Group specialising in modelling and simulation, plus a Committee dedicated to supporting the information management needs of the organization.

- AVT Applied Vehicle Technology Panel
- HFM Human Factors and Medicine Panel
- IST Information Systems Technology Panel
- NMSG NATO Modelling and Simulation Group
- SAS System Analysis and Studies Panel
- SCI Systems Concepts and Integration Panel
- SET Sensors and Electronics Technology Panel

These Panels and Group are the power-house of the collaborative model and are made up of national representatives as well as recognised world-class scientists, engineers and information specialists. In addition to providing critical technical oversight, they also provide a communication link to military users and other NATO bodies.

The scientific and technological work is carried out by Technical Teams, created under one or more of these eight bodies, for specific research activities which have a defined duration. These research activities can take a variety of forms, including Task Groups, Workshops, Symposia, Specialists' Meetings, Lecture Series and Technical Courses.

The content of this publication has been reproduced directly from material supplied by STO or the authors.

Published May 2022

ISBN 978-92-837-2385-1

Table of Contents

List of Figures

List of Tables

List of Acronyms

1,3-MAP	1-methyl-3-acetylpyridine
1,4-DMP	1,4-dimethylpyridine
AFRRI	Armed Forces Radiobiology Research Institute
AGRS	Acute Gastrointestinal Radiation Syndrome
ALC	Absolute Lymphocyte Count
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AML	Acute Myeloid Leukemia
ANG2	ANGiopoietin 2
ARS	Acute Radiation Syndrome
ASC	Adipocyte Stem Cell
AST	Aspartate Aminotransferase
BAT	Biodosimetry Assessment Tool
BMT	Bone Marrow Transplantation
BRP	Biodosimetry Research Program
CBLB613	Naturally Occurring Mycoplasma-derived lipopeptide ligand for the Toll-like Receptor 2/6
CBMN	Cytokinesis Block Micronucleus
CBRE	Chemical, Biological, Radiological, and Environmental
CE	Cell Equivalent
CIPRO	CIPROfloxacin
CK	Creatine Kinase
COMEDS	The Committee of the Chiefs of Military Medical Services in NATO
CRP	C-Reactive Protein
CRS	Cutaneous Radiation Syndrome
DCA	Dicentric Chromosome Assay
DIC	Dicentric
DoD	U.S. Department of Defense
DOREMI	Low Dose Research towards Multi-disciplinary Integration Network of Excellence
DRF	Dose Reduction Factor
DT3	Delta-Tocotrienol
DTPA	Diethylene triamine pentaacetic acid
DTRA	Defense Threat Reduction Agency
DU	Depleted Uranium
EACK	Emergency Antiapoptotic Cytokine therapy
EGF	Epidermal Growth Factor
EURADOS	The European Radiation Dosimetry Group
FDA	U.S. Food and Drug Administration
FISH	Fluorescence in situ Hybridation
FRAT	The First-Responders Radiological Assessment Triage
G-CSF	Granulocyte Colony-Stimulating Factor
GFP	Green Fluorescent Protein
GT3	Gama Tocotrienol

HARS	Hematological Acute Radiation Syndrome
HENRE	Health Effects from Nuclear and Radiological Environments
HFM	Human Factors and Medicine
HSC	Hematopoietic Stem Cell
HSPC	Hematopoietic Stem and Progenitor Cell
HTO	Tritiated water
HUMN	Human Micronucleus project
HUVEC	Human Umbilical Vein Endothelial Cell
ICRP	International Commission on Radiological Protection
IL	Interleukin
IND	Improvised Nuclear Device
LDH	Lactico Dehydrogenase
LDR	Low Dose Radiation
LET	Linear Energy Transfer
LLC	Louis Lang Carcinoma
MAD	Mean of the Absolute Differences
MAPK	Mitogen-Activated Protein Kinase
MCV	Mean Corpuscular Volume
MedCM	Medical Countermeasure
MELODI	The Multi-disciplinary European Low Dose Initiative
METREPOL	Medical Treatment Protocols for Radiation Accident Victims
Mfrat	Mobile First-responders Radiological Assessment Triage (for smart phones)
MMO	Military Medical Operations
MN	Micronucleus
MNA	1-Methylnicotinamide
MONO	Monocyte count
MSC	Mesenchymal Stem Cell
MULTIBIODOSE	Multi-Disciplinary Biodosimetric Tools to Manage High Scale Radiological Casualties
MURAD	Moscow-Ulm Radiation Accident Database
NA	Nicotinamide
NAc	Nicotinic Acid
NATO	North Atlantic Treaty Organization
NHP	Nonhuman Primate
NIHR	The National Institute for Health Research
NK	Natural Killer (Cell)
NLR	Neutrophil-to-Lymphocyte Ratio
NO	Nitric oxide
NRPB	The National Radiological Protection Board
NTE	Non-Targeted radiation Effect
PB	Phenylbutyrate
PBI	Partial Body Irradiation
PCC	Premature Chromosome Condensation
PDGF	Platelet-Derived Growth Factor
PHE	Public Health England
PI	Proliferation Index
PLSDA	Partial Least Square Discriminant Analysis
PLT	Platelets
POC	Point-Of-Care diagnostic
PrB	Prussian Blue

RANET	Radiation Assistance Network
RBC	Red Blood Cells
RBE	Relative Biological Effectiveness
RC	Response Category
RCI	Radiation Combined Injury
RDD	Radiation Dispersal Device
REAC/TS	Radiation Emergency Assistance Center/Training Site
RED	Radiation Exposure Device
RENEB	Realizing European Network in Biodosimetry
RES	Radiation Exposure Status
RIBE	Radiation-Induced Bystander Effect
RIMODS/MOF	Radiation-Induced Multi-organ Dysfunction Syndrome / Multi-organ Dysfunction Failure
RIPD	Radiation-Induced Performance Decrement
RN	Radiological and Nuclear
RTG	Research Task Group
SAA	Serum Amyloid Protein
SEARCH	System for Evaluation and Archiving of Radiation accidents based on Case Histories
SFT3	Stem Cell factor, FLT-3 ligand and interleukin-3 Combination
SIRS	Systemic Inflammatory Response Syndrome
SOLO	Epidemiological Studies of Exposed Populations in the Southern Urals
SOM23	Somatostatin analog
TBI	Total Body Irradiation
TBSA	Total Body Surface Area
TNF	Tumor Necrosis Factor
TRL	Technology Readiness Level
US	United States
WBC	White Blood Cells
WBI	Whole Body Irradiation
winFRAT	First-Responders Radiological Assessment Triage (for Windows)
WMD	Weapons of Mass Destruction

Preface

Almost forty years ago when the proceedings of the 1985 workshop of the NATO Research Study Group (RSG-5) on the Assessment of ionizing Radiation Injury in Nuclear Warfare [Ottawa, Canada, DS/A/DR (86) 191] was published, the main concern of both the NATO Military Command and the Medical Advisor was to predict the performance decrement and medical outcome of irradiated troops following a nuclear detonation. Since the end of the Cold War, the Radiological/Nuclear (RN) threat has changed but is still present and problematic. Nuclear weapons remain Weapons of Mass Destruction (WMD) whereas the emergence of technologically skilled terrorist groups and rogue states facilitates diverse kinds of scenarios comprising malevolent ionizing radiation exposure. Regarding the number of victims involved in such RN situations, small size to mass-casualty events may happen. Radiation Dispersal Devices (RDD), Radiation Exposure Devices (RED) and Improvised Nuclear Devices (IND) are plausible threat scenarios.

Forces must be prepared to execute and support prevention, protection, and recovery measures and operations during a CBRN incident that affects both civilian populations and military forces. The RN threat remains a critical concern for all NATO military commanders and medical advisors. Medical radiation preparedness requires a full-spectrum understanding of the mechanisms of action of ionizing radiation, rapid diagnosis of exposure, and how to most effectively manage their effects using medical countermeasures – either pre- or post-exposure. In the context of defence operations in an RN environment, whatever the mission to be fulfilled, any efforts should contribute to prevent fatalities, better take care of radiation casualties, and optimize return to duty. The HFM-291 RTG has therefore aimed at optimizing available countermeasures and bridging medical gaps.

The main focus of this RTG was to continue work on diagnosis and prediction of ARS. During previous RTG life-cycles several deployable software tools were developed and updated during this RTG. Conversion of pre-versions into Apps facilitated their use for point-of-care diagnostic. The usefulness of this approach was proved within two previous exercises based on RED scenarios and exploited by the RTG (2011 – 2012 and 2015-2016). The first one resulted in five 2013 publications in Radiation Research with a front cover in this journal, and another international publication for the second exercise. Based on this previous work it was agreed to organize a NATO workshop for introduction into these tools. The NATO workshop entitled “Software Tools for Triage of the Acute Radiation Syndrome9999999a practical workshop (StTARS)” was organized as one of the key deliverables of our RTG. Within this workshop we described the purpose and function of software tools. These tools either allowed an integrated estimation of dose (BAT, WinFRAT), or the prediction of ARS severity based on changes in blood cell counts (H-module) in the first days after exposure to ionizing radiation. Importantly, participants became introduced to these tools primarily by those who developed them, so that participants received first-hand tuition about their strengths and limitations. The first workshop took place in Paris in 2019. The feedback was excellent and a second workshop was planned in the USA. Caused by the coronavirus pandemic, the second NATO StTARS workshop planned in 2020 had to be postponed. It is now agreed to take presumably place in 2021 at Oak Ridge Tennessee, hosted by REACT/TS.

Modelling is very helpful to unravel complex threats and consequent injuries. As already described, RTG produced different mature and deployable software tools dealing with the assessment triage (i.e., update of Mobile-FRAT App and the first version of H-Module App, both on an Android and IOS based application).

Comprehensively, to ensure Forces health protection, pre-incident, during the incident and post-incident medical care, and then rehabilitation, appropriate medical countermeasures have to be designed, developed, validated, and made available to the Forces. Regarding defence operations in an identified RN environment, the use of radioprotectants may be a good option warranting dedicated research and development studies.

Candidate molecules have been evaluated by this RTG. Unfortunately, when considering terrorists attacks that are unexpected in nature, the prophylactic approach is of low utility and all biomedical research efforts should be focused on diagnosis, mitigation, and treatment of RN damage. Significant efforts were made to mitigate external irradiation toxicity and several studies are included in the Technical Report. Future research should be focused on deployable, well tolerated, and effective mitigators and therapeutics. Moreover, always regarding high-level radiation exposure, new combined injury models and a better knowledge of the development of RIMODS/MOF are needed.

Another new challenge had to be faced with respect to the societal evolution and population concerns about low-level radiation issues. On one hand, anti-nuclear activists challenge the civil nuclear energy and the nuclear deterrent, on the other, low-level radiation issues have become an increasing matter of concern after the Fukushima catastrophe. The understanding of RN health challenges embraces not only acute and delayed toxicity of high-level radiation but also low-level radiation toxicity, in particular carcinogenesis. The matter for this RTG was to identify late deterministic or stochastic adverse effects of radiation doses below the ARS threshold for soldiers and populations exposed to an accidental or malevolent RN environment. Another contribution in this regard comprised RTG activities towards the development of an App to improve the risk communication in particular after LLR. CRRis (comparing radiation exposure risks with daily life risks), a NATO App for improving risk communication in an RN event, should be developed. The first steps and a concept in this direction were published by RTG as a group.

Exchanges with NATO non-medical RN experts as well as non-NATO medical experts are already taking place and urgently required in future, in order to better take into account all aspects of the RN threat that may affect the Forces and civilian populations. The joint efforts of the HFM-291 RTG must certainly be pursued in a follow-on RTG that would include more nations to fill the remaining gaps.

To conclude, significant work has been made by the HFM-291 RTG and useful internationally acknowledged deliverables have been produced. Collaborations were facilitated by this NATO scientific platform to dampen undesired reductions of an already decreased radiobiological scientific society. Moreover, we advise the reader to consult the 85 scientific publications produced by the members of the RTG during the 2017 – 2021 period, which are listed in Chapter 6.

However, this progress does not imply the final resolution of the medical management after low and high radiation exposures. Importantly, milestones successfully achieved over this RTG life cycle and many more have to be accomplished along with a forthcoming RTG. It is like fighting cancer; development of innovative diagnostic or therapeutic advancements adds to the arsenal of countermeasures, but it does not solve the problem entirely, implying further vigorous efforts being executed.

Foreword

Inordinate exposure to ionizing radiation continues to pose a threat to military operations. This persistent risk has long been recognized by the Human Factors and Medicine Panel of the NATO's Science and Technology Organization (STO/HFM) as reflected by its decision to set up the Research Task Group 291 (HFM-291 RTG) entitled Ionizing Radiation Bioeffects and Countermeasures, a follow-on from the HFM-222 RTG and two or three earlier similar RTGs. In contrast to its predecessors, HFM-291 due to the SARS-CoV-2 pandemic was active longer spanning a five-year period of 2017 – 2021. Representatives from eight NATO countries (CZE, DEU, FRA, GBR, ITA, NDL, POL, USA) participated in the activity of the RTG holding one meeting per year. Throughout its life-span the functioning of the HFM-291 RTG was skillfully arranged and led by its chairman, Col Prof Michael Abend (DEU), Deputy Director of the Bundeswehr Institute of Radiobiology in Munich. Under his leadership the RTG provided a venue to convene seventeen radiobiology experts from a broad spectrum of research to share pertinent knowledge.

Primarily, HFM-291 elaborated on, updated, and expanded the research and accomplishments of HFM-222, especially with respect to the diagnosis, management, and prognosis of the Acute Radiation Syndrome (ARS) including application of stem cells in the treatment of the cutaneous and hematopoietic presentations of this disease. With respect to the low-level radiation scenarios one group of the RTG experts conducted research devoted to biomedical effects of irradiation or contamination at low doses of ionizing radiation.

I praise the HFM-291 RTG for its efforts and the valuable content of the Final Report which reflects considerable progress in research on health effects of high levels of ionizing radiation focusing on biological dosimetry (diagnostic and predictive indicators), radioprotectors, mitigators, management of ARS, and prevention of late effects. Especially commendable are the tangible deliverables of the Task Group such as improved deployability of the previously designed software tools such as Mobile-FRAT and H-Module, providing the basis for a NATO App to improve risk communication in a radiological/nuclear event called CRRis (comparing radiation exposure risks with daily life risks), the organization and execution of the highly acknowledged NATO StTARS workshop on software tools for triage of ARS and last but not least the eighty-five joint peer-reviewed publications.

Certainly, not all radiobiology gaps pertinent to the NATO operations have been addressed and filled by the HFM-291 RTG. Particularly disturbing is the paucity of participants (only one representative) and of research devoted to various aspects of low-level radiation the exposure to which constitutes the most probable contingency during the present-day military operations. Indeed, additional work is needed devoted to this and other aspects of radiobiological research, especially in terms of translating the results of the state-of-the-art science into deployable, workable standard procedures of, e.g., rapid and reliable diagnostics, allocation of scarce medical resources, post-evacuation care, handling of combined injuries, as well as detection and exploitation of beneficial effects of low-level exposures. These and other relevant challenges underscore the rationale for continuing the activities of the HFM-291 RTG by a future installment of a task group devoted to Radiation Bioeffects and Countermeasures.

Col (ret) Prof Marek K. Janiak, MD, PhD

HFM-222 RTG Mentor (retired)

Terms of Reference

General Remarks

The NATO HFM 222 Research Task Group (RTG) on "Ionizing Radiation Bioeffects and Countermeasures" follows two different tasks:

1) The HFM-222 RTG represents a platform to connect military and non-military Institutions worldwide dealing with the same scientific issues of military relevance. By doing so synergistic effects and collaborations emerge in order to jointly work on urgent military issues. For instance, the recent HFM-222 RTG facilitated a French-German military collaboration. France took care of a sophisticated animal model and Germany provided a specific methodology. As a result of that collaboration the base of a new early and high-throughput diagnostic tool for prediction of the Acute Radiation Syndrome (ARS) could be developed. In order to enable these synergistic effects, it is necessary providing broad terms in our TOR so that different Institutions will become attracted and might decide to participate. These TOR by nature will not change over time, since e.g., the diagnostic or the therapy of the ARS will be constantly improved even after inventing a new diagnostic tool. Also, it takes decades for the development of new diagnostics or therapeutic regimens. The 3-year time frame of an RTG cannot cover these periods of development and, therefore, the broader TOR will remain the same over many RTGs.
2) In order to highlight the progress performed in different fields and to show what will be performed within the next RTG we introduced examples into the TOR (details of the previous work are reflected in the report of the RTG). Here, we extrapolate from that what has been performed in the previous RTG and what is planned to be done as the next steps within the requested RTG. Prerequisite of that is the constant membership of the nations involved which, unfortunately, changes over time and is motivated mainly by budget restrictions.

Research Area and Scope

Based on different exposure scenarios we have to deal with low- or high radiation exposures and associated health effects (Figure i). A continuous improvement on the prevention (prophylaxis, mitigators) and medical management (diagnosis, triage, therapy, decisions on the occupation of medical resources) of these health effects represents the core task of this RTG (Figure i).

Also, radiation risks from low dose radiation are not well known and operational guidance today is unclear. Balancing operational requirements against precautionary thresholds will present challenges in any contested battlespace or disaster-response theater of operations involving radiological contamination of even quite low dose.

In order to bridge the gap in appropriate judgement of radiation exposure health risk, the RTG intends to extend their work on risk communication (Figure i). This is one measure dealing with psychological effects, which represents another highly relevant topic of the new RTG. Also, this topic will improve the civilian-military cooperation. Another innovation of the new RTG will be a teaching class, where RTG members introduce their recently developed innovative prototype software tools to support medical management of acute health effects. This at the same time is intended as another measure for attracting students to our area.

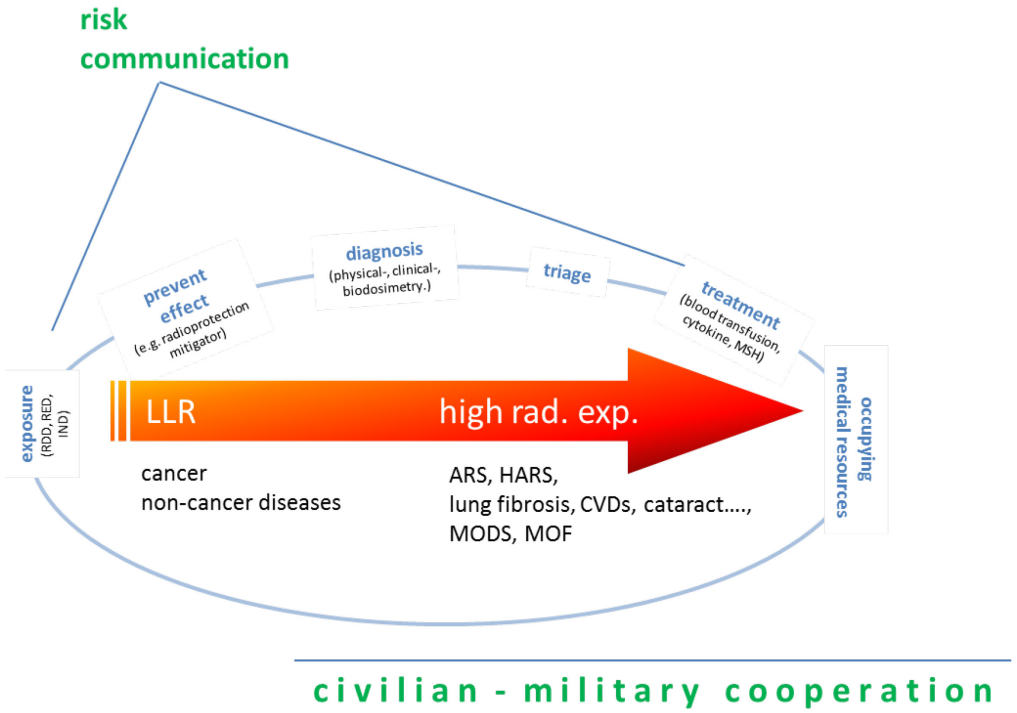


Figure i: Research Scope of the HFM-291 RTG on "Ionizing Radiation Bioeffects and Countermeasures."

Specific Goals

1) Research into mechanisms of ionizing radiation-induced injury:
   - Establishment of origins and mechanisms of acute radiation-induced pathology.
   - Establishment of origins and mechanisms of delayed Multi-Organ Dysfunction Syndrome and Failure (MODS/MOF).
   - Development and characterization of suitable animal models for the study of acute radiation syndrome and Multi-Organ Dysfunction Syndrome and Failure (MODS/MOF).
   - Identification of biomarkers of ionizing radiation exposure which correlate between animals and human.
   - Establishment of origins and mechanisms of low dose radiation pathology.
   - Develop specific models that are amenable for a better understanding on the impact of low dose or delayed radiation effects.
   - Examinations on radiotoxicology after incorporation of radionuclides.

In this context the research of the previous RTG did lead to further tasks for the requested RTG such as:

- *France examined the time-dependent relevance of radiation biomarkers to distinguish partial body from total body exposures in baboons. Currently these extensive data are published and a validation of these biomarkers of exposure on human samples is planned for the requested RTG.*
- *Noteworthy, in the future, Czech republic will perform a study on TBI patients in order to reveal new protein biomarkers in blood serum using mass spectrometry.*

- *USA has established new animal models i.e., mini-pig that can more effectively address delayed radiation effects.*
- *USA has established novel in vitro and in vivo models and discovered new biomarkers that may be useful for late arising pathologies.*

2) RDT&E into agents that protect (administered before exposure), mitigate (administered after or during exposure), and/or treat ionizing radiation-induced injury (caused by external/internal exposures):
   - Mechanisms of action to include surrogate markers of protection.
   - Relative efficacy and limitations of promising classes of radioprotectants/radiomitigators/ radiotreatments.

In this context research of the previous RTG did lead to further tasks for the requested RTG, such as:

- *Poland administered several derivatives of nicotinic acid to mice before and after sublethal irradiation. Of the five compounds that were tested, four prolonged survival of the mice when given both 7 days before and 7 days after the irradiation i.e., they exerted both protective and mitigative effects. Stimulation of hematopoiesis appeared not the likely explanation of the enhanced survival. Instead, other mechanisms such as mitigation of radiation-induced inflammation, thrombosis, and/or depressed endothelial function need to be considered. Further insight into the mechanism during the requested RTG will provide the measure to potentially improve classes of radioprotectants/radiomitigators.*
- *France examined the role of mesenchymal stem cells in the pathology of the ARS as well as the development of MODS and MOF. First research in this direction appeared very promising, albeit only limited numbers of treated victims have been injected with autologous cells. Gene therapy is still in its infancy but work is going on to determine whether it could represent a valuable approach in the future. This work will be continued within the requested RTG.*
- *USA provided an overview on their research related to that topic. USA was heavily involved in research on the treatment of the ARS using cytokines (e.g., G-CSF). Recently and after decades of intensive research, G-CSF got approved by the FDA as the first drug for treatment of the hematological ARS in humans. Future research will e.g., focus on the treatment of the gastrointestinal syndrome of the ARS.*
- *Czech Republic examined the effect of Epidermal Growth Factor (EGF) and Bone Marrow Transplantation (BMT) on Gastrointestinal (GI) damage after high-dose irradiation of mice. This combination was identified as a promising strategy in the treatment of the GI syndrome. Future work has to validate these results in another animal model.*

3) Research and development on biodosimetric methods to estimate the level of radiation exposure and the severity of radiation injury in order to facilitate and support diagnostic/triage of health effects:
   - Standardized rapid dose assessment and radiation injury severity for triage of potentially exposed personnel and mass-casualty management.
   - Development of high-throughput and early biodosimetry tools for sensitive, accurate and reliable dose estimation and prediction of late developing radiation-associated injuries. Consider a multiparameter approach to assist in the prediction of clinical outcome.

In this context research of the previous RTG will lead to further tasks for the requested RTG such as:

- *Italy provided an overview on their capacity for biodosimetry using, for example, the dicentric chromosomal assay and the Cytokinesis Block MicroNucleus assays or using dogs as sentinel of environmental risk for detection of radiation in operational theaters. Future work will, for example, focus on the establishment of further calibration curves using other radiation qualities.*

- *USA developed a biodosimetry tool available as an App for smart phones. This release will be updated in future work.*
- *HFM-222 RTG members performed a joint exercise to examine the potential of clinical signs and symptoms for the medical management after a radiological event. These data are currently published as an "invited manuscript" and will be used as the base of a teaching class for the requested new RTG.*

4) Identification of predictors, indicators and prophylactic or therapeutic treatments for radiation-induced late effects such as cancer, cardiovascular disease, cataractogenesis or fibrosis occurring after absorption of, for example, moderate or low dose and low dose rate exposures.

In this context research of the previous RTG will lead to further tasks for the requested RTG such as:

- *Germany identified certain candidate genes associated with the development of cardiovascular disease in radiation victims (Mayak worker). A validation in another radiation exposed cohort is planned for the requested next RTG.*
- *USA has established novel in vitro and in vivo models and discovered new biomarkers (i.e., histone modifications) that may be useful for late arising pathologies.*

5) Integration of medical expertise and RDT&E to provide specific recommendations to NATO COMEDS. Guidance to inform field commanders and deployed forces.

In this context research of the previous RTG will lead to further tasks for the requested RTG such as, for example, Germany developed a so called "H-Module". It predicts the hematological ARS based on changes in blood cell counts within the first three days after exposure. It is planned to distribute this tool under the umbrella of the NATO and to introduce students and researchers to this tool as a teaching class in the context of the requested RTG.

6) Developing tools for improving risk communication and dealing with psychological effects.

   This provides a new task as already shown.

7) Delivering and introducing newly developed innovative tools of the RTG to the public and military Institutions.

   This provides a new task as already shown.

Deliverables

1) Update on the state-of-the-art in basic research, prevention, and medical management (diagnosis/triage, therapy, requirement/occupation of clinical resources) of health effects and reporting about it via RTG reports (some more specific outputs are shown as examples above).
2) Developing tools for improving risk communication, e.g., converting/comparing radiation exposure risks with daily life risks (CompRadRisk NATO App).
3) Organizing and teaching a NATO course on the medical management of acute health effects with the introduction into state-of-the-art newly developed RTG's software tools in order to maintain the civilian-military cooperation.

Acknowledgements

Pr. Dr. Marek K. Janiak (POL) served as the Referee for the Ionizing Radiation Bioeffects and Countermeasures HFM-291 RTG until his retirement in 2020. He provided helpful guidance to this RTG. Sine 2020 PD Dr. Kai Kehe (DEU) replaced his position and provided his support as well. The HFM-291 RTG chair also extends thanks to all members who contributed to the success of the RTG tasks. There were three annual on-side HFM-291 RTG meetings, two online-meetings and several sub-meetings of two Task Forces build by RTG members in order to work out details regarding NATO StTARS workshop and CRRis development. Progress and necessary decisions were presented to the whole RTG at their annual meetings. The chair is grateful to the respective host Nations, Institutes' leadership, and gives credit to the local hosts, who organized the meetings and provided excellent social activities. In addition, the excellent editorial, publishing, and graphic support services by the NATO STO Publication Office and Editor are gratefully acknowledged.

Michael Abend (DEU)

HFM-291 RTG Chair

HFM-291 Membership List

CHAIR

Col Prof. Michael ABEND
Bundeswehr Institute of Radiobiology
GERMANY
Email: michaelabend@bundeswehr.org

MEMBERS

Mr. Christophe BADIE
Public Health England
UNITED KINGDOM
Email: christophe.badie@phe.gov.uk

Dr. William BLAKELY
Armed Forces Radiobiology Research Institute (AFRRI)
UNITED STATES
Email: william.blakely@usuhs.edu

Dr. Stefania DE SANCTIS
Italian Military Research Center
ITALY
Email: stefania.desanctis@gmail.com

BGen Dr. Michel DROUET
IRBA
FRANCE
Email: michel1.drouet@intradef.gouv.fr

Dr. Crawford FOSTER
Institute of Naval Medicine
UNITED KINGDOM
Email: crawford.foster381@mod.uk

Ms. Valeria FRANCHINI
Italian Military Research Center
ITALY
Email: valeriafrn@gmail.com

Capt John GILSTAD
Armed Forces Radiobiology Research Institute (AFRRI)
UNITED STATES
Email: john.gilstad@usuhs.edu

Col Lester A. HUFF
USUHS Armed Forces Radiobiology Research Institute (AFRRI)
UNITED STATES
Email: lester.huff@usuhs.edu

Dr. Juliann KIANG
Armed Forces Radiobiology Research Institute (AFRRI)
UNITED STATES
Email: juliann.kiang@usuhs.edu

Mr. Tjerk Pier KUIPER
CEAG
NETHERLANDS
Email: tp.kuipers.01@mindef.nl

Col Dr. Florigio LISTA
Italian Military Research Center
ITALY
Email: romano.lista@gmail.com

Dr. Mathew MCCAULEY
DCPS, DPHC, JFC Ministry of Defence
Ireland
Email: drmmccauley@yahoo.com

Dr. Alexandra MILLER
AFRRI/USUHS
UNITED STATES
Email: alexandra.miller@usuhs.edu

Dr. Ewa M. NOWOSIELSKA
Military Institute of Hygiene and Epidemiology
POLAND
Email: enowosielska@wihe.waw.pl

Col. PD Dr. Matthias PORT
Bundeswehr Institute of Radiology
GERMANY
Email: matthiasport@bundeswehr.org

Major Diane RICCOBONO
IRBA
FRANCE
Email: diane.riccobono@intradef.gouv.fr

Dr. Daniela STRICKLIN
Applied Research Associates, Inc.
UNITED STATES
Email: dstricklin@ara.com

Assoc. Prof. Ales TICHÝ
University of Defence
CZECH REPUBLIC
Email: ales.tichy@unob.cz

LTC Mitchell WOODBERRY
Armed Forces Radiobiology Research Institute (AFRRI)
UNITED STATES
Email: mitchell.woodberry@usuhs.edu

PANEL/GROUP MENTORS

Prof. Marek K. Janiak (ret. Sept. 2020)
Military Institute of Hygiene and Epidemiology
POLAND
Email: *not available*

Col Prof. Dr. Kai KEHE
Bundeswehr Medical Academy
GERMANY
Email: kaikehe@bundeswehr.org

Ionizing Radiation Bioeffects and Countermeasures

(STO-TR-HFM-291)

Executive Summary

In a situation of radiological or nuclear NATO military defensive operations, the HFM-291 RTG on "Ionizing Radiation Bioeffects and Countermeasures" addressed medical-related challenges associated to these scenarios. HFM-291 RTG contributed to diagnostic aspects by widening the biomarker panel for exposure (dose) reconstruction to include the aspect of Acute Radiation Syndrome (ARS) effect prediction, improved the deployability and point-of-care diagnostic of previously developed software tools (Mobile-FRAT, H-Module), and organized one internationally highly acknowledged NATO workshops (NATO StTARS workshop on software tools for triage of the ARS). The second workshop is in progress.

Medical therapeutic developments included stem cell research with implications for future treatment strategies of multiple organ systems (i.e., hematopoietic, cutaneous) ARS. Regarding Low-Level Radiation (LLR) scenarios (e.g., a radiological dispersible device), the RTG conducted research to fill in gaps regarding LLR and developed the base of a NATO App for improving risk communication in a radiological and nuclear event called CRRis (comparing radiation exposure risks with daily life risks).

Shortages in personnel, financial resources, and research projects, underlined the significance of this NATO RTG as a powerful platform to counteract the erosion of the radiobiological society via fruitful collaborations. The total of 85 joint publications by this group is an impressive demonstration of this kind of collaboration. Each peer-reviewed publication represents an independent validation of NATO research, reflecting its significance.

Effets biologiques du rayonnement ionisant et contre-mesures

(STO-TR-HFM-291)

Synthèse

Le RTG HFM-291 intitulé « Effets biologiques du rayonnement ionisant et contre-mesures » s'est intéressé aux défis d'ordre médical associés aux scénarios d'opération radiologique ou nucléaire de défense militaire de l'OTAN. Le RTG HFM-291 a contribué aux aspects de diagnostic en élargissant la palette des biomarqueurs servant à reconstituer l'exposition (la dose), afin d'inclure la prédiction de l'effet sous la forme du syndrome aigu d'irradiation (SAI), a amélioré la déployabilité et le diagnostic sur le lieu de soin par des outils logiciels précédemment mis au point (Mobile-FRAT, H-Module), et a organisé un séminaire de l'OTAN reconnu au niveau international (séminaire OTAN StTARS sur les outils logiciels de triage du SAI). Le deuxième séminaire est en cours.

Les développements concernant les thérapies médicales ont inclus les recherches sur les cellules souches et leurs implications dans les futures stratégies de traitement du SAI de systèmes incluant plusieurs organes (autrement dit, hématopoïétique, cutané). Dans les scénarios à faible niveau de rayonnement (LLR) (par exemple, un engin radiologique), le RTG a mené des recherches pour combler les lacunes sur le LLR et a développé la base d'une application OTAN visant à améliorer la communication du risque dans un événement RN, appelée CRRis (comparant les risques d'exposition au rayonnement avec les risques de la vie quotidienne).

Le manque de personnel, de ressources financières et de projets de recherche a souligné l'importance du RTG de l'OTAN, comme tremplin formidable pour lutter contre l'érosion de la société radiobiologique, au moyen de collaborations fécondes. Les 85 publications conjointes de ce groupe expriment et démontrent cela de manière impressionnante. Chacune de ces publications approuvées par des pairs valide de manière indépendante les recherches de l'OTAN et reflète leur importance.

Chapter 1 – NATIONAL STATUS REPORTS (2017 – 2021)

1.1 CZECH REPUBLIC NATIONAL STATUS REPORT 2017 – 2021, A. TICHÝ, UNOB

The capacity of the Department of Radiobiology, Faculty of Military Health Sciences, University of Defence remained unchanged within 2017 – 2021. The staff comprises 1 professor, 1 associate professor and 4 post-doctoral fellows supported by 3 lab technicians. The staff is organized into 1 group of Radiation Pathology. The main tasks of the department are teaching and research activities, which are closely connected.

In the field of research, the experimental work includes histology and cytology, in vitro methods, methods of proteomic analysis and methods of flow cytometry. Individual technological units allow in vitro and in vivo observation of post radiation mechanisms on molecular, cellular, and organ levels. Military research is focused on early diagnosis and therapy of post radiation damage as the main objective of the department. The aim of investigation in the medium-term horizon is discovery and practical introduction of biodosimetric markers, study of molecular mechanisms of radiation-induced DNA damage repair and autophagy, development of radioprotection agents as well as continuous renewal of decontamination agents for the Army of the Czech Republic. Mutual cooperation with other CBRN research workplaces world-wide also remains an integral part of our research activities. Cooperation with civilian workplaces at the Faculty of Medicine and the University Hospital in Hradec Králové is focused on radiation oncology. The Department of Radiobiology takes part in military medical-specialist education in the form of pre-graduate and post-graduate education, mainly in doctoral studies. The main educational activity is lecturing on military radiobiology. The main topics are the effects of nuclear weapons on the living organism; the possibilities of the protection; and medical treatment of irradiated persons. Other specific military issues are disaster medicine, CBRN protection, etc., which are taught at the Faculty of Military Health Sciences, where instructors from our department give lectures on these topics.

For the last four years our team was engaged with a plethora of topics within the field of radiobiological research. Hereafter is a brief description of individual research topics with further detailed description of three sub-areas given in sections of Chapter 2 “New Approaches in Diagnostics and Therapy of ARS” (Section 2.1.1), “Radiation Bioeffects Modulation by a Small Molecule Inhibitor” (Section 2.2.2), and “Development of Small Molecules for Enhanced Radioprotection” (Section 2.2.4).

One of the research problems was modulation of radiation-induced pulmonary fibrosis by hyaluronic acid nanoparticles was studied. The purpose of the study was to determine effect of hyaluronic acid nanoparticles on the development and/or mitigating of Radiation-Induced Lung Injuries (RILI). The combination of exceptional physicochemical properties of Hyaluronic acid (HA) and advanced options of nanostructures has shown excellent potential for new treatment strategies. Also, HA is a major component of ECM in lung and plays an important role in process of tissue injury and tissue repair. In this study, we used female C57BL/6J mice with whole thorax irradiation by 17 Gy. Hyaluronic Acid Nanoparticles (HANPs) of two different sizes (84.5 and 124 nm) were delivered by intratracheal instillation directly into lung before irradiation. Samples of blood and lung tissue were collected in three time-points regarding the development of radiation-induced lung injuries.

The results indicate that significant effects were caused in molecular and cellular levels. In the blood, populations of B-lymphocytes and neutrophils were significantly changed. On the other hand, level of TGF-β, crucial factor of lung tissues fibrosis after irradiation, were significantly affected by HANPs treatment and population of T helper and neutrophils in the lung during intermediate and fibrotic phases. Also, significant differences were observed on tissue levels during fibrotic phase in both groups treated with HANP-s compared with only 17 Gy partial irradiated group. According to our findings, HA-NPs application seems promising to attenuate RILI, mainly during chronic, fibrotic phase.

The next research area was a novel approach in biological dosimetry, voltammetric detection of oxidative DNA damage after irradiation. Advances and modernization of laboratory methods and increasing demands on retrospective biodosimetry force us to search for new approaches for accurate and fast quantification of absorbed dose in cases of accidental exposure to ionizing radiation. The most appropriate voltammetric detection mode appears to be in conjunction with screen-printed carbon electrodes to study and analyze the effects of ionizing radiation on nucleic acids.

Differential Pulse and Square Wave Voltammetry (DPV and SWV) were used to investigate the electrochemical behavior of 8-hydroxyguanine (8-OHG) at screen-printed carbon electrodes chemically modified by multi-walled carbon nanotubes. 8-OHG arises in response to DNA damage by oxidation of base guanine and it is the most prominent of oxidative lesions contributed to mutagenesis, carcinogenesis, and aging.

Disposable screen-printed carbon electrodes modified with carboxy-functionalized multi-walled carbon nanotubes were successfully utilized for enhanced electrochemical detection of 8-hydroxyguanine by differential pulse voltammetry. The method was optimized and validated for detection and quality control of 8-OH-Gua in model samples. The highest sensitivity of the detection was achieved in phosphate buffer in pH range of 7–8. The limit of detection was calculated as 0.57 μM. For practical purposes, the samples containing 8-OH-Gua must be kept light protected at 4 °C for 24 h only.

In order to increase the detection sensitivity for the analysis in biological samples, the square wave voltammetry approach was selected using a modified sensor with gold nanoparticles. The sensitivity of 8-OH-Gua detection using SWV was significantly higher than by DPV. Moreover, 8-OH-Gua was detected in a biological urine sample of the irradiated experimental mouse model only by changing the detection mode. This fact contributes to the idea of using electrochemical detection as a tool for assessment of DNA damage caused by ionizing radiation.

The last research area was engaged with the role of primary cilia in IR-induced molecular signaling of mesotheliomal cells. Pleural mesothelioma is an aggressive and malignant disease that develops from metastatic lung tissue cells. This type of tumor is known for its poor prognosis, low survival, radio- and chemoresistance. By its nature, IR affects cells at several levels. Its impact on DNA and other molecular mechanisms have been also extensively studied at our department. IR also affects cytoskeletal stability and thus the morphological structure of the cell. The primary cilium, a sensory organelle found on most human body cells, usually serves as a mechanical sensory tool interacting with the cellular microenvironment. The primary cilium could be considered as a candidate with predictive value regarding various forms of cell damage based on their morphological changes, such as number, length, in addition to its molecular characteristics.

MSTO-211H cell line was irradiated (5 Gy) or treated with chloral hydrate (1 mM CH) for 48 hours. Cell viability was determined through a WST-1 test. Immunofluorescence was used to assess the presence of primary cilia in the cells. For qPCR were used primers PTCH1, SMO, and GLI1. The expression of key Hedgehog signaling components (PTCH1, SMO, and GLI1) was calculated using the Livak method ($2^{-\Delta\Delta Ct}$). The statistical analyses were performed using a one-way ANOVA followed by a post hoc Tukey test using the GraphPad Insta software ($p < 0.05$).

Primary cilia occur in MSTO-211H cells and their incidence becomes increased after a dose of 5 Gy. Chemical deciliation with 1 mM CH does not affect the proliferation and viability of MSTO-211H cells. However, both cell proliferation and viability were decreased after irradiation with 5 Gy. Chemical deciliation with 1 mM CH in combination with a 5 Gy irradiation dose resulted in a significantly increased proliferation capacity in the treated MSTO-211H cells. Our results also show that the co-treatment of mesothelioma cells with 1 mM CH+5 Gy results in the downregulation of PTCH1 and the upregulation of SMO compared to non-treated cells. Interestingly, GLI1 expression was significantly upregulated in co-treated MSTO-211H cells when compared against non-treated cells, an observation that was not made under single treatment with either CH or a 5 Gy irradiation dose.

This work aims to determine whether the presence or absence of primary cilia has a direct role in the activation of the Hh signaling pathway in cancer cells after exposure to ionizing radiation, thus contributing to their radioresistant capacity.

1.2 FRANCE NATIONAL STATUS REPORT 2017 – 2021, M. DROUET, IRBA

The "Institut de Recherche Biomédicale des Armées" (IRBA) is the multidisciplinary research center of the French Armed Forces Sanitary Service located near Paris (Brétigny sur Orge). The Department "Effets Biologiques des Rayonnements" (EBR) belongs to IRBA CBRN Defense Division. It is about 20 scientists and technicians strong, military as well as civilian, and comprises 2 units: Radiobiology and Risques Technologiques Emergents (RTE).

The EBR department has expertise in various preclinical models including rodents and minipig and NHP if required. In vitro as well as in vivo irradiations can be managed with a 60cobalt gamma source and an x-ray small animal radiation research platform SARRP device. Cytogenetic biodosimetry analysis is routinely performed using a Metasystem platform. Usual technical platforms (genomic, flow cytometry, etc.) are shared with other research units. Specific sources are used for EMF studies and RTE unit has also developed an expertise in RMN.

DEBR is working with civilian laboratories with special links with Commissariat à l'énergie atomique (CEA) and Institut de Radioprotection et Sécurité Nucléaire. Management is ISO 9001 certified (IRBA as a whole) and DEBR is part of the 1296 Inserm unit (UMR).

At the military level, IRBA belongs to a proactive network which includes Percy military hospital – the military trauma center of the Paris area – "Service de Protection Radiologique des Armées", which handles accidents in the Forces, and the centre de transfusion sanguine des armées, which has a special expertise in cell therapy.

2020 has been especially challenging for DEBR due to the COVID-19 crisis which significantly hampered international exchanges (IMRIS symposium 2020 cancelled). Radiobiologists made a contribution to hospital support and gave advice when required proposing new therapeutic approaches.

DEBR main research topics are:

- Biodosimetry studied from a multiparametric approach.
- New therapeutic approaches of ARS which focus on hematopoiesis and CRS.
- Radiotoxicology represents a new research area in collaboration with CEA.
- Biological effects of EMF focusing on non-thermal effects.

PhD candidates can graduate at DEBR/IRBA under the umbrella of Paris Diderot University

Finally, the radiobiology teaching is mainly delivered in Val de Grace School and DEBR was very proud to host in 2019 the first StTARS symposium.

1.3 GERMANY NATIONAL STATUS REPORT 2017 – 2021, M. PORT, BIR

The staff of the Bundeswehr Institute of Radiobiology (BIR) has not changed in recent years. It comprises a total of 48 experts of whom one third are scientists (n = 16) and twice as many technicians (n = 32).

BIR is one of three Institutions (including Technical University Munich as well as Helmholtz Zentrum) involved in a Master's in Radiobiology. The number of students who are doing a practical (2 – 6 weeks), a master's degree or a PhD is about 8 – 12 for an internship, 2 – 4 for a master's degree and 1 – 2 for a PhD per year.

BIR comprises laboratories with a methodological focus as outlined below (Figure 1-1). Assays established in the laboratories are used to augment the mobile Task Force (TF) and at the same time represent the reach-back TF capability of the Institute. These laboratories require support regarding e.g., occupational safety, IT, and others (Figure 1-1). In order to guarantee a high qualitative standard, the whole Institute has been certified according to the DIN EN ISO 9001 since 2008. Standard operating protocols and many other documents were developed and become updated annually in order to reflect the current flow of developing technologies, experiences and knowledge.

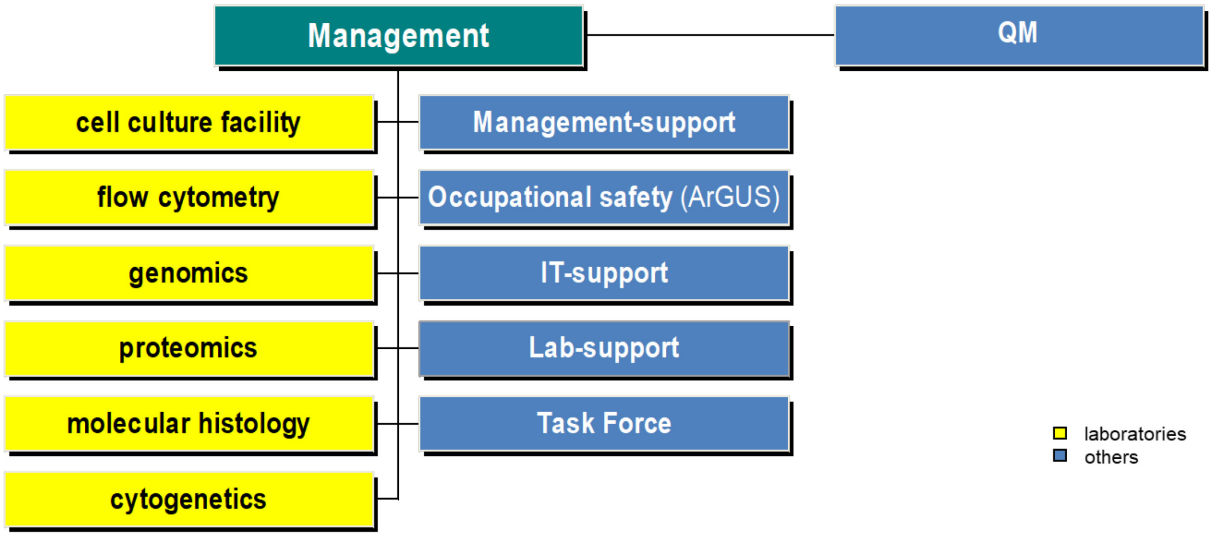


Figure 1-1: Structure of the Bundeswehr Institute of Radiobiology.

BIR as a scientific and military Institution has to both reflect scientific freedom and follow restrictions caused by security reasons. For instance, BIR shares a centralized IT-structure with all other military Institutions. Hence, BIR received standard office computers and software, however, no designated procedure to establish and sustain research specific IT-infrastructure exists. Also, BIR has restricted access to the central file service. Therefore, a separate network and file service for research data has to be maintained. Also, official mobiles with difficult to access email accounts exist. Due to COVID-19, access to online seminars and organizing online workshops has been required but has been challenging to realize within the structures of a military Institution. Finally, due to high security standards, data exchange with national and international cooperation partners was and is problematic. Improving this situation is a demanding task of BIR's directorate.

Laboratories' primary products per year will not be discussed here. However, the overall goals of BIR are:

1) Further improvement of acute (late) health effect diagnosis with emphasis on high-throughput and point of care diagnostics as a main column of BIRs current portfolio.

2) Extending BIRs portfolio into the field of therapeutic strategies, in particular for acute health effects. Here, collaborations with other Institutions and participation in ongoing and established animal models is required.

3) Further tasks include:

   a) Deeper insight into electromagnetic field exposure and identification of EMF-induced biological effects using e.g., a "Mode-Stirred Chamber";

   b) Continuing the co-organizer/teaching of intern. Master's degree course "Radiobiology";

   c) Improving the interface with military/civilian clinics via joint exercises simulating radiological scenarios; and

   d) Stimulating NATO and the German Military regarding the storage of radionuclide decorporation drugs to be prepared for a radiological event.

1.4 GREAT BRITAIN NATIONAL STATUS REPORT (TWO INSTITUTIONS) 2017 – 2021, C. FOSTER, C. BADIE

C. Foster, NAVYINM

In the UK Armed Forces, the Royal Navy is the lead Service for the provision of Radiation Medicine.

The Institute of Naval Medicine, based in Gosport in Hampshire, is the Royal Navy's center of excellence for occupational and environmental health. It brings together scientists and medical professionals to promote health and safety and maximize the operational effectiveness of military personnel. Radiation Medicine expertise sits within the Underwater Medicine Division which also supports submarine and diving operations. A team of six clinicians, primarily occupational physicians, deliver expertise in these areas supported by a small number of administrative staff. In Radiation Medicine the prime outputs are around the clock on call service to provide advice on the management of nuclear or radiological incidents or emergencies, medical policy advice, and training of wider Defence Medical Services personnel in recognizing and managing the effect of exposure to ionizing or non-ionizing radiations.

The work of the Division primarily involves the application of knowledge into military scenarios. To this end, the division relies on collaboration with, and contributions from, many other organizations such as the Defence Science and Technology Laboratory, Public Health England, and NATO partners.

C. Badie, PHE

Public Health England (PHE) was established on 1 April 2013 to bring together public health specialists from more than 70 organizations into a single public health service. Its mission is to protect and improve the nation's health and to address inequalities, working with national and local government, the NHS, industry, academia, the public and the voluntary and community sector. The Cancer Mechanisms and Biomarkers group has 6 members of staff + students is part of the Radiation effects department strong of 31 members of staff and is localized at the Centre for Radiation, Chemical and Environmental Hazards (CRCE) on the Harwell campus near Oxford.

The Cancer Mechanisms and Biomarkers group studies the fundamental mechanisms by which ionizing radiation causes cancer, conducting research on chromosomal and molecular mechanisms that underlie radiation-induced leukemia initiation and development and cell of origin using experimental mouse models. Moreover, the group aims to identify, develop, and validate biological markers of radiation exposure, radiation-induced cancer, and cancer risk assessment; identifying bio-indicators/biomarkers of long-term risks aiming at identifying and characterizing genes that influence individual susceptibility to radiation-induced cancer. Importantly, the group undertakes research to identify genes responsive to radiation exposure at the transcriptional level.

In terms of equipment the group owns three Tissue Culture laboratory Flow cytometers, a cell sorter (4 lasers) and immunohistochemistry, microscopy, biochemistry equipment (including Western blotting, magnetic beads sorting (RoboSep)), animal facilities, radiation exposure systems (X-rays, Gamma-rays for low dose-rates). For molecular biology endpoints, Microarrays, CGH, PCRs, MQRT-PCR. More specifically:

1) A molecular counting analysis system (NanoString® Technologies nCounter®) in which color-coded barcodes represent single target molecules -Barcoded probes which hybridize directly to a target molecule in solution like mRNAs (Reporter Probe carries the signal and the Capture Probe allows the complex to be immobilized for data collection). There is no enzymatic reaction required and each probe is individually counted without the need for amplification. It provides very sensitive digital data; and
2) a Digital PCR, Third' generation of PCR which create at least 10 million droplets allowing to perform absolute quantification without standard curve. The analysis can use at least 2 fluorescent dyes and a detection range of at least 5 orders of magnitude allowing the detection of very rare events (1/100000).

1.5 ITALY NATIONAL STATUS REPORT 2017 – 2021, V. FRANCHINI

The Scientific Department of Army Medical Center in Rome performs studies and research for the health activities useful for every military operational need, especially in case of health emergencies. Other main issues concern toxicology and food/water hygiene for the military community and the management of assistance and medical rehabilitation of Italian veterans.

This Department consists of three branches and each branch has three sections.

We carry out our activities in the first branch dedicated to studies, research, and advanced training in the field of *Biomedicine* and *Biotechnology for CBRN defence* collaborating with several National and International Institutes and Universities.

In particular the two main topics of this branch are:

1) Biological warfare agents and unusual pathogens (bacteria and viruses);
2) Genotoxicity and biodosimetry.

The personnel of the section that deals with genotoxicity/biodosimetry is composed of one military officer, 4 civilian biologists and a civilian lab technician. This research group studies biological and clinical effects of ionizing radiation and is involved in the NATO Panel HFM-291. The other research field of this group concerns the genotoxic and cytotoxic effects of non-ionizing radiation. These researchers are also members in the NATO Panel HFM-298.

Due to the pandemic, all the usual activities of the first branch have been considerably reduced since February 2020, and all the lab personnel has been involved in the research and diagnostics of SARS-CoV-2 supporting the virology laboratory.

The Scientific Department of the Army Medical Center is on the list of the Reference Laboratories by Italian Ministry of Health with analyses of 800 – 1500 swabs per day. In addition, recently, hundreds of samples from positive people have been sequenced using Next Generation Sequencing, in order to classify and identify the SARS-CoV-2 variants.

In this scenario, several people (more than 60) of different military forces attended our laboratories to learn methods and protocols with the aim of establishing SARS-CoV-2 satellite laboratories in every Italian region.

1.6 NETHERLANDS NATIONAL STATUS REPORT 2017 – 2021, T. KUIPERS, MOD

The report is divided into three sections related to programs, research and capabilities as follows.

1.6.1 Programs

1.6.1.1 V1802 CBRN Research

The main focus of this research program is on chemical and biological hazards. The research program consciously excludes radiological and nuclear related research since the national availability of possible research connections seems to be lacking. The need for radiological and nuclear related research is diverted to international cooperation like SMART CBRN and NATO HFM.

1.6.1.2 SMART CBRN

The Strategic Mutual Assistance in R&T (SMART) initiative to collaborate on topics like CBRN with Norway has gradually left the pilot phase and is reaching a quid pro quo status. Via this route the C and B research results can be exchanged with R and N research results.

1.6.1.3 Future V2207 CBRN Research

More and more the need for national radiological and nuclear related research is heartfelt within the MoD of The Netherlands and the search for (inter)national research institutes on R and N has started.

1.6.2 Research

1.6.2.1 Cosmic Radiation Exposure of Dutch Military Aviation Personnel

In the context of new radiological protection legislation inspired by ICRP Publication 132 “Radiological Protection from Cosmic Radiation in Aviation” the need arose for the Dutch Ministry of Defence to review the cosmic radiation dose received by military aviation personnel.

According to new legislation (Bbs 2018) the annual cosmic radiation dose of military aviation personnel working above sea level has to be monitored, where in former legislation the dose levels below 8 kilometers above sea level were exempted (Bs 2012).

The need for compliancy with new legislation made it necessary to review all flight movements and perform a dose estimate for all military aviation personnel within the Dutch Ministry of Defence. In addition, the target groups that are legally eligible for special attention and information provision were updated.

The Royal Netherlands Air Force (RNLAF) provided data of flight movements (duration and altitude) that was processed by the CARI-6M computer program by the Defence Radiation Protection Unit (SBD).

Calculations show that for a small part of the military aviation personnel, the annual dose level does exceed the annual 1 mSv dose limit. These individuals receive special attention in the form of personal dose registration. Other groups are exempted but all are eligible to cosmic radiation information in their curriculum amongst other employer obligations.

1.6.2.2 HAWK-Exposures

Several scientific reports have been reviewed in the context of radiation exposure (non-ionizing and ionizing radiation) from the former used HAWK air defence system. The results have been used in court.

1.6.2.3 Future Research Possibilities

The covenant between the Dutch MoD and National Institute for Public Health and the Environment (RIVM) show possibilities to connect to scientific research on low dose radiation effects but needs to be explored.

1.6.3 Capabilities

- Operational CBRN Training Centre in Vughtfor CBRN training of military CBRN Response team, national police, civil medical personnel, and civil fire brigade.
- Fully equipped fieldable mobile radiation measurement laboratories in line with civil capabilities.
- ISO-17025 certified radiation detection equipment calibration lab.

1.7 POLAND NATIONAL STATUS REPORT 2017 – 2021, E.M. NOWOSIELSKA, M.K. JANIAK

The staff of the Department of Radiobiology and Radiation Protection, Military Institute of Hygiene and Epidemiology, currently consists of six persons. Until his retirment on August 31, 2020, the Department was led by Prof. Marek K. Janiak, and on September 1, 2020, Prof. Paweł Szymański was appointed head of the DRRP.

From 2018 to 2021 the following studies were carried out at the DRRP:

- "Effect of internal contamination with tritiated water on the innate anti-tumor and anti-inflammatory reactions in radioresistant and radiosensitive mice", granted by the Polish National Research Center. The aim of the project was to evaluate whether internal deposition of HTO in mice from strains of different radiosensitivities and immune phenotypes can affect the immune system and hence the development of pulmonary tumor colonies and whether such effects can be linked to alterations in the functions of NK lymphocytes and Mφ, production of pro- and anti-inflammatory cytokines, and/or the structure of the haematopoietic system. The results of this study were published in 2018 (Nowosielska, E.M., Cheda, A., Zdanowski, R., Lewicki, S., Scott, B.R., Janiak, M.K. Effect of internal contamination with tritiated water on the neoplastic colonies in the lungs, innate anti-tumour reactions, cytokine profile, and haematopoietic system in radioresistant and radiosensitive mice. Radiat Environ Biophys 2018, 57:251-264. doi: 10.1007/s00411-018-0739-4);
- "Nicotinic acid derivatives as potential radio-protective and/or radio-remedial agents", granted by the Polish National Research Center. The aim of the project was to investigate the potential radio-protective and/or radio-remedial effects of the selected nicotinic acid derivatives exhibiting anti-thrombotic and anti-inflammatory properties. The results of this study were published in 2021 (Cheda, A., Nowosielska, E.M., Gebicki, J., Marcinek, A., Chlopicki, S., Janiak, M.K. A derivative of vitamin B3 applied several days after exposure reduces lethality of severely irradiated mice. Sci Reports 2021, 11: 7922. doi: 10.1038/s41598-021-86870-3);
- "Preclinical evaluation of whole-body irradiations with low doses of X-rays combined with inhibition of immune checkpoints and a heat shock protein as a novel therapy for lung cancer", granted by the Polish Ministry of Defence. In this study we aimed to evaluate the therapeutic efficacy of whole-body exposures at low-level ionizing radiation used along with the blockade of various pro-neoplastic mechanisms with the view of providing preclinical grounds for innovative therapy of lung cancer. The results of this study were published in 2021 (Nowosielska, E.M., Cheda, A, Pociegiel, M., Cheda, L., Szymański, P., Wiedlocha, A. Effects of a unique combination of the whole-body low dose radiotherapy with inactivation of two immune checkpoints and/or a heat shock protein on the transplantable lung cancer in mice. Int J Mol Sci 2021, 22:12, 6309. doi: 10.3390/ijms22126309);

- "Effects of low doses of low-LET radiation on impaired vascular endothelium", granted by the EU Network of Excellence DoReMi. The aim of the project was to evaluate the effects of low doses of X-rays on the structure and function of vascular endothelium in diabetic (db/db) mice before and during the development of hyperglycaemia-induced vascular complications in *diabetes mellitus*. The rational for this study was based on the presumption that endothelial disfuction in diabetes may be prevented or mitigated by exposures at low doses of low-LET radiation. The manuscript of the results of this study is under preparation.

Other tasks of the DRRP include participation in oversight of the radiological protection rules in the MoD units that are using sources of Ionizing Radiation (IR) by the on-site visits, reviewing of applications submitted to the Military Units of Preventive Medicine for permission to use IR sources, and assessment of the permanent shields around new x-ray generators. Additional tasks include elaboration of model documents and procedures for units that use IR sources, as well as education and training of medical and other personnel in the effects of IR and radiation protection.

Future research activities of the DRRP include:

- Investigation of the biological activity and multi-organ biodistribution of new potential radiopharmaceuticals;
- Further studies of biomedical effects of ionizing radiation with special emphasis on the effects of whole-body exposures of mice at different irradiation doses and schemes;
- Development of innovative therapeutic strategies using a combination of immunotherapy and different irradiation schemes;
- Investigation of new radio-protective and radio-remedial agents for the prevention and treatment of diseases induced by the absorption of high doses of ionising radiation; and
- Evaluation of the usability of radiomarkers for studies of oxidative stress, cell survival (cytotoxicity), neuroprotection, the blood-brain barrier permeability for biologically active substances, and the central nervous system blocking power of neurotoxic substances acting via the cholinergic system.

Moreover, there are plans to set up in Poland the National Center for Radiobiology and Biological Dosimetry (RADBIODOZ) which, as a unique entity in Poland, will use various cytogenetic and molecular methods to estimate doses of IR absorbed during radiation accidents. The Center will also pull the largest group of radiobiologists in Poland to study the effects of low dose radiation, DNA repair, abscopal effects, new techniques of biological dosimetry, and toxicity of radiopharmaceuticals. Such a team is crucial for the development and sustainment of competence in radiobiological research necessary for pursuance of the nuclear power programme that has recently been started in Poland.

1.8 UNITED STATES OF AMERICA NATIONAL STATUS REPORT 2017 – 2021, A. MILLER, B.J. BENE, W.F. BLAKELY, J.G. KIANG, L. SENCHAK, V.K. SINGH

As the United States' only military radiobiology laboratory, AFRRI continues to fulfill unique responsibilities to advance the science and technology and support readiness for radiological and nuclear operations. AFRRI is a joint entity of the U.S. military departments charged with executing the U.S. Department of Defense (DOD)'s Medical Radiological Defense Research Program. Its civilian and active-duty military personnel conduct exploratory and developmental research to address core areas of study that include prevention, assessment, and treatment of radiological injuries. AFRRI collaborates with other government facilities, academic institutions, civilian laboratories, and other countries, including our NATO colleagues to research the biological effects of ionizing radiation.

There are currently five major research areas at AFRRI that encompass the prevention, assessment, and treatment of injuries resulting from the effects of ionizing radiation:

1) Low Dose Radiation Doses and Rates,
2) Radiation Countermeasures,
3) Internal Contamination/Toxic Metals,
4) Radiation Combined Injury, and
5) Biological Dosimetry.

These lines of research are bolstered by an educational component to broadcast and increase awareness of the medical effects of ionizing radiation, in particular mass casualty after nuclear detonation or radiological accidents. AFRRI as a part of the U.S.'s only military medical school, Uniformed Services University of Health Sciences (USUHS), which is in the final stages of graduating its first radiobiology-trained military PhD scientist through the Molecular and Cellular Biology doctoral program at USUHS. AFRRI's five radiation research programs have remained extremely active over the last four years producing more than 55 peer-reviewed publications in journals including *Radiation Research, Military Medicine, PlosOne, Cell Biosciences*, and many others.

1.8.1 Low Dose/Chronic Radiation Program

During this RTG291 cycle AFRRI has developed a chronic low dose radiation program. AFRRI is currently conducting a number of studies examining potential adverse health effects of prompt low or chronic low doses of ionizing radiation; however low dose exposure has not historically been one of the Institute's defined research areas until recently. This new program is a welcome addition to a strong high-dose radiation project portfolio. A low dose or chronic radiation research focus is very necessary because during Operation Tomodachi, USPACOM took a conservative approach to ALARA, limiting individual cumulative radiation exposure to the equivalent of 3 mGy (Miller et al. 2018). Radiation avoidance measures necessary to meet this target included mission-impacting constraints such as deferred maintenance to reduce crew exposures and increased ship standoff distances. After-action analyses identified lack of consistent guidelines to translate detectable radionuclide levels to protective actions, and widespread lack of preparation to implement ALARA decision-making within an evolving emergency (Miller et al. 2018). An understanding of chronic low dose exposure is even more critical when considering that military operations following a nuclear event will need to occur in a chronic or low dose nuclear environment. Currently AFRRI is increasing its research staff, enhancing its infrastructure, and focusing new methodologies to investigate whether low dose prompt/intermittent or chronic radiation can induce cardiovascular, neurological, or hematological organ damage. These adverse health effects could result in unexplained hypertension or early organ failure, or significant changes in anxiety and personality that could affect combat operations. Neurological studies examining low dose effects on neurological tissues are underway to assess whether those effects that could lead to behavior modification. Military resilience could be affected and could potentially impact combat operations particularly under the combined stress of battlefield operations in a nuclear environment. A multi-organ system approach is being established and will be able to address relevant questions regarding military operations in a chronic low dose radiation Fallout-like environment.

1.8.2 Radiation Countermeasures

Currently, three white blood cell growth factors (Neupogen, Neulasta and Leukine) and 1 platelet growth factor (Nplate) have been approved by the U.S. Food and Drug Administration (FDA) for mitigating hematopoietic acute radiation syndrome (H-ARS). However, these growth factors may be associated with side effects such as bone pain and their survival efficacy only observed by giving the drug within 24 h post-irradiation in irradiated mice and nonhuman primates. The efficacy is limited (Kiang and Olabisi, 2019). The chance of receiving treatment after injury-depends on availability of the medical support especially during military action, most

likely beyond 24 h post radiation exposure. Thus, new medical countermeasures with high efficacy and wider therapeutic window beyond 24-h after radiation exposure are required to mitigate/treat the ARS. AFRRI was the first research center to demonstrate that a proinflammatory factor IL-18 plays a key role in radiation-induced cell and tissue damage and dysfunction (Xiao, 2016; Kiang et al. 2020); IL-18 binding protein (IL-18BP) as a natural antagonist of IL-18 countered IL-18 activation and therefore mitigated radiation-induced hematopoietic, gastrointestinal and cardiovascular multiple-organ injuries and increased animal survival with a wider therapeutic window from 24 h and beyond after lethal doses of total-body radiation exposure (Li et al. 2020). Treatment with bone marrow transplant, Gentamicin, or WR-151327 shows highly significant improvement on survival after irradiation as well (Ledney and Elliott, 2010). It is reported that amifostine (Srinivasan et al. 1997), GT3 (Kumar et al. 2020), 5-AED (Whitnall et al. 2002) administered prior to irradiation are effective to protect mice from mortality. Because the survival efficacy of Neupogen and Neulasta is limited, AFRRI has been initiating polypharmacy approaches. Ghrelin is a 28 amino acid peptide produced and released by stomach during hunger. Co-therapy of Ghrelin along with Neulasta significantly enhances Neulasta's efficacy to improve the survival (Kiang et al. 2021) and brain hemorrhage (Kiang et al. 2019) after lethal radiation exposure. Similar enhancement is also evident with combinational therapy of Ciprofloxacin and Neulasta (Kiang et al. unpublished data).

AFRRI collaborated with private companies to develop a thrombopoietin mimetic, pegylated IL-11, AUR1402, and a placenta-derived stromal cell product. Both compounds are being investigated with different strains of mice after irradiation (Ghosh et al., Kumar et al. 2020). The results are unpublished but could have an impact on development of new partial body countermeasures.

AFRRI has made significant strides establishing a partial body radiation model. Partial body radiation on lungs or GI has been explored (Ghosh et al., Kumar et al. 2020) and results demonstrated that the partial body irradiation model was achieved by sparing 2.5% bone marrow by avoiding radiation exposure to tibia, foot, and tail. Further studies are ongoing as the model is amenable to studying long-term radiation effects.

1.8.3 Radiation Combined Injury

Approximately 65% of injuries suffered from nuclear explosions combine radiation injury with other forms of injury, such as burn, wound, hemorrhage, blast, trauma, and/or sepsis, which greatly increases the risk of morbidity and mortality when compared to that of radiation injury alone. To date, no U.S. FDA-approved countermeasures are available specifically treating Radiation Combined Injury (RCI) (Kiang and Olabisi, 2019).

AFRRI has established experimental mouse models with radiation followed by penetrating wound, burned wound, hemorrhage, and bacterial infection (Kiang and Olabisi, 2019). The underlying mechanisms involve multiple levels. Due to the mechanistic complexity, the drugs or remedies are yet to be fully defined. In the past 15 years, many attempts have been made. Treatment with Alx4100TPO (a TPO receptor agonist) (Kiang et al. 2017a), bone marrow transplant (Ledney and Elliott, 2010), mesenchymal stem cells (Kiang and Gorbunov, 2014a), Ghrelin (Kiang et al. 2014b, 2018, 2020), Ciprofloxacin (Fukumoto et al. 2014), WR-151327 and Silvadene (Ledney and Elliott, 2010) resulted in increases in survival after radiation combined injury. The underlying mechanisms involves the inhibition of miR-34a and cytokines in specific tissues and circulation, several signaling pathways, and apoptosis.

Combinational therapies of 5-TDCM plus Gentamicin (Ledney and Elliott, 2010) Neulasta plus Alxn4100TPO (Kiang et al. 2017b), or Neulasta plus citrulline (Wang et al. 2021) are also effective for enhancing the survival after irradiation combined injury, likely by enhancing survival of the hematopoietic stem/progenitor cells, GI repair, or accelerating recovery of cutaneous wounds. The mechanism of this enhanced survival benefit is unknown and is currently under investigation.

AFRRI research has also made a contribution to the recent investigations regarding the human virus SARS-CoV-2, COVID-19. AFRRI radiation mitigation studies with ketone ester have indicated that a ketone-based metabolic therapy may not only prevent oxidative damage and radiation-induced cytokine alterations (Miller, unpublished) but could be used as a treatment to blunt the cytokine storm associated with COVID-19 (Bradshaw et al, 2020). AFRRI is not known for its infectious disease research but this finding regarding the ketone ester may have relevance to other fields including emerging infectious diseases that result in a cytokine storm.

1.8.4 Internal Contamination/Depleted Uranium/Heavy Metals

A multi-institutional study assessing the health effects of embedded metal fragments and blast injury is continuing with our colleagues at the U.S. Department of Veterans' Affairs (DVA), U.S. Food and Drug Administration (FDA), and University of Kentucky. AFRRI's role in the project is to assess the short- and long-term health effects of embedded military-relevant metals, including depleted uranium. Metals were selected from the U.S. DOD and U.S. Department of Veterans Affairs (DVA) list of "metals of concern." In conjunction with the U.S. DVA, AFRRI is proposing a battery of urinary biomarker assays to potentially detect early changes in metal excretion and renal damage that may affect the fragment removal policy for particular metals.

A preliminary investigation on the use of molecularly imprinted polymers and dendrimeric complexes for internal radionuclide decorporation has been initiated. The results are not completed yet but also foreshadow new research directions potentially conducted at AFRRI.

Depleted uranium (DU), the radioactive heavy metal used primarily in military applications, has been shown to be both neoplastically transforming and genotoxic. In vivo studies have also demonstrated that DU is leukemogenic and genotoxic. While DU possesses both a radiological (alpha particle) and chemical (metal) component it is generally considered a chemical biohazard. However recent studies have shown that alpha particle radiation does play a role in DU's toxic effects and that radiation "bystander effects" are also part of DU's radioactive activity damaging nearby cells not exposed to DU (Miller et al. 2017). This novel effect of DU exposure could have implications for radiation risk and for health risk assessment associated with DU exposure.

1.8.5 Biodosimetry

AFRRI's Biodosimetry program was conceived to transition research efforts to address critical gaps in the U.S. DoD's medical readiness to respond to radiological threats. AFRRI staff members have sustained research efforts involving several components of multiple parameter biodosimetry. These efforts contribute for both field- as well as reach-back reference laboratories biodosimetry applications. The initial approach was to establish a reference cytogenetic biodosimetry laboratory to support radiation dose assessment using reach-back analysis capabilities. The program was soon expanded to include the development of diagnostic approaches to support applications in far-forward environments. Use of multiple parameters-based biodosimetry is justified due to the need to fill critical gaps in the performance criteria for both far-forward and reach-back applications. The multi-parameter approach continues to involve cytogenetic, proteomic, and gene expression methodologies (1). Specific details of this multiparametric effort are detailed below.

Cytogenetics. Research efforts involved optimization and validation studies using a variety of dose assessment — cytogenetic assays:

1) Dicentric Chromosome Aberration (DCA) (Subramanian et al. 2020, Gregorie et al. 2021),
2) Cytokinesis-Blocked Micronucleus Assay (CBMN) (Goh et al. 2021),
3) Fluorescence in situ hybridization translocation assay (Goh et al. 2019), and

4) Premature Chromosome Condensation (PCC) assay (Sebastian et al. in review; Blakely and Bolduc, 2019).

In addition, we sustained our participation in exercises to demonstrate AFRRI's cytogenetic biodosimetry laboratory competency (Gregorie et al. 2021).

Hematology: The use of hematology data from large-animal radiation models and human radiation accident database were used to characterize radiation responses and develop predictive algorithms to assess risk for hematological acute radiation syndrome severity (H-ARS). These studies used:

1) Baboon (Blakely et al. 2018; Bolduc et al. 2019),
2) Rhesus monkeys (King et al. 2018), and
3) Human radiation accident databases (Blakely and Bolduc, 2020).

Clinical Signs and Symptoms: In collaboration with our NATO colleagues and in partnership with AFRRI's Military Medicine Operations (MMO) Department AFRRI staff participated in the 2017 NATO biodosimetry exercise using the suite of software tools (i.e., Biodosimetry Assessment Tool or BAT, mobile First-responder Assessment Triage or mFRAT, and H-module) (Dörr et al. 2017).

Proteomics: The use of proteomics for biodosimetry is gaining acceptance in the biodosimetry community. We reported on the use of early-phase proteomic biomarkers for assessment of H-ARS severity using a radiation baboon model (Blakely et al. 2018). In addition, one of our patents reporting on use of panel of biomarkers for radiation dose assessment was recently issued (Blakely et al. 2020). Radiation Risks. An ongoing effort, in partnership with AFRRI's MMO Departments, involves the development of a "NATO Radiation Risk Communication Card (RRCC)," representing USA's contribution to one of the focus areas of the current RTG (Blakely et al. in preparation).

Integration Algorithms: The integration of multiple parameter biodosimetry concept dictates the need to produce predictive algorithms that provides first-responders with the utility of clinician decision tools. AFRRI's staff in Biodosimetry are incorporating predictive algorithms in the majority of the biodosimetry components illustrated above (Blakely et al. 2018; Blakely and Bolduc, 2020; Blakely et al. 2020).

Networks: Response to surge requests for dose assessment by cytogenetics in the case of a radiological-mass casualty incident relies on the ability to cooperate with partners in networks. AFRRI sustained its efforts in this area by contributing to the development of concepts of operations for a United States dosimetry and biodosimetry network (Dainiak et al. 2019).

1.8.6 Military Medical Operations

The Military Medical Operations (MMO) department continues to be the home of AFRRI's Medical Radiobiology Advisory Teams (MRAT) which provide health physics and medical subject matter expertise to Combatant Commanders, DoD agencies, allied forces, federal agencies, state, and local governments dealing with accidents and incidents involving nuclear weapons, nuclear reactors, radiological dispersal devices, and industrial and/or medical sources. MRAT's support spans across radiation protection, incident modelling for plume prediction and health physics interpretation of the results, personnel dose estimation, detection of ionizing radiation and radionuclide identification, contamination control, decontamination, medical countermeasures, diagnosis and treatment of acute radiation syndrome and internal contamination and radiation risk communication. AFRRI currently maintains three MRAT teams that are deployable on short notice and provide reach-back capability calling on the skills of radiobiologists, biodosimetrists and other research professionals at AFRRI as well as access to advanced biodosimetry techniques. Most recently MRAT supported Joint Task Force – Civil Support during the Vibrant Response Lite 20 and Sudden Response 21 exercises as part of the J34 CBRN Force Protection Cell.

MMO also provides an educational and training course developed in response to the development of nuclear weapons post WWII and the uncontrolled proliferation of nuclear material and technology. The Medical Effects of Ionizing Radiation (MEIR) was initially developed 45 years ago and is continuously revised to provide relevant up-to-date information on a variety of topics including radiological and nuclear threats, radiation physics, physical- and biodosimetry, nuclear weapons effects, RADIAC equipment, effects of ionizing radiation on biological systems, signs and symptoms of acute radiation syndrome, late effects, management of casualties, internal contamination, radiation accidents, psychological factors and incident response. The training culminates in a discussion based workshop on a simulated radiological or nuclear scenario. The course is delivered by health physicists and radiation trained physicians over 3 or 4 days based on audience and location and is officially approved to provide Continuing Medical Education (CME) and Continuing Nursing Education (CNE) credits. The course is designed to educate providers, nurses, medical planners, CBRN emergency response personnel, Civil Support Teams and decon team members.

1.8.7 Acknowledgements, Disclosure and Disclaimer

The authors acknowledge and thank the co-contributors and collaborators to the AFRRI's scientists in this effort. The authors want to particularly recognize the support of COL Mohammed Naeem, AFRRI Director; Dr Bruce Doll, USUHS, Assistant Vice-President for Technology, Research, Innovation and AFRRI Scientific Director; and LT Elih Velazquez, Head, AFRRI Science Research Department (SRD). A special thanks to former AFRRI Director CAPT Danielle Wooten and former SRD Head, LTC Mitchell Woodberry for their recognition and support of the importance of USUHS/AFRRI involvement in this NATO RTG. The assistance of CPT Gavriella Simantov has been critical to the success of this endeavor. The views expressed are those of the authors and do not necessarily reflect the official policy or position of the AFRRI, USUHS, DoD, nor the U.S. Government. Support for this research is from AFRRI project numbers RBB43523, RBB44313, AFR-B4-10971, RAB33529/34363/310934, NIAID AI080553. NIAID-AFRRI IAA, and JPC-7.

1.8.8 References

Blakely, W.F. et al. (2018). Use of proteomic and hematology biomarkers for prediction of hematopoietic acute radiation syndrome severity in baboon radiation models. Health Physics, Jul 115(1): 29-36.

Blakely, W.F., Bolduc, D.L. (2019). Methodology for assessment of the fraction and dose of the body exposed to life-threatening ionizing radiation exposure. Invention disclosure, 17 Sept 2019.

Blakely, W.F., Bolduc, D.L. et al. (2020). Radiation injury index algorithm based on hematology changes for rapid early-phase radiological triage applications. U.S. Provisional Patent Application No. 62/867,471, filed on 27 June 2019; U.S. patent filed (HJF 534-19 US) on 26 June 2020.

Bolduc, D.L., Blakely, W.F., (2019). Baboon radiation quality (mixed field neutron and gamma, gamma alone) dose -response model systems: Assessment of H-ARS severity using hematologic biomarkers. Radiat Prot Dosimetry, Dec 31, 186(1): 15-23.

Blakely, W.F., Glezer, E.N., Kenten, J., Kumar, S., Matthew, A., Ossetrova, N.I., Sigal, G. (2020). Biodosimetry Panels and Methods. U.S. Patent No 2000011866, published 9 January 2020.

Blakely, W.F. Bolduc, D.L., Clasp, T., Barrera, C., Senchak, L. NATO HMF-291 RTG Radiation Risk Communication Card. Military Medicine (USUHS cleared for submission 2021).

Bradshaw, P., Seeds, J., Miller, A.C., Mahajan V, Curtis W. (2020). COVID-19: Proposing a ketone-based metabolic therapy as a treatment to blunt the cytokine storm, Oxidative Medicine Cellular Longevity, Sep 9:6401341, doi: 10.1155/2020/6401341.

Dainiak, N., Albanese, J., Kaushik, M., Balajee, A.S., Romanyukha, A., Sharp, T.J., and Blakely, W.F. (2019). Concepts of operations for a US Dosimetry and Biodosimetry Network. Radiat Prot Dosimetry, Doi:10.1093/rpd/ncy294.

Dörr, H., Abend, M., Blakely, W.F., Bolduc, D.L., Boozer, D., Costeira, T., Dant, T., De Amicis, A., De Sanctis, S., Dondey, M., Drouet, M., Entine, F., Francois, S., Gagna, G., Guitard, N., Hérodin, F., Hoefer, M., Lamkowski, A., La Sala, G., Lista, F., Loiacono, P., Majewski, M., Martigne, P., Métivier, D., Michel, X., Pateux, J., Pejchal, J., Reeves, G., Riccobono, D., Sinkorova, Z., Soyez, L., Stricklin, D., Tichý, A., Valente, M., Woodruff, C.R. Jr, Zarybnicka, L., Port, M. (2017). Using clinical signs and symptoms for medical management of radiation casualties – 2015 NATO exercise. Radiat Res, Mar 187(3):273-286. Doi: 10.1667/RR14619.1. Epub 2017 Feb 20.

Gregoire, E., Barquinero, J.F., Gruel, G. Benadjaoud, M., Ainsbury, E., Beinke, C., Balajee, A., Beukes, P., Blakely, W.F., Dominguez, I., Duy, P.N., Flegal, F.N., Gil, O.M., Güçlü, I., Guogyte, K., Hadjidekova, V., Hadjidekova, S.P., Hande, P., Jang, A., Lumniczky, K., Martinez, J.G., Meschini, R., Milic, M., Montoro, A., Moreno, M., Oestreicher, U., Pajic, J., Ricoul, M., Sabatier, L., Sebastia, N., Sommer, S., Szkarlat, Z., Testa, A., Terzoudi, G., Valente, M., Venkatachalam, P., Vral, A., Wilkins, R.C., Wojcik, A., Zafiropoulos, D., Kulka, U. (2021). RENEB Inter-Laboratory Comparison 2017: Limits and pitfalls of ILCs. Int J Radiat Biol Published on-line 25 May 2021.

Goh, V.S.T., Fujishima, Y., Abe, Y., Sakai, A., Yoshida, M.A., Ariyoshi, K., Kasai, K, Wilkins, R.C., Blakely, W.F., Miura, T. (2019). Construction of fluorescence in situ hybridization (FISH) translocation dose-response calibration curve with multiple donor data sets using R, based on ISO 20046:2019 recommendations. Int J Radiat Biol, Dec; 95(12):1668-1684.

Goh, V.S.T., Nakayama, R., Blakely, W.F., Abe, Y., Chua, C.E.L., Nakata, A., Fujishima, Y., Yoshida, M.A., Kasai, K., Ariyoshi, K., Miura, T. (2021). Improved harvest and fixation methodology for isolated human peripheral blood mononuclear cells in cytokinesis-block micronucleus assay. Int J Radiat Biol 97 (2): 197-207, 2021.

Kiang, J.G., Anderson, M.N., Smith, J.T. (2018). Ghrelin therapy sustains granulocyte colony-stimulating factor and keratinocyte factor to mitigate hematopoietic syndrome and spleen after whole-body ionizing irradiation combined with wound. Cell Biosci, 8:27.

Kiang, J.G., Gorbunov, N.V. (2014a). Bone marrow mesenchymal stem cells increases survival after ionizing irradiation combined with wound trauma: Characterization and therapy. J Cell Sci Ther, 5:190.

Kiang, J.G., Olabisi, A.O. (2019). Radiation: A poly-traumatic hit leading to multi-organ death. Cell Biosci, 9:25.

Kiang JG, Smith JT, Anderson MN, Umali MV, Ho C, Zhai M, Lin B, Jiang S. (2019). A novel therapy, using Ghrelin with pegylated G-CSF, inhibits brain hemorrhage from ionizing radiation or combined radiation injury. Pharm Pharmacol Int J, 7(3):133-145.

Kiang, J.G., Smith, J.T., Cannon, G., Anderson, M.N., Ho, C., Zhai, M., Cui, W., Xiao, M. (2020). Ghrelin, a novel therapy, corrects cytokine and NF-κB-AKT-MAPK network and mitigates intestinal injury induced by combined radiation and skin-wound trauma. Cell Biosci, 10:63.

Kiang, J.G., Zhai, M., Lin, B., Smith, J.T., Anderson, M.N., Jiang, S. (2021). Co-therapy of Pegylated-G-CSF and Ghrelin for enhancing survival after exposure to lethal radiation. Front Pharmacol, 2021:628018.

Kiang, J.G., Zhai, M., Liao, P.-J., Ho, C., Gorbunov, N.V., Elliott, T.B. (2017a). Thrombopoietin receptor agonist mitigates hematopoietic acute radiation syndrome and improves survival after whole-body ionizing irradiation followed by wound trauma. Mediators of Inflammation 2017, 7582079.

Kiang, J.G., Zhai, M., Liao, P.-J., Elliott, T.B., Gorbunov, N.V. (2014b). Ghrelin therapy improves survival after whole-body ionizing irradiation combined with wound or burn: Amelioration of leukocytopenia, thrombopenia, splenomegaly, and bone marrow injury. Oxid Med Cell Longev, 2014:215858.

Kiang, J.G., Zhai, M., Bolduc, D.L., Smith, J.T., Anderson, M.N., Ho C, Lin B, Jiang S. (2017b). Combined therapy of pegylated-G-CSF and Alx4100TPO improves survival and mitigate acute radiation syndrome after whole-body ionizing irradiation alone and followed by wound trauma. Radiat Res 188:476-490.

King, G.L., Sandgren, D.J., Mitchell, J.M., Bolduc, D.L., Blakely, W.F. (2018). System for scoring severity of acute radiation syndrome response in Rhesus Macaques (Macaca mulatta). Comp Med, Oct 10. doi: 10.30802/AALAS-CM-17-000106. [Epub ahead of print].

Kumar, V.P., Stone, S., Biswas, S., Sharma, N., Ghosh, S.P. (2020). Gamma Tocotrienol protects mice from targeted thoracic radiation injury. Front Pharmacol 11:587970.

Ledney, G.D., Elliott, T.B. (2010). Combined injury: Factors with potential to impact radiation dose assessments. Health Phys, 98(2):145-152.

Li, X., Cui, W., Hull, L., Wang, L., Yu, T., Xiao, M. (2020). IL-18 binding protein (IL-18BP) as a novel radiation countermeasure after radiation exposure in mice. Radiation Research 10(1):18674.

Miller, A.C., Gilstad, J., Brenner, D.J. (2018). Global health engagement of low dose radiation, Military Medicine, 182, 9/10:1677.

Miller, A.C. et al. (2017). Radiation effects of depleted uranium: Radiation bystander effects, Toxicology and Applied Pharmacology, 331, 135-141.

Sebastian, R., Subramanian, U., Hsiao, K.-H., Bolduc, D.L., Blakely, W.F. Premature chromosome condensation (PCC) assay with chromosomal length ratio (LR) and cell-cycle progression index (CPI) Analyses, Radiat Res (in review).

Subramanian, U., O'Brien, B., McNamara, M., Romanyukha, L., Bolduc, D.L., Olsen, C., Blakely, W.F. (2020). Automated dicentric aberration scoring for triage dose assessment: ^{60}Co gamma rays dose-response at different dose rates. Health Physics, July: 119(1): 52-58.

Srinivasan, V., Weiss, J.F., Kumar, S. (1997). Radioprotection by misoprostol (PGE1 methyl analog) in combination with vitamin E, selenomethionine and WR-3689794. Adv Exp Med Biol, 400B:791-797.

Wang, L., Zhai, M., Lin, B., Cui, W., Hull, L., Li, X., Anderson, M.N., Smith, J.T., Umali, M.V., Jiang, S., Kiang, J.G., Xiao, M. Peg-G-CSF and L-Citrulline combinational therapy for mitigating skin wound combined radiation injury in a mouse model. Radiat Res 2021 (in 2nd review).

Whitnall, M.H., Elliott, T.B., Landauer, M.R., Wilhelmsen, C.L., McKinney, L., Kumar, K.S., Srinivasan, V, Ledney, G.D., Seed, T.M. (2002). Protection against gamma-irradiation with 5-androstenediol. Mil Med, 167(2 Suppl):64-65.

Xiao, M. (2016). The role of proinflammatory Cytokine Interleukin-18 in radiation injury. Health Phys 111(2):212-217.

Chapter 2 – HIGH LEVEL RADIATION BIOEFFECTS

2.1 BIOMARKERS OF EXPOSURE AND EFFECTS

2.1.1 New Approaches in Diagnostics and Therapy of the Acute Radiation Syndrome, A. Tichý, CZE, 2017

The aim of the project was to evaluate and design a new biodosimetric approach in terms of the efficiency of practical usage for the civilian population and for military personnel exposed to ionizing irradiation emergencies.

The large animal mammal model for hematopoietic acute radiation syndrome was studied extensively. We used minipigs for whole body gamma irradiation. First, the lethal dose LD50/30 was determined at 2.4 Gy. Then we screened for suitable parameter applicable as a triage tool at different time points (0, 1, 8, 24, and 48 hours). According to changes in hematological parameters occurring during the first 15 days after irradiation (especially changes in total lymphocytes and neutrophils in the peripheral blood), the animals were divided into five categories H0, H1, H2, H3 and H4, differing with the prognosis according to the human leukocyte deprivation model by Andrews.

Our data indicate that i) Absolute leukocyte counts; ii) The level of caspase-3 protein; iii) Immunophenotyping of major lymphocyte populations and their subsets; and iv) Quantitative determination of gamma-H2AX in peripheral blood lymphocytes could be used for retrospective categorization of irradiated minipigs within the range of 0 – 4 Gy. In conclusion, we have shown that minipigs represent a convenient large animal model for in vivo studies on peripheral blood leukocyte radiosensitivity, with possible practical biodosimetry implications.

Furthermore, we were engaged with the discovery of now proteomic biomarkers of radiation as 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. Notably, the current methods are time-consuming and lacking capacity. As IR triggers a 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 was to verify this hypothesis in blood of Total Body Irradiated (TBI) leukemia patients and to identify and quantify plasma proteins before and after irradiation using mass spectrometry targeted analysis.

Peripheral blood was taken before and 24 hours after TBI. Plasma samples of leukemic patients (n = 15) were pooled and immuno-depleted using MARS Hu-14 column (Agilent). Healthy donors (n = 15) of corresponding sex and age were sampled in parallel to reduce bias caused by oncological condition and temporal effects. Proteins were reduced, alkylated, and digested. 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). Proteotypic peptides for targeted Single Reaction Monitoring (SRM) were selected using PeptideAtlas (ISB, Seattle, USA) and subsequent analysis was carried out using Skyline freeware (University of Washington, USA).

We acquired a list of plasmatic proteins with statistically significant up-regulation (ratio $\geq$ 1.2) or down-regulation (ratio $\leq$ 0.83) 24 hours after irradiation. We ruled out proteins significantly up-regulated in non-irradiated patients when compared to healthy donors as possibly associated with the disease. We also discriminated proteins that have changed in healthy donors within 24 hours as the unstable ones. Finally, we obtained 23 proteins from label-free and 19 proteins from iTRAQ analysis, and assessed their radiobiological relevance. As a result, the top 15 candidates were selected for further analysis together with another 5 radiation-responsive proteins that were added based on literature search.

In conclusion, 20 candidates were subjected to targeted analysis by SRM in 30 individual TBI samples. As this is an ongoing project, the validation is still in the process. To our best knowledge, this is the first attempt to identify radiation biomarkers from human plasma proteome of oncological patients with potential implications to biological dosimetry.

In the area of ARS therapy, we studied mitigation effect of insulin growth factor-1 (IGF-1), which stimulates epithelial regeneration, but it may also induce life-threatening hypoglycemia. Thus, we first assessed its safety. Subsequently, we examined the effect of IGF-1 administered in different dose regimens on gastrointestinal damage induced by high doses of gamma radiation.

The fasting C57Bl/6 mice were injected subcutaneously with IGF-1 in a single dose of 0, 0.2, 1, and 2 mg/kg to determine the Maximum Tolerated Dose (MTD). The glycemic effect of MTD (1 mg/kg) was additionally tested in non-fasting animals. Subsequently, a survival experiment was performed. Animals were irradiated (60Co source with dose 14, 15, or 16 Gy; shielded head), and IGF-1 was administered subcutaneously at a dose of 0.2 or 1 mg/kg 1, 24, and 48 h after irradiation. Finally, we evaluated the effect of six different IGF-1 dosage regimens (at 1 mg/kg, one to five doses starting at 1 [five regimens] or 24 h [one regimen] after irradiation) administered subcutaneously on gastrointestinal damage and peripheral blood changes in mice six days after irradiation (12 and 14 Gy; shielded head).

MTD was established at 1 mg/kg. This dose mitigated both acute and late lethality of IR exposure. However, different dosing regimens showed different efficacy, with three- and four-dose regimens (administered 1, 24, and 48 hours and 1, 24, 48, and 72 hours after irradiation, respectively) being the most effective. The three-dose regimens supported intestinal regeneration; however, when administered 24 h after irradiation, its potency decreased.

We conclude that IGF-1 seems promising in the treatment of high-dose irradiation damage but the dose and selected dosage regimen affect its efficacy.

2.1.2 BAT/FRAT in the Context of Field Biodosimetry, W.F. Blakely, USA, 2017

The Armed Forces Radiobiology Research Institute (AFRRI) Biodosimetry Research Program (BRP) has a mission goal to develop rapid, high-precision analytical methods that can assess radiation exposure doses from clinical samples. These methods will aid in the triage of personnel during a radiological scenario. They will guide treatment decisions, and aid in monitoring recovery from injury and effectiveness of treatments (Blakely and Meineke, 2012). At present our research objectives to: i) automate, field-deployable, biological dosimetry capabilities for rapid battlefield dose assessment, ii) establish reference biological dosimetry for definitive analysis of biological samples from theatre operations, and iii) identification and validation of biomarkers for acute and late radiation effects.

Biodosimetry Research Strategy

Current effects underway are in automating and transitioning cytogenetic biodosimetry assays into clinical service activities with appropriate clinical laboratory certifications (Subramanian et al. 2020). Additional effects underway are on identifying novel biomarkers amenable to accelerated analysis of non-invasive samples. A variety an animal (i.e., rodents, minipigs, nonhuman primates) and human-model systems are employed in the AFRRI BRP studies involving radiation exposures (i.e., total- and partial body) to acute and low-dose rate with gamma, x-rays, and neutrons. Ongoing efforts to develop methods of rapidly assessing radiation exposure to assure appropriate medical treatment involve activities to:

- Establish tissue archives from radiation accidents victims,
- Develop animal-model based ARS severity scoring system,

- Evaluate multiple candidate biomarkers in large animal radiation models,
- Focus on biomarkers of ARS severity, and
- Produce integrating and validated prognostic algorithms to predict radiation injury severity and/or dose from biological indicators.

These research efforts are directed to establish an integrated diagnostic system to provide robust applied biodosimetry capability (Blakely et al. 2016). No single assay is sufficient to address potential radiation exposure scenarios that can be complex and involve mass casualties (Blakely, 2013). Triage, clinical, and definitive radiation biodosimetry all require multiple bioassays and analytical technologies designed for use in Chemical, Biological, Radiological, and Environmental (CBRE) diagnostics and general medical care. Our research studies are based on the general hypothesis that use of multiple parameter biodosimetry assessment based on measurement of hematology changes, blood enzyme activities and/or protein will enhance early diagnostic triage for acute radiation exposures (Blakely 2012). The research program maximizes it efforts by collaboration (Dorr et al. 2017; Goh et al. 2017, 2019; Blakely et al. 2011; 2018, Bolduc et al. 2019; Dainiak et al. 2019; Gregoire et al. in press). Worksheets and software developed have been deployed to DoD website to aid in assessment of radiation injury severity by integrating a variety of medical data available to health workers in the field (Salter et al. 2010; Blakely et al. 2010; Blakely 2013).

Medical Recording – Worksheets and Software Tools

Since the conception of a research team concept at AFRRI the BDP has collaborated with the AFRRI Military Medical Operations (MMO) Department to transition Biodosimetry worksheets and software applications.

Medical data forms (Accessible at website: www.usuhs.edu/afrri/biodosimetrytools):

- AFRRI Adult/Pediatric Field Medical Record (AFRRI Form 330) – Provides a convenient one-page form for gathering emergency medical information in the field. Applicable to both adult and pediatric cases.
- AFRRI Biodosimetry Worksheet (AFRRI Form 331) – Provides a place for recording the facts about a case of radiation exposure, including the source and type of radiation, the extent of exposure, and the nature of the resulting injuries. Applicable to both adult and pediatric cases, and
- AFRRI Radiocesium Worksheet (AFRRI Form 335) – Flowchart details steps for screening patients suspected of having been or confirmed to have been exposed to radiocesium during a Radiation Dispersal Device (RDD) event.

Software applications (Accessible at website: www.usuhs.edu/afrri/biodosimetrytools for BAT and winFRAT):

- **BAT – Biodosimetry Assessment Tool version 1.06**. A computer-based software diagnostic tool for use by health-care providers early after a radiation incident. Assists providers in identifying individuals with significant radiation exposures and in making appropriate treatment decisions. BAT is NOT a substitute for treatment decisions by physicians and other trained health-care professionals. Selected features in the BAT application include:
  - Runs on Windows XP, Vista, or 7, 10;
  - Uses templates to collect, integrate, and archive data obtained from patients accidentally exposed to ionizing radiation;
  - Includes an interactive human body map to easily indicate location of radioactivity detected by an appropriate device;
  - Delivers diagnostic information (clinical signs and symptoms, physical dosimetry, etc.);
  - Generates diagnostic indices for the development of a multi-parameter dose assessment;
  - Assesses clinical parameters useful for casualty management;

- Displays concise, relevant patient diagnostic information; and
- Archives information for further use in radiation protection management.

- **WinFRAT – First-responders Radiological Assessment Triage (for Windows) version 0.7.6.0 beta:**
  - Enables first-responders to triage suspected radiation casualties based on the initial, or prodromal, features listed in the Emergency Radiation Medicine Response – AFRRI's Pocket Guide (https://www.usuhs.edu/sites/default/files/media/afrri/pdf/afrri-pocket-guide.pdf).

    Selected features include:

    - Provide, with minimum text entry, signs and symptoms, blood lymphocyte counts, and dosimetry data;
    - Assess the multi-parameter triage dose or the exposure without an assigned dose or indicate no evidence of overexposure;
    - Provide dose-specific messages addressing reliability and diagnostic information, hospitalization estimations, and mortality projections.
- **mFRAT – Mobile First-responders Radiological Assessment Triage (for smart phones) Version 1.0** (accessible for iOS at https://itunes.apple.com/us/app/mfrat/id1068173990?ls=1&mt=8 and for Androids at https://play.google.com/store/apps/details?id=edu.usuhs.frat).

Mass-Casualty Radiological/Nuclear Incident – Biodosimetry Algorithm

The U.S. concept of operations for radiological response is based on the use of a multi-parameter biodosimetry approach (Milner et al. 2016) and is more mature for reach-back application. In the case of field biodosimetry applications there is recognition for the need to enhance the biodosimetry tools to provide effective early-phases diagnostic triage (Blakely 2008; 2011). Here we introduce a biodosimetry algorithm based on the use of AFRRI biodosimetry tools and existing available commercial equipment (Figure 2-1).

In the case of an Improved Nuclear Device (IND) incident, which would result in mass casualties, many individuals are expected to have combined injuries resulting from radiation burns and wounding from broken glass. The severity of combined injuries can be used as a gauge of relative distance from the epicenter of the nuclear explosion and a ready means to sort individuals for immediate radiation burn therapy. Current thermal burn therapy includes procedures identical to current approaches to treat individuals with life-threatening radiological injuries (Figure 2-1).

AFRRI's biodosimetry software tool (i.e., mFRAT) provides a convenient hand-held recording system to capture relevant clinical signs and symptoms and location-based estimate of radiation dose to perform an initial triage radiation dose assessment. Those individuals with suspected exposures >2 Gy could then be further biosampled for a complete blood cell count and blood proteomic analysis (i.e., C-reactive protein), again using hand-held diagnostic equipment with U.S. Food and Drug Administration (FDA) approval. AFRRI's biodosimetry worksheet (i.e., AFRRI Form 331) provides a convenient medical recording system to document relevant medical data for individuals. The constellation of clinical signs and symptoms along with hematology blood cell counts and proteomic results can provide diagnostic information to medical providers to develop an initial medical treatment strategy. Follow-up biosampling for definitive analysis by a qualified laboratory to perform dose assessment by cytogenetics and emerging molecular bioassays (i.e., proteomic, gene expression) is recommended.

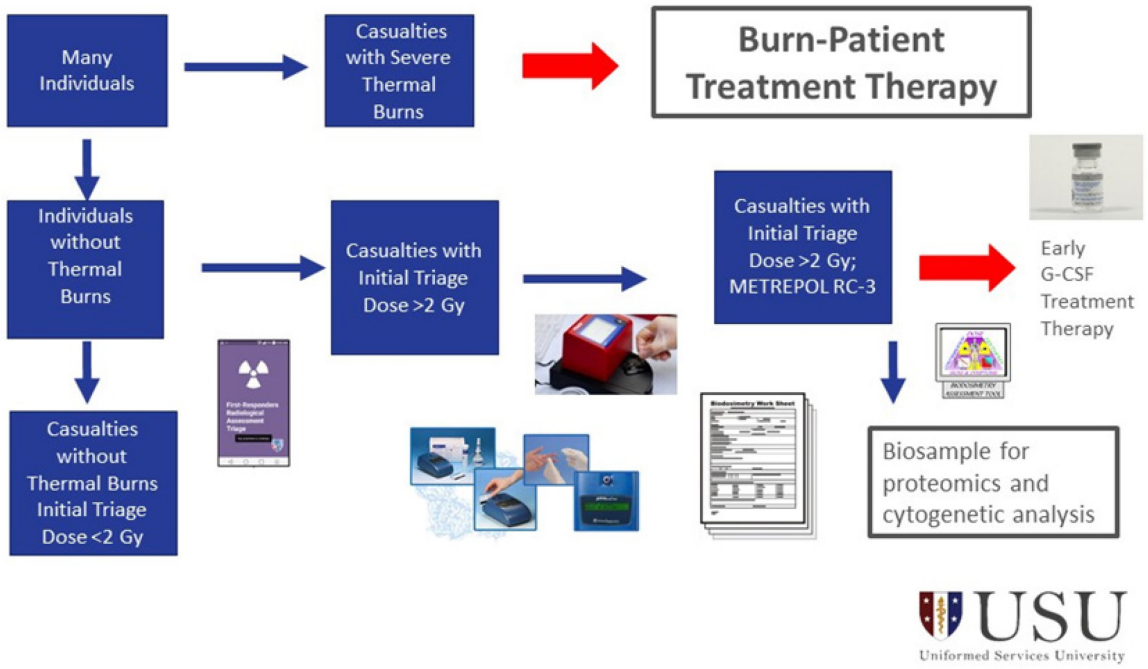


Figure 2-1: Mass-Casualty Radiological/Nuclear Incident: Biodosimetry Algorithm.

Summary

AFRRI's BRP, since its conception, has contributed to enhance both reference and field-deployable biodosimetry. These use of biodosimetry worksheets and software provides a platform to integrate the suite of multiple parameter biodosimetry diagnostics medical data to enable first-responders and medical care providers to develop appropriate medical treatment strategies. These tools also contribute to operational personnel to assess for potential radiation exposure levels of personnel to guide operational decisions.

Acknowledgements, Disclosure and Disclaimer

The author acknowledges and thanks the co-contributors and collaborators to the AFRRI's BRP studies as well as the Institute's leadership in their support of this effort. The views expressed are those of the author and do not necessarily reflect the official policy or position of the AFRRI, USUHS, DoD, nor the U.S. Government. Support for this research is from AFRRI project numbers RBB43523, RBB44313, and AFR-B4-10971.

References

Blakely, W.F. (2010). Biodosimetry medical recording-use of the Biodosimetry Assessment Tool. Health Physics, 99 Suppl 5, S184-91.

Blakely, W.F. (2011). Early biodosimetry response: Recommendations for mass-casualty radiation accidents and terrorism. In "Radiation Bioeffects and Countermeasures" (Chapter 7, pp: 7-1 to 7-32), Blakely, W.F., Janiak, M.K., Edwards, K., Duffy, F. (Eds.), Technical Report, North Atlantic Treaty Organization, Research and Technology Organization, Human Factors and Medicine-099 RTO-TR-HFM-099, AC/323(HFM-099)TP/356, Neuilly-sur-Seine, France: RTO.[1]

[1] Available from: http://www.rto.nato.int (Use the Publications drop-down menu to select RTO-TR Technical Reports. Enter "Blakely" into Author(s) field, then click Search. Click "Radiation Bioeffects and Countermeasures"; 2011.

Blakely, W.F., King, G.L., Ossetrova, N.I. Port M. (2011). Molecular biomarkers of acute radiation syndrome and radiation injury. In "Radiation Bioeffects and Countermeasures" (Chapter 5, pp: 5-1 to 5-22), Blakely WF, Janiak MK, Edwards K, Duffy F (Eds.), Technical Report, North Atlantic Treaty Organization, Research and Technology Organization, Human Factors and Medicine-099 RTO-TR-HFM-099, AC/323(HFM-099)TP/356, Neuilly-sur-Seine, France: RTO.[2]

Blakely, W.F. and Meineke, V. (2012). Perspectives on translational biological dosimetry research programs: Strategy for military operational applications. RTO HFM Symposium on "Biological Effects of Ionizing Radiation Exposure and Countermeasures: Current Status and Future Perspectives," held in Ljubljana, Slovenia 8 – 10 October 2012, and published in RTO-MP-HFM-223.

Blakely, W.F. (2012). Early-phase biological dosimetry, In Medical Consequences of Radiological and Nuclear Weapons (Senior Editor: A.B. Mickelson), Textbook of Military Medicine Publication, Borden Press, Chapter 6, 101-125.

Blakely, W.F. (2013). Recommended approach for use of multi-parameter biodosimetry. Medical Basis for Radiation Accident Preparedness, D. Christensen, S. Sugarman, F. M. O'Hara, Jr. (Editors), Oak Ridge, TN, Oak Ridge Universities, 33-50.

Blakely, W.F., Sandgren, D.J., Levine, I.H., Livingston, B.E., Goans, R.E. (2013). Integrated medical recording for multiple radiation bioassays. In: D. Christensen S. Sugarman, F. M. O'Hara, Jr. (Eds.) The Medical Basis for Radiation-Accident Preparedness: New York: Parthenon; 401-403.

Blakely, W.F., Romanyukha, A., Hayes, S.M., Reyes, R.A., Stewart, H.M. Jr, Hoefer, M.H., Williams, A., Sharp, T., Huff, L.A. U.S. Department of Defense multiple-parameter biodosimetry network. Radiat. Prot. Dosimetry. 2016 Nov 24. [Epub ahead of print]

Blakely, W.F., Bolduc, D.L., Debad, J., Sigal, G., Port, M., Abend, M., Valente, M., Drouet, M., Hérodin, F. (2018). Use of proteomic and hematology biomarkers for prediction of hematopoietic acute radiation syndrome severity in baboon radiation models. Health Physics Jul; 115(1):29-36. doi: 10.1097/HP.0000000000000819.

Bolduc-D.L., Blakely, W.F. (2019). Baboon radiation quality (mixed field neutron and gamma, gamma alone) dose -response model systems: Assessment of H-ARS severity using hematologic biomarkers. Radiat. Prot. Dosimetry, Dec 31, 186(1): 15-23.

Dainiak, N., Albanese, J., Kaushik, M., Balajee, A.S., Romanyukha, A., Sharp, T.J., and Blakely, W.F. (2019). Concepts of operations for a US Dosimetry and Biodosimetry Network. Radiat. Prot. Dosimetry, 186 (1): 130-138. Doi:10.1093/rpd/ncy294.

Dörr, H., Abend, M., Blakely, W.F., Bolduc, D.L., Boozer, D., Costeira, T., Dant, T., De Amicis, A., De Sanctis, S., Dondey, M., Drouet, M., Entine, F., Francois, S., Gagna, G., Guitard, N., Hérodin, F., Hoefer, M., Lamkowski, A., La Sala, G., Lista, F., Loiacono, P., Majewski, M., Martigne, P., Métivier, D., Michel, X., Pateux, J., Pejchal, J., Reeves, G., Riccobono, D., Sinkorova, Z., Soyez, L., Stricklin, D., Tichý, A., Valente, M, Woodruff, C.R. Jr, Zarybnicka, L., Port, M. (2017). Using clinical signs and symptoms for medical management of radiation casualties – 2015 NATO exercise. Radiat Res. Mar; 187(3):273-286. doi: 10.1667/RR14619.1. Epub 2017 Feb 20.

[2] Available from: http://www.rto.nato.int (Use the Publications drop-down menu to select RTO-TR Technical Reports. Enter "Blakely" into Author(s) field, then click Search. Click "Radiation Bioeffects and Countermeasures"; 2011.

Goh, V.S.T., Fujishima, Y., Abe, Y., Sakai, A., Yoshida, M.A., Ariyoshi, K., Kasai, K., Wilkins, R.C., Blakely, W.F., Miura, T. (2019). Construction of fluorescence in situ hybridization (FISH) translocation dose-response calibration curve with multiple donor data sets using R, based on ISO 20046:2019 recommendations. Int. J. Radiat. Biol. Dec; 95(12):1668-1684.

Goh, V.S.T., Nakayama, R., Blakely, W.F., Abe, Y., Chua, C.E.L., Nakata, A., Fujishima, Y., Yoshida, M.A., Kasai, K., Ariyoshi, K., Miura, T. Improved harvest and fixation methodology for isolated human peripheral blood mononuclear cells in cytokinesis-block micronucleus assay. Int. J. Radiat. Biol. (accepted Oct 2020).

Gregoire, E., Barquinero, J.F., Gruel, G., Benadjaoud, M., Ainsbury, E., Beinke, C., Balajee, A., Beukes, P., Blakely, W.F., Dominguez, I., Duy, P.N., Flegal, F.N., Gil, O.M., Güçlü, I., Guogyte, K., Hadjidekova, V., Hadjidekova, S.P., Hande, P., Jang, S., Lumniczky, K., Martinez, J.G., Meschini, R., Milic, M., Montoro, A., Moreno, M., Oestreicher, U., Pajic, J., Ricoul, M., Sabatier, L., Sebastia, N., Sommer, S., Szkarlat, Z., Testa, A., Terzoudi, G., Valente, M., Venkatachalam, P., Vral, A., Wilkins, R.C., Wojcik, A., Zafiropoulos, D., Kulka, U. RENEB inter-laboratory comparison 2017: limits and pitfalls of ILCs. Int/ J. Radiat. Biol. (in review).

Milner, E.E., Daxon, E.G., Anastasio, M.T., Nesler, J.T., Miller, R.L., Blakely, W.F. (2016). Concepts of operations (CONOPS) for biodosimetry tools employed in operational environments. Health Physics; 110(4):370-9. doi: 10.1097/HP.0000000000000470.

Salter, C.A., Sandgren, D.J., Levine, I.H., Blakely, W.F. (2010). New biodosimetry tools to support mental health in nuclear/radiological accidents or terrorism, The 71F Advantage: Applying Army Research Psychology for Health and Performance Gains, Washington D.C., National Defense University Press, 357-370.

Subramanian, U, O'Brien, B., McNamara, M., Romanyukha, L., Bolduc, D.L., Olsen, C., Blakely, W.F. (2020). Automated dicentric aberration scoring for triage dose assessment: ^{60}Co gamma rays dose-response at different dose rates. Health Physics, July: 119(1): 52-58.

2.1.3 Biomarkers of Radiation Exposure Developed at PHE, C. Badie, UK, 2017

Ionizing Radiation Exposure and Gene Expression

Exposure of cells to IR activates multiple signal transduction pathways which result in complex alterations in gene expression. Out of the ~25,000 human protein-coding genes, a few % can have their expression changed in response to IR. The response may depend on the dose, the dose rate, the radiation quality, the lapse between stress and analysis, the individual and the tissue analyzed, making gene expression analysis complicated but a great source if information. In our first study, we aimed to identify mRNA biomarkers in peripheral blood lymphocytes for dose estimation and prediction of individual response after exposure to ionizing radiation (KABACIK et al. International Journal of Radiation Biology, 2011). The purpose was to establish a panel of highly radiation responsive genes suitable for biological dosimetry and to explore inter-individual variation in response to ionizing radiation exposure. Analysis of gene expression in response to radiation was carried out using three independent techniques (Microarray, Multiplex Quantitative Real-Time Polymerase Chain Reaction (MQRT-PCR) and nCounterAnalysis System) in human dividing lymphocytes in culture and peripheral blood leukocytes exposed ex vivo from the same donors. Variations in transcriptional response to exposure to ionizing radiation analyzed by microarray allowed the identification of genes which can be measured accurately using MQRT PCR and another technique allowing direct count of mRNA copies. We have identified genes which are consistently up-regulated following exposure to 2 or 4 Gy of X-rays at different time points, for all individuals in blood and cultured lymphocytes. Down-regulated genes including cyclins, centromeric and mitotic checkpoint genes, particularly those associated with chromosome instability and cancer could be detected in dividing lymphocytes only. The data provided evidence that there are a number

of genes which seem suitable for biological dosimetry using peripheral blood, including sestrin 1 (SESN1), growth arrest and DNA damage inducible 45 alpha (GADD45A), cyclin dependent kinase inhibitor 1A (CDKN1A), cyclin G1 (CCNG1), ferredoxin reductase (FDXR), p53 up-regulated mediator of apoptosis (BBC3) and Mdm2 p53 binding protein homolog (MDM2). These biomarkers could potentially be used for triage after large-scale radiological incidents and for monitoring radiation exposure during radiotherapy. The group also studied human genetic variation to assess how it contributes to the observed range of radiosensitivity as there are few quantitative estimates of heritability available.

The group then focused on the dose-responses of transcriptionally responsive genes, especially at low doses and investigated these dose-responses and assessed inter-individual variability for high dose (0.5 – 4 Gy) and low dose (5 – 100 mGy) gene expression responses at 2 h and 24 h using 13 biomarkers transcriptionally regulated through the DNA damage response by the tumor suppressor p53 were investigated. High dose-response curves were best constructed using a polynomial fit while the low dose-response curves used a linear fi t with linear R 2 values of 0.841 – 0.985. These findings identify genes that fulfil some of the requirements of a good exposure biomarker even at low doses, such as sensitivity, reproducibility and simple proportionality with dose (Manning et al. International Journal of Radiation Biology 2013)

The full transcriptional response to radiation is very complex since it also involves epigenetic mechanisms triggered by radiation exposure such as modifications of expression of noncoding RNA such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) that have not been fully characterized. To improve the understanding of the transcriptional response to radiation, the simultaneously monitored the expression of ten protein-coding genes, as well as 19 miRNAs and 3 lncRNAs in a time- and dose-dependent manner in stimulated human T lymphocytes obtained from healthy donors and one patient with Ataxia Telangiectasia (AT), a well characterized radiosensitivity disorder. After 2 Gy X irradiation, expression levels were monitored at time points ranging from 15 min up to 24 h post irradiation. The majority of genes investigated responded rapidly to radiation exposure, with the peak up-regulation (CDKN1A, SESN1, ATF3, MDM2, PUMA and GADD45A) or down-regulation (CCNB1) occurring 2–3 h post irradiation, while DDB2, FDXR and CCNG1 responded with slower kinetics reaching a peak of expression between 5 and 24 h. A significant modification of expression after radiation exposure was observed for miR-34a-5p and miR-182-5p, with an up-regulation occurring at late time points reaching two to threefold at 24 h. Among the three lncRNAs studied, TP53TG1 demonstrated a weak up-regulation, reaching a maximum of 1.5-fold at 24 h after radiation exposure. Conversely, FAS-AS1 was up-regulated up to fivefold by 5 Gy irradiation. results indicate that expression of the protein-coding genes allows discrimination of the AT from healthy donors when analyzed at 2 h. However, differences in expression between AT and healthy donors are no longer detectable 24 h post irradiation although, interestingly, linear dose responses for some of the genes studied are obtained at this time point. miRNAs miR-34a-5p and miR-182-5p are responsive to radiation exposure in a dose- and time-dependent manner. Additionally, this is the first study to report that FAS-AS1 lncRNA is up-regulated by radiation exposure in an ATM-dependent fashion in human T lymphocytes (Kabacik et al. 2015).

The group also discussed the potential of samples for studies in radiation research (Pernot et al. 2014). Salivary biomarkers have important potential to facilitate breakthroughs in epidemiologic studies, management of emergency situations, and detection and surveillance of diseases by medical staff. During the last decade, an increasing number of studies on salivary biomarkers have been published as a consequence of the impressive development of new high-throughput technologies. Although the use of saliva samples is not without drawbacks, it represents an ideal non-invasive alternative to blood, particularly in children. The group lead the second RENEB gene expression study (Manning et al. 2016) where comparable dose estimates of blinded whole blood samples were obtained independently of culture conditions and analytical approaches.

Finally, during this period, the group studied radiotherapy-associated long-term modification of expression of inflammatory biomarkers (Manning et al. 2017). Little was known about the transcriptionally responsive genes which play a role in the inflammation response. In order to improve our understanding of such transcriptional response to radiation in vivo, simultaneously monitored the expression of 249 genes associated with the

inflammation response over the course of the radiotherapy treatment in blood of patients treated for endometrial or head and neck cancer was performed. The group identified genes whose transcriptional expression is either up-regulated (ARG1, BCL2L1) or downregulated (MYC) several fold in vivo. These modifications were consistently detected across patients and further confirmed by quantitative real-time polymerase chain reaction (QRT-PCR); they were specifically significant toward the end of the radiotherapy treatment, 5 weeks following the first radiation fraction and more pronounced in endometrial patients (respectively, 2.9, 4.1, and 1.8 times). Importantly, in an attempt to correlate expression levels with normal tissue reaction to IR, the group also identified three other genes CD40, OAS2, and CXCR1 whose expression level fluctuations during radiotherapy were more pronounced in patients developing late normal tissue responses to curative radiotherapy after the end of the radiotherapy treatment. Overall inflammation-associated genes which are promising biomarkers of IR exposure and susceptibility to radiation-induced toxicity.

References

Kabacik, S., Manning, G., Raffy, C., Bouffler, S., Badie, C. (2015). Time, dose and ataxia telangiectasia mutated (ATM) status dependency of coding and noncoding RNA expression after ionizing radiation exposure. Radiat Res, Mar, 183(3):325-37. doi: 10.1667/RR13876.1. Epub 2015 Mar 4.

Manning, G., Macaeva, E., Majewski, M., Kriehuber, R., Brzóska, K., Abend, M., Doucha-Senf, S., Oskamp, D., Strunz, S., Quintens, R., Port, M., Badie, C. (2017). Comparable dose estimates of blinded whole blood samples are obtained independently of culture conditions and analytical approaches. Second RENEB gene expression study. Int J Radiat Biol. Jan, 93(1):87-98. doi: 10.1080/09553002.2016.1227105. Epub 2016 Sep 14.

Manning, G., Tichý, A., Sirák, I., Badie, C. (2017). Radiotherapy-associated long-term modification of expression of the inflammatory biomarker genes ARG1, BCL2L1, and MYC. Front Immunol. Apr 10, 8:412. doi: 10.3389/fimmu.2017.00412. eCollection 2017. PMID: 28443095.

Pernot, E., Cardis, E., Badie, C. (2014). Usefulness of saliva samples for biomarker studies in radiation research. Cancer Epidemiol Biomarkers Prev. Dec, 23(12):2673-80. doi: 10.1158/1055-9965.EPI-14-0588.

2.1.4 New Ongoing Developments for Improvement of Transcription-Based Biological Dosimetry, C. Badie, UK, 2017

During this period, the group studied the influence of several potential confounding factors on radiation dose estimation using in vivo validated transcriptional biomarkers. For triage purposes following a nuclear accident, blood-based gene expression biomarkers can provide rapid dose estimates for a large number of individuals. Ionizing-radiation-responsive genes are regulated through the DNA damage-response pathway, which includes activation of multiple transcription factors. Modulators of this pathway could potentially affect the response of these biomarkers and consequently compromise accurate dose estimation calculations. In the present study, four potential confounding factors were selected: cancer condition, sex, simulated bacterial infection (lipopolysaccharide), and curcumin, an anti-inflammatory/ antioxidant agent. Their potential influence on the transcriptional response to radiation of the genes CCNG1 and PHPT1, two biomarkers of radiation exposure ex vivo, was assessed. First, both CCNG1 and PHPT1 were detected in vivo in blood samples from radiotherapy patients and as such were validated as biomarkers of exposure. Importantly, their basal expression level was slightly but significantly affected in vivo by patients' cancer condition. Moreover, lipopolysaccharide stimulation of blood irradiated ex vivo led to a significant modification of CCNG1 and PHPT1 transcriptional response in a dose- and time-dependent manner with opposite regulatory effects. Curcumin also affected CCNG1 and PHPT1 transcriptional response counteracting some of the radiation induction. No differences were observed based on sex. Dose estimations calculated using linear regression were affected by lipopolysaccharide and curcumin. In conclusion, several confounding factors tested in this

study can indeed modulate the transcriptional response of CCNG1 and PHPT1 and consequently can affect radiation exposure dose estimations but not to a level which should prevent the biomarkers' use for triage purposes (Cruz-Garcia et al. 2018).

Importantly, the group also validated a specific gene named FDXR as a biomarker of radiation exposure in humans in vivo. Previous investigations in gene expression changes in blood after radiation exposure have highlighted its potential to provide biomarkers of exposure. FDXR transcriptional changes in blood were investigated in humans undergoing a range of external radiation exposure procedures covering several orders of magnitude (cardiac fluoroscopy, diagnostic Computed Tomography (CT)) and treatments (total body and local radiotherapy). Moreover, a method was developed to assess the dose to the blood using physical exposure parameters. FDXR expression was significantly up-regulated 24 hr after radiotherapy in most patients and continuously during the fractionated treatment. Significance was reached even after diagnostic CT 2 hours post exposure. The group further showed that no significant differences in expression were found between ex vivo and in vivo samples from the same patients. Moreover, potential confounding factors such as gender, infection status and anti-oxidants only affect moderately FDXR transcription. Most importantly the group provided a first in vivo dose-response showing dose-dependency even for very low doses or partial body exposure showing good correlation between physically and biologically assessed doses. In conclusion, we report the remarkable responsiveness of FDXR to ionizing radiation at the transcriptional level which, when measured in the right time window, provides accurate in vivo dose estimates. (O'Brien et al. 2018).

References

Cruz-Garcia, L, O'Brien, G., Donovan, E., Gothard, L., Boyle, S., Laval, A., Testard, I., Ponge, L., Woźniak, G, Miszczyk, L., Candéias, S.M., Ainsbury, E., Widlak, P., Somaiah, N., Badie, C. (2018). Influence of confounding factors on radiation dose estimation using in vivo validated transcriptional biomarkers. Health Phys. Jul;115(1):90-101. doi: 10.1097/HP.0000000000000844. PMID: 29787434.

O'Brien, G., Cruz-Garcia, L., Majewski, M., Grepl, J., Abend, M., Port, M., Tichý, A., Sirak, I., Malkova, A., Donovan, E., Gothard, L., Boyle, S., Somaiah, N., Ainsbury, E. Ponge, L., Slosarek, K., Miszczyk, L., Piotr, W., Green, E., Patel, N., Kudari, M., Gleeson, F., Vinnikov, V., Starenkiy, V., Artiukh, S., Vasyliev, L., Zaman, A., Badie, C. (2018). FDXR is a biomarker of radiation exposure in vivo. Sci Rep. Jan 12;8(1):684. doi: 10.1038/s41598-017-19043-w.

2.1.5 Development of a Rapid Gene Expression Based Dose Estimation for Radiological Emergencies: Ongoing Work, C. Badie, PHE, 2018

Gene expression assays have shown great potential for rapid individual radiation dose exposure assessment and the group developed a project to optimize gene expression-based biological dosimetry protocols for radiological emergencies. The group carried out experiments to validate a newly developed protocol where several steps were optimized and to compare it with the current validated protocol in place in the laboratory. Several donor blood samples from were exposed ex vivo to of the following doses: 0, 0.5, 1, 2 Gy x-rays. Concomitant measurement of transcription level of genes FDXR, P21, PHPT1, CCNG1 and SESN1 plus HPRT (control) was performed. The group showed that both protocols provided similar dose estimates, the existing protocol being completed in 7 hours while the new protocol in merely 4 hours. Thus, a significant time shortening is achievable leading to a potential increase of throughput capacity. Hence, this new protocol can be recommended for mass radiation casualties triage purposes (Polozov et al. 2019).

During this period, the group also aimed to compare the induction and persistence of different radiation exposure biomarkers in human peripheral blood in vivo, hence. Blood samples of patients with Indicated Radiotherapy (RT) undergoing Partial Body Irradiation (PBI) were obtained soon before the first treatment and then after 24 h, 48 h, and 5 weeks; i.e., after 1, 2, and 25 fractionated RT procedures. Circulating peripheral blood was collected from ten patients with tumor of endometrium (1.8 Gy per fraction) and eight patients with

tumor of head and neck (2 Gy per fraction). Incidence of dicentrics and micronuclei was monitored as well as determination of apoptosis and the transcription level of selected radiation-responsive genes. Since mitochondrial DNA (mtDNA) has been reported to be a potential indicator of radiation damage in vitro, we also assessed mtDNA content and deletions by novel multiplex quantitative PCR. Cytogenetic data confirmed linear dose dependent increase in dicentrics and micronuclei in peripheral blood mononuclear cells after PBI. Significant up-regulations of five previously identified transcriptional biomarkers of radiation exposure (PHPT1, CCNG1, CDKN1A, GADD45, and SESN1) were also found. No statistical change in mtDNA deletion levels was detected; however, our data indicate that the total mtDNA content decreased with increasing number of RT fractions. Overall, these data represented the first study providing a multiparametric comparison of radiation biomarkers in human blood in vivo, which have potential for improving biological dosimetry (Tichý et al. 2018).

References

Polozov, S., Cruz-Garcia, L., Badie, C. (2019). Rapid gene expression based dose estimation for radiological emergencies. Radiat Prot Dosimetry, Dec 31;186(1):24-30. doi: 10.1093/rpd/ncz053.

Tichý A., Kabacik, S., O'Brien, G., Pejchal, J., Sinkorova, Z., Kmochova, A., Sirak, I., Malkova, A., Beltran, C.G., Gonzalez, J.R., Grepl, J., Majewski, M., Ainsbury, E., Zarybnicka, L., Vachelova, J., Zavrelova, A., Davidkova, M., Markova Stastna, M., Abend, M., Pernot, E., Cardis, E., Badie, C. (2018). The first in vivo multiparametric comparison of different radiation exposure biomarkers in human blood. PLoS One. Feb 23;13(2):e0193412. doi: 10.1371/journal.pone.0193412. eCollection 2018. PMID: 29474504.

NATO RTG GOSPORT September 2019

The main ongoing development during this time period was to assess nanopore sequencing analysis to determine if the technology can be used to detect radiation-inducible genes in human Peripheral Blood Mononuclear Cells (PBMCs) and generate a transcriptional radiation exposure signature in human blood. The technology offers not only long-read sequencing but also a portable device which can overcome issues involving sample shipment and provide faster results. For this goal, blood from nine healthy volunteers was 2 Gy ex vivo X irradiated. After PBMC isolation, irradiated samples were incubated along with the controls for 24 h at 370C. The sequencing analysis identified a radiation signature consisting of 46 differentially expressed genes which included 41 protein-coding genes, a long non-coding RNA and four pseudogenes, five of which have been identified as radiation-responsive transcripts for the first time. The genes in which transcriptional expression is most significantly modified after radiation exposure were APOBEC3H and FDXR, presenting a 25- and 28-fold change on average, respectively. These levels of transcriptional response were comparable to results we obtained by quantitative polymerase chain reaction (qPCR) analysis. Moreover, in vivo exposure analyses showed a transcriptional radio response at 24 h post irradiation for both genes together with a strong dose-dependent response in blood irradiated ex vivo. Future improvements, in sample processing and bioinformatic pipeline for specific radiation-responsive transcript identification, will allow the provision of a portable, rapid, real-time biodosimetry platform based on this new sequencing technology. In summary, our data show that nanopore sequencing can identify radiation-responsive genes and can also be used for identification of new transcripts (Cruz-Garcia et al. 2019).

References

Cruz-Garcia, L., O'Brien, G., Sipos, B., Mayes, S., Love, M.I., Turner, D.J., Badie, C. (2020). Generation of a transcriptional radiation exposure signature in human blood using long-read nanopore sequencing. Radiat Res, Feb;193(2):143-154. doi: 10.1667/RR15476.1. Epub 2019 Dec 12.

2.1.6 Dose Estimate for Prediction of Acute Effects, a Challenge, M. Abend, GER, 2017

Acute health effects after high radiation exposures are summarized as the Acute Radiation Syndrome (ARS). From the mechanistic point of view a dramatic and sudden cell death leads to organ function failure and consecutive to multi-organ failure. Several kinds of cell death are discriminated, but the reproductive cell death characterized by the reduction of radiosensitive stem cells and lost clonogenicity predominates regarding e.g., the hematopoietic or gastrointestinal syndrome. Both syndromes are comprised in the ARS.

Radiation Exposure Comprises Many Characteristics/Facets/Factors

The relation of radiation exposure (dose) with ARS depends on many factors as recently published (Port et al. 2019). Those facets or factors comprise e.g., radiation quality, dose rate, and homogeneity of exposure. They are known to affect outcomes in cellular and mammalian systems. For instance, given the same absorbed dose of, for example, 2 Gy of neutrons, this exposure will result in a substantially reduced clonogenicity of, for example, cultured cells in comparison to 2 Gy of X- or gamma-rays. Irradiation with neutrons is associated with an increased Radiobiological Effectiveness (RBE). Depending on the neutron energy (linear energy transfer), and the biological endpoint examined, the effect on living tissue might be 20-fold higher (Hall and Giaccia 2018). Fractionation of a single dose into two or more fractions with lower exposures and some hours of no exposure in between results in a several-fold increase in survival over a single dose exposure (Elkind and Sutton 1960). Similarly, a decreased dose rate and a corresponding increased exposure time of the same dose will result in increased survival of cultured cells due to repair of sublethal damage (Bedford and Mitchell 1973). The same absorbed dose given whole body versus partial body as well as a homogenous or inhomogeneous exposure will result in differences regarding e.g., survival. External compared to internal radionuclide contamination represents another scenario for which a dose estimate alone is even more challenging with respect to predicting acute health effects. Again, all these different dose characteristics/factors can cause several log-scale differences in cell survival. The surviving cell fraction is essential to organ function leading to different degrees of acute radiation-related health effects. Hence, providing an absorbed dose is insufficient since many other exposure characteristics are not considered but are urgently required for an effect prediction. Radiation exposure has many characteristics and providing an absorbed dose only is of limited clinical significance for effect prediction (Figure 2-2).

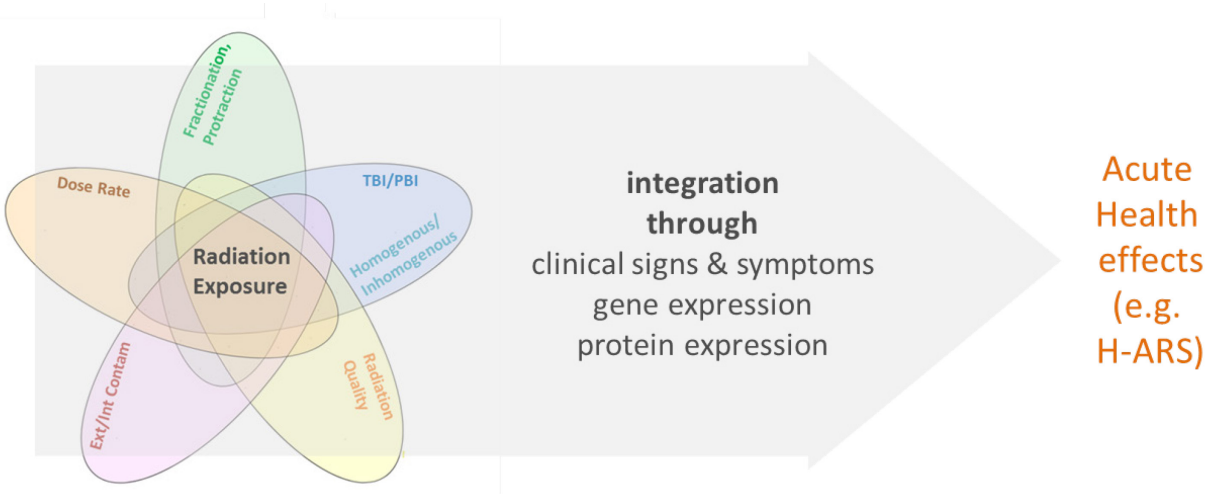


Figure 2-2: Radiation Exposure has Multiple Characteristics and All of them Combined have to be Considered in Order to Predict a Health Effect After Radiation Exposure. In addition, biological processes determine radiation-related acute effects (not shown). Biological processes, responses such as vomiting or blood cell count changes (clinical signs and symptoms) and others might integrate these different factors. Abbreviations: TBI/PBI (total/partial body irradiation); ext/int contam external/internal radionuclide contamination; H-ARS hematological acute radiation syndrome.

Biological Response to Radiation Comprises Many Aspects

Cells and tissues respond differently to the same radiation dose. In radiobiology, several important biological processes have been identified with strong impact on cell survival or cell death, finally leading to the configuration of radiotherapy as it is used nowadays (Hall and Giacia 2018). Again, several log-scale differences in survival can be expected due to differences in cell type radiosensitivity. For instance, bone marrow stem cells are more radiosensitive than cells originating from the breast, testis or thyroid (Hall and Giacia 2018). Also, inter-individual variation in radiosensitivity plays a role. During radiotherapy, high patient-to-patient variability in normal tissue reactions are observed (Bentzen and Overgaard 1994; Andreassen and Alsner 2009). Depending on the cell cycle phase, cells are known to be more (G2, M phase) or less radiosensitive (late S-phase), resulting in survival differences exceeding several log-scales (Sinclair 1968). Tissues respond to the same radiation exposure differently depending on the oxygenation status and the release of free radicals and associated cell damage. Hypoxic cells are more radioresistant so that irradiated cells survive under hypoxic compared to normal oxygen conditions (Palcic and Skarsgard 1984). Taking into account variation in biological factors, effect prediction is obviously challenging.

Integration of Radiation Exposure Characteristics and Biological Responses via Bioindicators for Effect Prediction

Radiation exposure along a “causal or deterministic pathway” results in an event, e.g., an acute health effect that increases in severity with dose. So called “intermediates” can represent cytological or even molecular biological changes (occurring in genes, RNA-species, proteins or metabolites) preceding the effect and causally related to it Figure 2-2). They are called bioindicators of effect (Abend and Port 2016) and might enable the integration of exposure and biological characteristics. For instance, an “intermediate” such as the reduction in lymphocyte counts and a transient increase and later decrease in granulocyte counts observed within the first 1 – 3 days after radiation exposure allow for H-ARS severity prediction (Port et al. 2017). The altered peripheral blood cell counts are caused by cell death in irradiated radiosensitive bone marrow stem cells. If partial and not the whole body irradiation occurred with either neutrons or gamma-rays, bone marrow stem cell death would be reduced. The number of surviving bone marrow stem cells would directly affect peripheral blood cell counts and the subsequent events of HARS severity, thus integration differences in radiation exposure. Likewise biological factors, such as cell type or individual radiosensitivity would be covered (integrated) by measuring bioindicators of effect (Figure 2-2). This has been recently shown by our group using gene expression analysis in the peripheral blood of animals (Port et al. 2016, 2017a,b) and by others using proteomic and hematology biomarkers (Blakely et al. 2018).

In summary, many different characteristics related to radiation exposure as well as the irradiated biological systems have to be considered for a meaningful prediction of acute radiation health effects (Figure 2-2). Bioindicators of effect prediction (e.g., H-ARS severity) could be used early after radiation exposure to integrate these different factors. This strategy holds promises to result in aggregated and robust predictors of clinical outcomes in exposed individuals.

References

Abend, M., Port, M. (2016). Combining radiation epidemiology with molecular biology – Changing from health risk estimates to therapeutic intervention. Health Phys. Aug;111(2):183-5.

Andreassen, C.N., Alsner, J. (2009). Genetic variants and normal tissue toxicity after radiotherapy: A systematic review. Radiother Oncol. Sep;92(3):299-309.

Bedford, J.S., Mitchell, J.B. (1973). Dose-rate effects in synchronous mammalian cells in culture. Radiat Res. May;54(2):316-27.

Bentzen, S.M., Overgaard, J. (1994). Patient-to-patient variability in the expression of radiation-induced normal tissue injury. Semin Radiat Oncol. Apr;4(2):68-80.

Blakely, W.F., Bolduc, D.L., Debad, J., Sigal, G., Port, M., Abend, M., et al. (2018). Use of proteomic and hematology biomarkers for prediction of hematopoietic acute radiation syndrome severity in baboon radiation models. Health Phys. Jul;115(1):29-36.

Elkind, M.M., Sutton, H. (1960). Radiation response of mammalian cells grown in culture. 1. Repair of X-ray damage in surviving Chinese hamster cells. Radiat Res. Oct;13:556-93.

Hall, E.J., Giaccia A.J. (2018). Radiobiology for the Radiologist, International Eights Edition. Lippincott Williams and Wilkins.

Palcic, B., Skarsgard, L.D. (1984). Reduced oxygen enhancement ratio at low doses of ionizing radiation. Radiat Res. Nov;100(2):328-39.

Port, M., Hérodin, F., Valente, M., Drouet, M., Lamkowski, A., Majewski, M., et al. (2016). First generation gene expression signature for early prediction of late occurring hematological acute radiation syndrome in baboons. Radiat Res. Jul;186(1):39-54.

Port, M., Hérodin, F., Valente, M., Drouet, M., Lamkowski, A., Majewski, M., et al. (2017). Gene expression signature for early prediction of late occurring pancytopenia in irradiated baboons. Ann Hematol. Feb 24;96(5):859-70.

Port, M., Hérodin, F., Valente, M., Drouet, M., Ullmann, R., Majewski, M., et al. (2017). Pre-exposure gene expression in baboons with and without pancytopenia after radiation exposure. Int J Mol Sci. Mar 2;18(3):541.

Port, M., Pieper, B., Knie, T., Dörr, H., Ganser, A., Graessle, D., et al. (2017). Rapid prediction of hematologic acute radiation syndrome in radiation injury patients using peripheral blood cell counts. Radiat Res. Aug;188(2):156-68.

Port, M., Majewski, M. Abend, M. (2019). Radiation dose is of limited clinical usefulness in persons with acute radiation syndrome. Radiation Protection Dosimetry, 186(1):126-129.

Sinclair, W.K. (2012). Cyclic X-ray responses in mammalian cells in vitro. 1968. Radiat Res. Aug;178(2):AV112-24.

2.1.7 Using mRNA and Small RNA Gene Expression Changes in the Peripheral Blood for Easy Detection of Ra-223 Incorporation, P. Ostheim, BIR, 2018

Radioactive sources are widely used in industry, research and medicine and it seems relatively easy to get access of such material (IAEA 2005, NCRP Report No. 166, 2012). The find of abandoned radioactive material is regularly reported (IAEA 2004). Those “orphan sources” of radioactivity have resulted in large-scale scenarios in several cases in the past as for example in Goiania (IAEA 1988). Considering the harmful effect of radioactive sources, it is not far-fetched that this could also be of interest for terroristic attacks. A Radiological Dispersal Device (RDD, “dirty bomb”) is a conventional explosive combined with radioactive material. There are a couple of examples, which show that the attack by a dirty bomb must be considered as a serious terrorist threat (Rosoff and Winterfeldt 2007, Hall and Giaccia 2012, Confer et al. 2018).

Radiation analytics is the established method for detection of incorporated alpha-emitting radionuclides. It is laborious and time-consuming. We wondered whether changes in gene expression measured in the human peripheral blood after radionuclide incorporation might serve as an easier and high-throughput biodosimetric approach for detection of incorporated alpha-radionuclides in a nuclear scenario. In collaboration with the

University Medical Center (Mainz, Germany), we measured gene expression in 24 peripheral blood samples of five patients suffering from multiple bone-metastatic, castration-resistant prostate cancer (without visceral or nodal involvement), who underwent treatment with the alpha-emitting isotope Radium-223 dichloride (Ra-223, Xofigo®) over an up to six month period. Patients received about 4 MBq per cycle and month and were treated ideally for six months.

Altogether 24 blood samples were collected for analysis. For the present study, we performed a whole genome screening and identified potential candidate genes on a transcriptional (protein coding mRNAs) as well as a post-transcriptional (non-coding small RNAs) level by differential gene expression relative to pre-treatment (DGE (≥|2|-fold, $p<0.05$). These RNA-species were then validated using qRT-PCR methodology.

In a first step, we employed Next Generation Sequencing (NGS) for a whole genome screening of mRNAs (RNA seq) and small RNAs in one patient at eight different time points during six cycles of Ra-223-therapy. Altogether 1900 mRNAs and 972 small RNAs (222 miRNAs) could be identified that were differentially up- or down-regulated over the whole period of time after the first treatment with Ra-223 (≥|2|-fold) or with peaking profiles (≥|5|-fold) at specific points in time. In a second step, 72 candidate mRNAs and 101 small RNAs (29 miRNAs) were chosen for methodological (n = 8 samples) and independent (remaining four patients n = 20 samples) validation, shifting to a qRT-PCR platform. Hereby, gene expression values among dose and time did not show a significant dose-to-gene expression association common for all patients and all points in time but individual radiation-induced gene expression pattern were observed. Regarding these inter-individual responses and adjusting time scale accordingly, 15 mRNAs could be validated by means of positive methodological validation as well as independent validation considering inter-individual points in time (Figure 2-3).

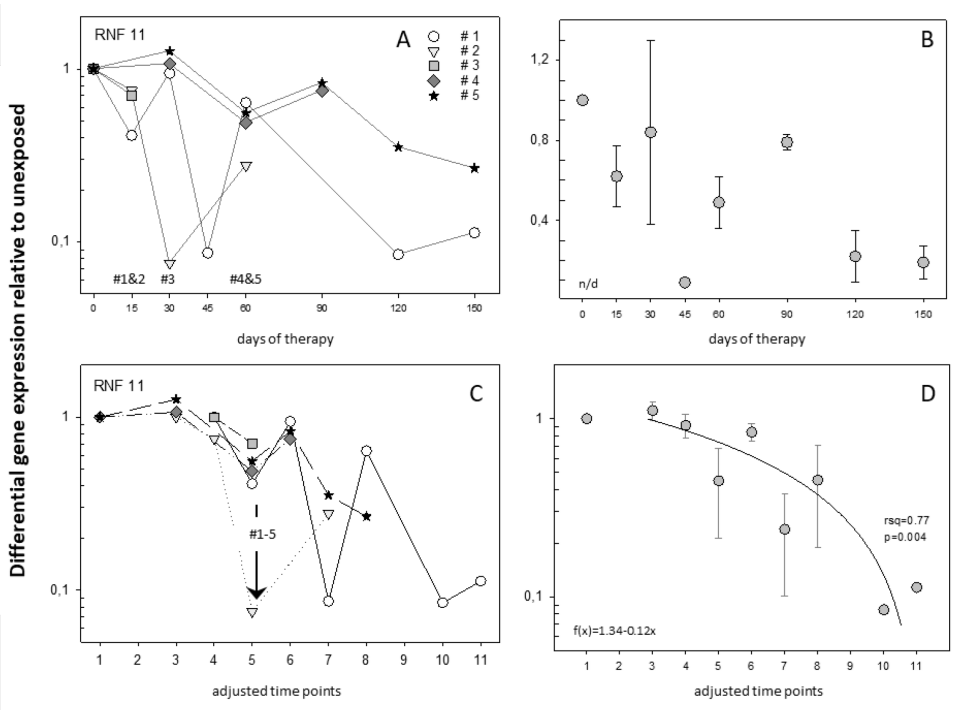


Figure 2-3: Inter-Individual Dose/Time-to-Gene Expression Pattern are Depicted for RNF11 (A), Corresponding Mean Values for Each Time Point Are Provided in (B), No Significant Association Over Time Could be Found. The time point, in which expected down-regulation of both genes for each patient was observed, is written above the x-Scales. Shifting Individual Dose/Time-Gene-Expression Pattern so that Expected Gene Expression Events Align into the Same Time Point (C) Resulted in Significant Dose-to-Gene Associations Based on the Time Adjusted Mean Values for All Patients (D). Error bars represent the standard deviation.

In conclusion, we identified radiation-induced transcriptional gene expression changes in whole blood associated to incorporated alpha-radionuclides. However, these associations were masked by gene expression changes differing over time in individuals which might be caused by patients' severe clinical conditions (two died during the follow-up). This highlights the challenges associated with examinations in patients suffering from severe and life-threatening diseases and analysis strategies on how to deal with it. We consider our results as an indication for a simplified diagnostic tool for identification of incorporated alpha-emitting radionuclides, but further research is required.

References

Confer, D., Chao, N. and Case, C. (2018). Are we prepared for nuclear terrorism? New England Journal of Medicine, Correspondence, doi:10.1056/NEJMc1805627.

Hall, E.J. and Giaccia, A.J. (2012). Radiobiology for the radiologist: Seventh edition. Radiobiology for the Radiologist: Seventh Edition.

IAEA – International Atomic Energy Agency (2005). Categorization of radioactive sources. IAEA Saf. Stand. Ser. No. RS-G-1, 70.

IAEA – International Atomic Energy Agency (2004). Strengthening control over radioactive sources in authorized use and regaining control over orphan sources.

IAEA – International Atomic Energy Agency (1988). The radiological accident in Goiânia. International Atomic Energy Agency.

Miller, K.L. (2012). NCRP Report No. 166, Population monitoring and radionuclide decorporation following a radiological or nuclear incident. Health Phys. doi:10.1097/hp.0b013e31822e57bc.

Rosoff, H. and Von Winterfeldt, D. (2007). A risk and economic analysis of dirty bomb attacks on the ports of Los Angeles and Long Beach. Risk Anal. doi:10.1111/j.1539-6924.2007.00908.x.

2.1.8 Application of Biodosimetry in Three Real Life Exposure Scenarios, C. Beinke, BIR, 2018

Industrial radiography, using sealed X- or γ-ray sources is widely used as a non-destructive testing method to inspect castings, welded assemblies and other engineering structures for internal defects such as blowholes or cracks. Despite the use of appropriate equipment in accordance with safe work practices poses a little risk of unintended radiation exposure to workers or members of the public, on average, industrial radiographers receive radiation doses exceeding those of other occupationally exposed workers. In this presentation the accidental gamma radiation exposure of a radiography worker and the cytogenetic examination of the worker's blood lymphocytes is described (Figure 2-4).

The presumed exposure of the worker was due to a malfunction in the shielding of the Ir192 source during operation. Because the source was sealed no additional beta radiation exposure was assumed. The TLD badge indicated an absorbed dose of 0.078 Sv which presumably took place in December 2013. No clinical symptoms were reported in the case history after the potential radiation exposure. In order to follow radiation protection aspects and to clarify the situation for the concerned worker, it was decided to perform biological dosimetry using dicentric chromosome and micronucleus analysis four month post exposure. Micronucleus frequency was not increased above the lab's control value of micronucleus background frequency of unexposed individuals (Figure 2-5).

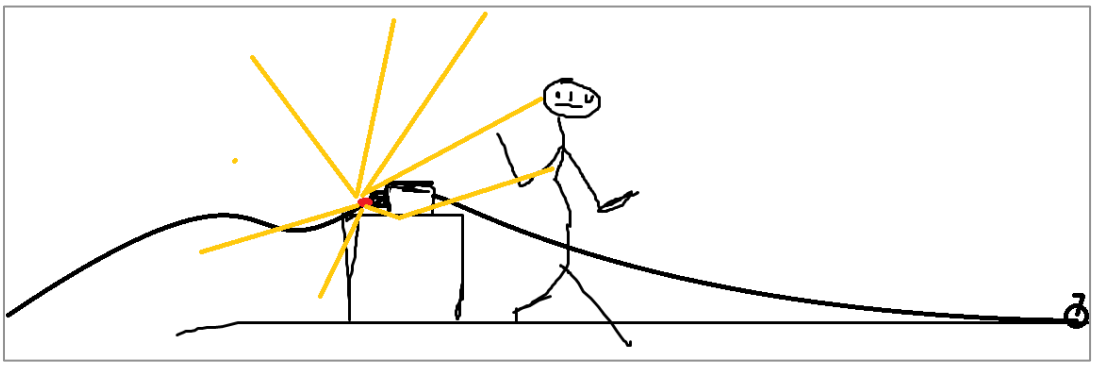


Figure 2-4: Location of W1 During Troubleshooting. Partial shielding of W1's body due to his position behind the device (dimensions: 35x15x25 cm, weight: 15 kg, located in 1m height on a box).

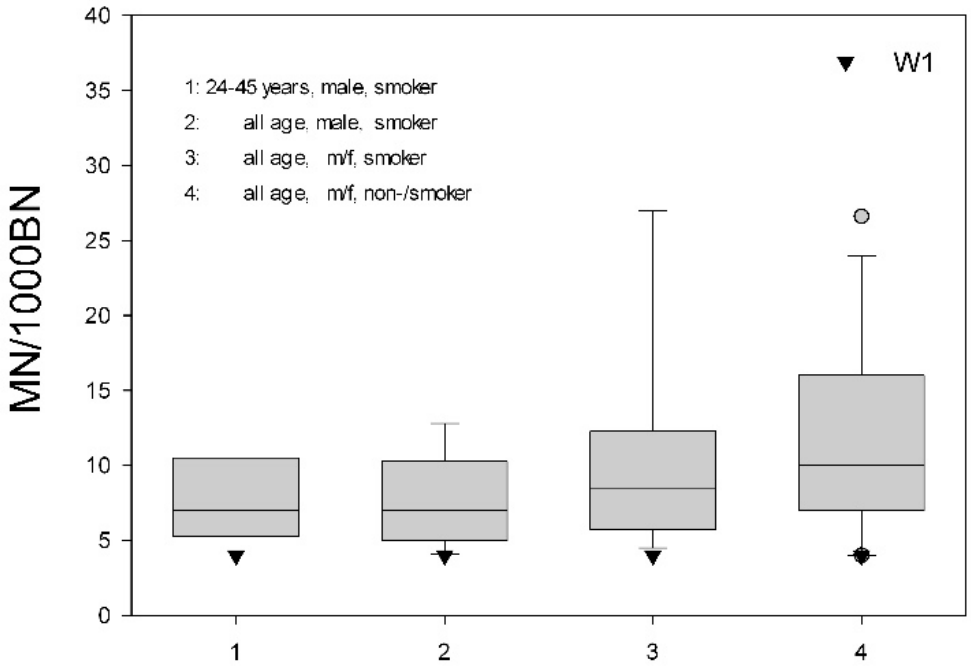


Figure 2-5: The MN Frequency of the Radiography Worker W1 (Triangle, W1 is Visualized within the Box Plots Representing the MN Frequency Distribution of Four Different Control Groups Following the Definitions Depicted within the Graph).

Dicentric analysis in 2,152 metaphase cells resulted in an estimated dose of no more than 0.159 Gy (95% upper confidence level) with a mean dose of 0.066 Gy (equivalent whole body exposure) based on interpolation from the lab's calibration curve for Co^{60} γ -radiation. The observed dicentric frequency (0.003 dicentrics/cell) differs significantly from the lab's background level of dicentric chromosomes in unexposed individuals (0.0007 dic/cell). As overdispersion of dicentric chromosomes (u = 9.78) indicated a heterogeneous (partial body) exposure, we applied the Dolphin method and estimated an exposure of 2.1 Sv affecting 21 % of the body volume. As the overdispersion of dicentric chromosomes is caused by only one cell containing two dicentrics, it is possible that this was an incidental finding. Thus, a very low-dose radiation overexposure of the radiography worker has to be assumed, although it remains uncertain, if in fact a heterogeneous exposure has occurred.

2.1.9 Radiotoxicology: Characterization of Inflammatory/Genotoxic Response after Irradiation in a THP1 Model, M. Drouet, IRBA, 2018

The presentation "Sharing and discussing about results related to an in vitro study of a pulmonary radio-contamination model" shown in Section 2.1.11 provides an updated version of the intermediate results shown herein.

2.1.10 Introduction of the H-Module App (Alpha Version), M. Majewski, BIR, 2018

Physicians require prompt guidance for diagnosis and therapeutic interventions of radiation injury patients after radiological or nuclear events (e.g., terrorist attacks, nuclear power plant accidents or use of an improvised nuclear device). Physical examination of patients and basic laboratory tests (Blood Cell Counts of lymphocytes, granulocytes and thrombocytes, BCC) are universally available and integrate the extent of irradiation (total or partial body) and individual response of each patient. Diagnostic guidance and treatment protocols for acute effects occurring after absorption of high radiation doses (to address ARS) have been established (Fliedner et al. 2001) and are frequently updated (Fliedner et al. 2001, Gorin et al. 2006). The Medical Treatment Protocols for Radiation Accident Victims (METREPOL) document serves as a resource for physicians. Hematologic changes, such as the development of severe immune deficiency (due to lymphocytopenia and granulocytopenia) or thrombocytopenia, represent one of the challenges in appropriate clinical management of HARS. Patient outcomes are improved as treatment decisions have benefited from early diagnosis (within the first three days post irradiation). For instance, recommended administration of cytokines should begin within the first day after exposure. Recently, filgrastim (G-CSF) was approved for the treatment of patients with HARS (Farese and MacVittie 2006, Hérodin and Drouet 2005).

In previous studies, we examined the utility of Blood Cell Counts (BCCs) in the first three days post irradiation to predict clinical outcome, specifically for Hematologic Acute Radiation Syndrome (HARS, Port et al. 2017). For the development of mathematical models (hypothesis generating) we analyzed 454 BCC samples from 267 individuals along with their clinical outcome HARS severity scores (H1-4). The BCC and HARS severity scores originated partly from radiation accident victims and were stored in the SEARCH database, the System for Evaluation and Archiving of Radiation Accidents based on Case Histories (SEARCH) database. In these previous analysis we created binary categories for severity scores, i.e., 1 (H0 vs. H1-4), 2 (H0-1 vs. H2-4) and 3 (H0-2 vs. H3-4), to assess the discrimination ability of BCCs using unconditional logistic regression analysis. The validation of the models was performed on a second independent group comprising another 275 BCCs from 252 individuals. Individuals with a score of H0 were easily separated from exposed individuals based on developing lymphopenia and granulocytosis. We found an almost complete discrimination of H0 vs. irradiated individuals during model validation (negative predictive value, NPV > 94%) for all three days, while the correct prediction of exposed individuals increased from day 1 (positive predictive value, PPV 78-89%) to day 3 (PPV > 90%). Hence, altogether 519 cases and 729 BCC were analyzed taking advantage of real case histories from radiation accidents and validation on clinical cases (Port et al. 2017). From there we developed a prediction model spread sheet to provide early and prompt diagnostic predictions and therapeutic recommendations including identification of the worried well, requirement of hospitalization or development of severe hematopoietic syndrome. These results improve the provisional classification of HARS. We tested this application during a NATO exercise with experts in the field and implemented the spread sheet in medical management class for radiobiology students with very good results. We showed that the clinical outcome of radiation injury patients can be rapidly predicted within the first three days post irradiation using peripheral BCC and our tool. However, students observed limitations of the spread sheet version. For instance, only blood cell counts from two consecutive days [first and second days] could be added to the spread sheet, but not day two only) and each day required another spread sheet, making it a little bit uncomfortable working with it. Also, when adjusting for patients suffering from acute/chronic infections again, another worksheet had to be used. That made the work with spread sheets confusing.

Large-scale events will probably overwhelm the capacity of few R/N experts. Here we see the requirement to broaden the accessibility of this knowledge to medical doctors who are not familiar with the ARS. This motivated us to transform the spreadsheet version into a smartphone application. The goal is to provide:

1) An App with some improvements over the existing spreadsheet version of the H-module (e.g., completeness and smoothened use);
2) Provide a simple interface;
3) Allow for easy distribution;
4) Provide a link to experts for support and diagnostic after triage. Novel treatment regimens for therapy of the Acute Radiation Syndrome (ARS) were developed over the last years. Their application relies on an early and high-throughput diagnosis.

Other than the collection of nine spread sheets (required to reflect different combination of daily BCCs and considering acute/chronic infections as a confounder on BCC counts) the H-module App integrates these inputs on one screen. The new architecture of the App and how it works is shown in Figure 2-6.

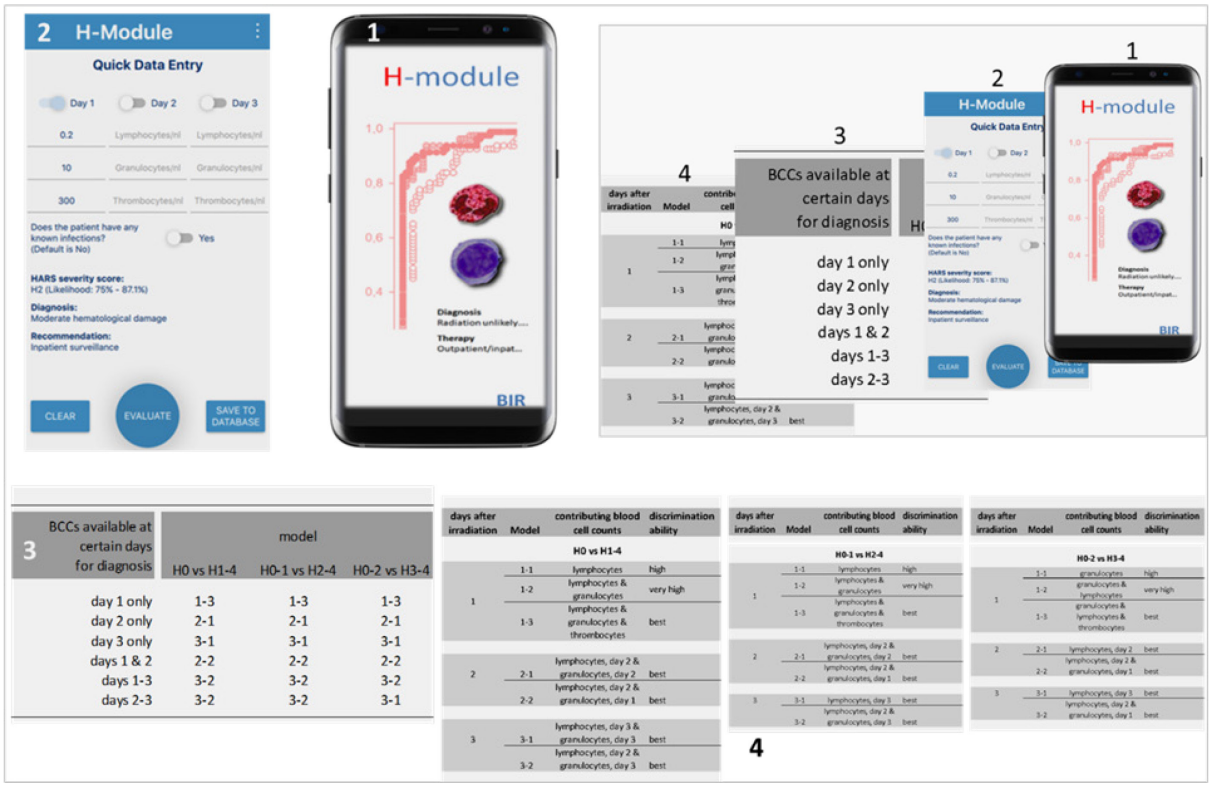


Figure 2-6: The H-module App Consists of Four Layers and Each Layer Provides the Base for the Next Step/Layer (Insert with Overlapping Layers to the Right). The layers are labeled (1 – 4) and shown in full on the left and below the insert.

References

Farese, A.M., MacVittie, T.J. (2015). Filgrastim for the treatment of hematopoietic acute radiation syndrome. Drugs Today (Barc), 51:537-48.

Fliedner, T.M., Friesecke, I., Beyrer, K. (2001). Medical Management of Radiation Accidents – Manual on the Acute Radiation Syndrome. Oxford: British Inst of Radiology.

Gorin, N.-C., Fliedner, T.M., Gourmelon, P., Ganser, A., Meineke, V., Sirohi, B., et al. (2006). Consensus conference on European preparedness for haematological and other medical management of mass radiation accidents. Ann Hematol, Oct; 85(10):671-9.

Hérodin, F., Drouet, M. (2005). Cytokine-based treatment of accidentally irradiated victims and new approaches. Exp Hematol, 33:1071-80.

Port, M., Pieper, B., Knie, T., Dörr, H., Ganser, A., Graessle, D., et al. (2017). Rapid prediction of hematologic acute radiation syndrome in radiation injury patients using peripheral blood cell counts. Radiat Res, Aug, 188(2):156-68.

2.1.11 A Quantitative Comparison of the Chemo- and Radiotoxicity of Uranium at Different Enrichment Grades, M. Port, A. Rump, BIR, 2019

Objective: The radiotoxic effects of uranium are often in the focus of the public fears, but the chemical toxic effects of uranium are reported to surpass radiation effects. As there is no uranium isotope that is not radioactive, it is not possible to study chemical effects fully independently from radiation effects. In order to quantitate and compare radio- and chemotoxicity, we determined the median lethal doses of uranium due to its chemical toxicity and calculated the absorbed radiological doses resulting from the ingestion or inhalation of corresponding amounts depending on the isotopic enrichment grade. Committed effective doses over 50 years are related to the stochastic health effects like cancer occurrence and can be converted to a loss of statistical life time (mean loss 0.4 day / mSv). The equivalent doses absorbed within a short time frame permits conclusion on the induction of deterministic effects (e.g., acute radiation sickness).

Method: Simulations were based on the biokinetic models of the International Commission for Radioprotection and performed using Integrated Modules for Bioassay Analysis software. Results were compared with the doses given by the calculator of the WISE uranium project. The fractions of the total doses absorbed within different time periods were derived from the respective areas under the activity-time curves in the whole body.

Results: The distribution of the total dose on the organs and tissues depends on the invasion pathway and the solubility of the compound. In the case of inhalation, the absorption of the total dose is more protracted than after ingestion. The incorporation of depleted or natural uranium in lethal amounts due to nephrotoxicity does not lead to deterministic radiation effects and is associated with committed effective doses reaching at most about 200 mSv (proposed possible threshold for therapeutic interventions after accidental radionuclide incorporation). The inhalation of low enriched uranium leads to higher effective doses up to 690 mSv, but they are still insufficient to cause acute deterministic effects. Even highly enriched uranium seems not to induce radiation nephropathy, but deterministic effects on the hematopoietic system cannot be excluded in particularly sensitive patients. But the equivalent doses to the lungs associated with the inhalation of poorly soluble compounds of highly enriched uranium are in a range that may induce radiation pneumonitis.

Conclusion: Our findings give clear evidence that for depleted and natural uranium chemical toxicity is much more marked than radiotoxicity. However, this conclusion must not be drawn for enriched and in particular highly enriched compounds that besides stochastic effects may even cause deterministic radiation effects.

This summary provides a shortened version of a recent publication and more details can be found in the following reference.

References

Rump, A., Eder, S., Lamkowski, A., Hermann, C., Abend, M., Port, M. (2019). A quantitative comparison of the chemo- and radiotoxicity of uranium at different enrichment grades. Toxicol Lett 313:159-168.

2.1.12 Sharing and Discussing about Results Related to an in vitro Study of a Pulmonary Radio-Contamination Model, D. Riccobono, IRBA, 2019

Lung exposure to actinides, α emitters, such as plutonium (^{239}Pu), can occur following a reactor incident in the nuclear industry, a malicious act targeting a nuclear power plant or a dirty bomb explosion (Yamamoto 2013). ^{239}Pu is known to induce pulmonary fibrosis and cancers after contamination (Sanders, Lauhala et al. 1993, Dudoignon, Guillet et al. 2003, Muggenburg, Guilmette et al. 2008, Griffiths, Van der Meeren et al. 2010). Injectable diethylenetriaminepentaacetic acid (DTPA), a chelating agent, is currently the reference treatment for ^{239}Pu and actinides contamination (IAEA 2018) allowing soluble form decorporation. DTPA is however poorly efficient on ^{239}Pu insoluble form at retention site (lungs after internal contamination by inhalation). There is therefore a need to improve therapeutic management following internal contamination with actinides. We made hypothesis that ^{239}Pu distribution can influence lung damages and that limitation of damages can modify ^{239}Pu accessibility to chelating agents. Based on these hypothesis, combination of DTPA with anti-inflammatory (dexamethasone (DEX) and mucolytic/expectorant (Ambroxol (AMB)) molecules were studied in order to optimize pulmonary ^{239}Pu decoporation and to limit lung damages.

THP-1 cells (human monocytes cell line, a reference macrophages model (Auwerx 1991)), were differentiated into macrophages using phorbol 12-myristate 13-acetate (PMA). Cells were then either irradiated with X-ray using Small Animal Radiation Research Platform (SARRP, 0-20 Gy) on standard culture plates or contaminated with ^{239}Pu colloids (300 Bq/well) on inserts (Figure 2-7). In the first case, cell viability, pro-inflammatory cytokines secretions and DNA Double-Strand Breaks (DSB) were measured from 1h to 72h post irradiation using respectively PrestoBlue® and CytotoxOne, ELISA dosing method and γ-H2AX foci staining to validate THP-1 model choice. In the second case, ^{239}Pu distribution between upper and lower compartments (retention in upper compartment, Figure 2-7) was assessed using Liquid Scintillation (LS) activity measurements up to 7 days after contamination in presence of different treatments (DTPA 50 µM, DEX 3.7 µM and/or AMB 50 µM) to identify most promising therapeutic strategies

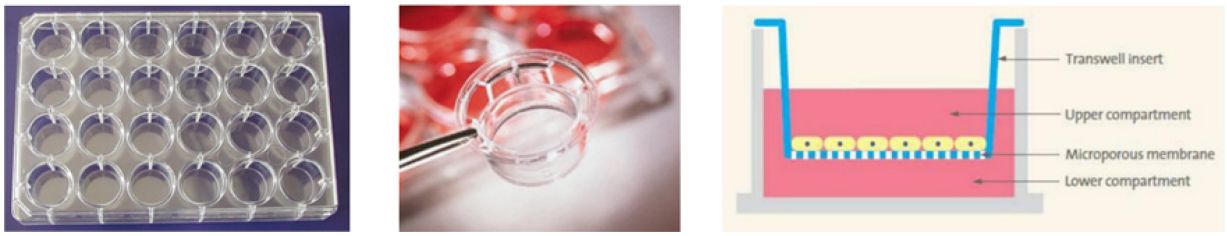


Figure 2-7: Transwell® Inserts Cell Culture Device. Cells were cultured on microporous membrane and ^{239}Pu colloids were deposited in the upper compartment. Activity measurements (liquid scintillation) were carried out on lower compartment media samples at different times post-contamination.

X-ray exposures up to 20 Gy did not decrease THP-1 cells viability. Nevertheless, high doses x-ray exposures (from 10 Gy) induced marked increases of IL-6 (interleukin-6) and MCP-1 (Monocyte Chemoattractant Protein 1) concentrations in cells supernatants that persisted up to 72h post irradiation. In the other experimental conditions, MCP-1 decreased progressively over time following irradiation. Cell exposure to DEX induced a significant decrease in MCP-1 quantities while AMB did not show any effect. Between 5h and 48h post-irradiations at 20 Gy, increases of DNA DSB (γ-H2AX foci) were observed in THP-1 cells.

After ^{239}Pu contamination in the upper compartment, THP-1 cells phagocyted part of ^{239}Pu particles. DTPA treatment promoted ^{239}Pu dissolution and its release from cells. On the contrary, DEX or AMB alone did not modify ^{239}Pu release rate. However, associations of DTPA with DEX seemed to increase ^{239}Pu release rate from cells compare to DTPA alone (Figure 2-8).

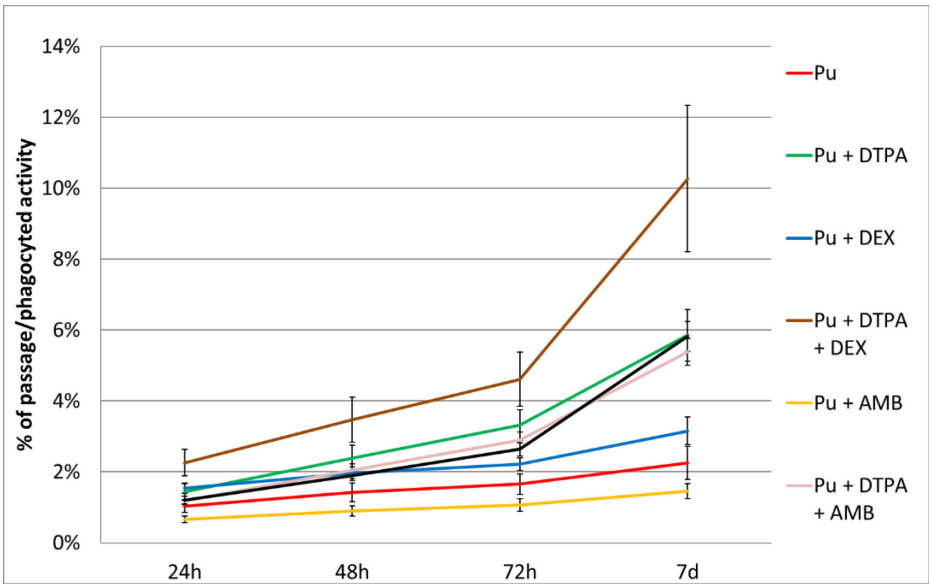


Figure 2-8: Dissolution of ^{239}Pu Colloid Particles after Treatment with DTPA, DEX and/or AMB. Percentage of activity passage in the lower compartment in relation to the cells phagocytized activity (%) 24h, 48h, 72h and 7 days (7d) post contamination ± standard deviation.

THP-1 cell line is a relevant model to assess efficiency and toxicity of potential actinides decorporation treatments combinations based on inflammation, genotoxicity and cell viability assessment. First results after ^{239}Pu contamination will have to be completed by pro-inflammatory cytokines ELISA dosing on cells supernatants, autoradiographies and immunostaining analyzes. Moreover, an *in vivo* study will be implemented to assess treatments efficiency on rats after lungs contamination with ^{239}Pu (intra-tracheal instillations). In this study, ^{239}Pu biodistribution, inflammatory response, lung macrophages activation and pulmonary genotoxicity will be studied.

References

Auwerx, J. (1991). The human leukemia cell line, THP-1: A multifacetted model for the study of monocyte-macrophage differentiation. Experientia 47(1): 22-31.

Dudoignon, N., Guillet, K. and Fritsch. P. (2003). Evaluation of risk factors for lung tumour induction in rats exposed to either NpO(2) or PuO(2) aerosols. Int J Radiat Biol 79(3): 169-174.

Griffiths, N.M., Van der Meeren, A., Fritsch, P., Abram, M.C., Bernaudin, J.F., Poncy, J.L. (2010). Late-occurring pulmonary pathologies following inhalation of mixed oxide (uranium + plutonium oxide) aerosol in the rat. Health Phys 99(3): 347-356.

IAEA (2018). Medical Management of Persons Internally Contaminated with Radionuclides in a Nuclear or Radiological Emergency. Vienna.

Muggenburg, B.A., Guilmette, R.A., Hahn, F.F., Diel, J.H., Mauderly, J.L., Seilkop, S.K., Boecker, B.B. (2008). Radiotoxicity of inhaled (239)PuO(2) in dogs. Radiat Res 170(6): 736-757.

Sanders, C.L., Lauhala, K.E., McDonald, K.E. (1993). Lifespan studies in rats exposed to 239PuO2 aerosol. III. Survival and lung tumours. Int J Radiat Biol 64(4): 417-430.

Yamamoto, L.G. (2013). Risks and management of radiation exposure. Pediatr Emerg Care 29(9): 1016-1026; quiz 1027-1029.

2.2 MEDICAL COUNTERMEASURES

2.2.1 Cutaneous Radiation Syndrome

2.2.1.1 New Therapeutic Approaches of the Cutaneous Radiation Syndrome at IRBA, D. Riccobono, FRA, 2017

Irradiation exposure may be accidental, in civilian or military context with industrial source for example or nuclear weapon accident but malicious as well with terrorism such as a Radiological Exposure Device (RED) which is a hidden source in a place with many people like the theatre, metro, etc. In all cases, the exposure to ionizing radiations leads to several symptoms depending on the dose and whether it is a partial or on total body irradiation. For total body irradiation, an acute radiation syndrome is observed with 3 components: gastrointestinal neurological and hematological symptoms. By contrast, local irradiation of the skin leads to Cutaneous Radiation Syndrome (CRS). CRS does not have any pathognomonic signs but its clinical course is very specific starting with a first erythema or a blister and then a latency period that will be shorter for higher doses. After this latency period, there are a second erythema, a hair loss, dry and then moist desquamation appear associated with extensive and unpredictable inflammatory waves. Due to this extensive inflammation, the final lesion is much larger than the initial injury. Finally, there is no complete healing and the development of fibrosis is observed with recurrent and painful inflammation for doses above 15Gy or a persistent and painful necrosis with an important disability for doses above 25 Gy (Peter 2013). Currently the reference treatment for IAEA is based on autologous Bone Marrow Mesenchymal Stem Cells (BM-MSC) injections. Mesenchymal stems cells are used in this therapeutic strategy, as a cellular medicine based on their tissue regeneration properties. The stem/stromal cells wherever they come from, have common properties. They are multipotent cells so they can differentiate in several specialized cells. They can be found in several tissues like skin, bone marrow, and fat tissue for example. They can migrate to injury sites and have immunomodulation properties. But one of the most important points is that they can secrete many paracrine factors such as VEGF, HGF, etc. involved in tissue repair (Spencer, Gimble et al. 2011). The IAEA reference treatment protocol is composed of dosimetry guided surgery, and autologous skin graft. It starts as soon as possible after irradiation with physical dosimetry, followed by a surgical excision of irradiated area associated with BM-MSC harvesting. Then autologous skin grafts are performed while BM-MSC are expanded. After cell quality control, iterative BM-MSC injections are administered in conjunction, or not, with skin graft according to repair evolution (IAEA 2009). Unfortunately, this treatment leads to a significant disability due to tissue excision. Moreover, BM-MSC harvesting is invasive and painful; a large number of cells is also required over a long period of long time so this treatment cannot be used in the case of mass casualties. So, there are many optimization strategies and 3 of them are evaluated at the French Armed Forces Biomedical Research Institute.

First, the use of another kind of stromal cell: Adipose Tissue Derived Stromal Cells (ADSC). Their harvesting is less invasive; they are up to 500 times more abundant than BM-MSC so the expansion time is reduced and their properties are equal or even more important than BM-MSC ones (Bochev et al. 2008). Many results have been reported using ADSC for the treatment of skin burns, chronic ulcer, and skin injury but also for muscle damage such as myocardic ischemia and wounded skeletal muscle (Suzuki et al. 2015, Condé-Green et al. 2016, Raposio et al. 2016). The studies are well advanced with animal model and phase I to 3 clinical trials. In the Radiobiology Unit of IRBA, a minipig model of CRS was developed to evaluate these cells on radiation skin injury (Figure 2-9).

Minipigs ADSC were harvested 30 days before irradiation and cells were expanded. On day 0 minipigs were irradiated. They received a local high-dose irradiation of 50 Gy in the lumbar area using a cobalt60 source. Five animals received 4 subcutaneous injections on days 25, 46, 76 and 96 post irradiation of 50 million autologous ADSC and 8 controls received the vehicle which is PBS. The clinical evolution of controls was very similar to human. After a latency period, large inflammatory processes were observed with dry and moist desquamations leading to the development of persistent and painful necrosis. By contrast, we observed

a transient inflammation after each injection for animals that received subcutaneous ADSC but it was not harmful and evolved to wound healing (Figure 2-10(A)).

So, subcutaneous ADSC injections improve skin wound healing in CRS. Moreover, histological studies were performed such as hematoxylin eosin staining and von Willebrandt immunostaining (Figure 2-10(B), Figure 2-10(C)). A major disorganization of epidermal layers without any revascularization markers in irradiated controls was detected whereas there was a complete epidermis recovery with a major revascularization in animals that received ADSC (Forcheron et al. 2012). Nevertheless, there was a subcutaneous defect. Necropsies have highlighted that it was due to subcutaneous muscles fibrosis.

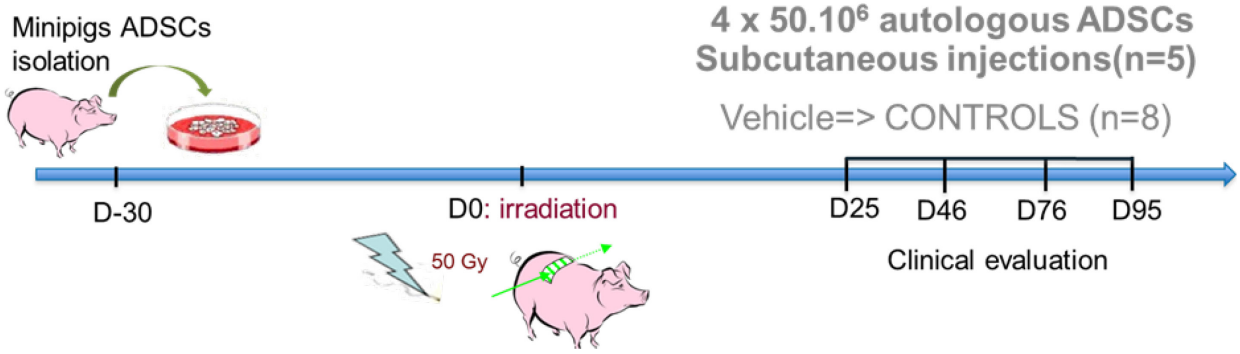


Figure 2-9: French Armed Forces Biomedical Research Institute Model of CRS on Minipigs.

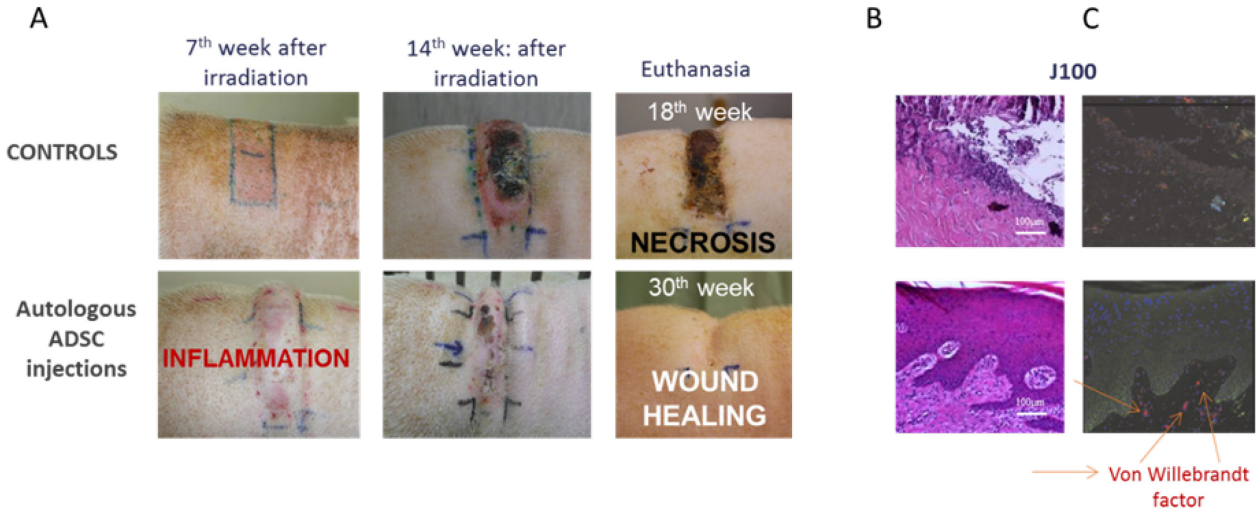


Figure 2-10: Minipigs' Skin Evolution After a 50 Gy Local Irradiation. (A) Clinical Evolution; (B) Hematoxylin Eosin Staining on Irradiated Cutaneous Tissues on Day 100 Post Irradiation; (C) von Willebrandt Immunostaining on Irradiated Cutaneous Tissues on Day 100 Post-Irradiation.

To reduce the radio-induced muscle defect, the same irradiation protocol followed by the association of subcutaneous and intramuscular autologous ADSC injections was evaluated in a short-term preliminary study. Three controls received PBS and 3 animals received 150 million autologous ADSC split into 3 subcutaneous injections and 75 million autologous ADSC split into 3 intramuscular injections on day 25, 46, 66. The observation of muscle tissue after necropsies performed on day 76 post irradiation highlighted more significant necrosis and mostly fibrosis areas in controls' muscles compared to animals that received SC and IM ADSC (Figure 2-11(A)). This was confirmed with Hematoxylin Phloxin Saffron (HPS) and immunofluorescent

staining. Large fibrosis was observed (HPS staining) confirmed by red collagen deposit specific of fibrosis in irradiated controls (Figure 2-11(B)) (Riccobono et al. 2016).

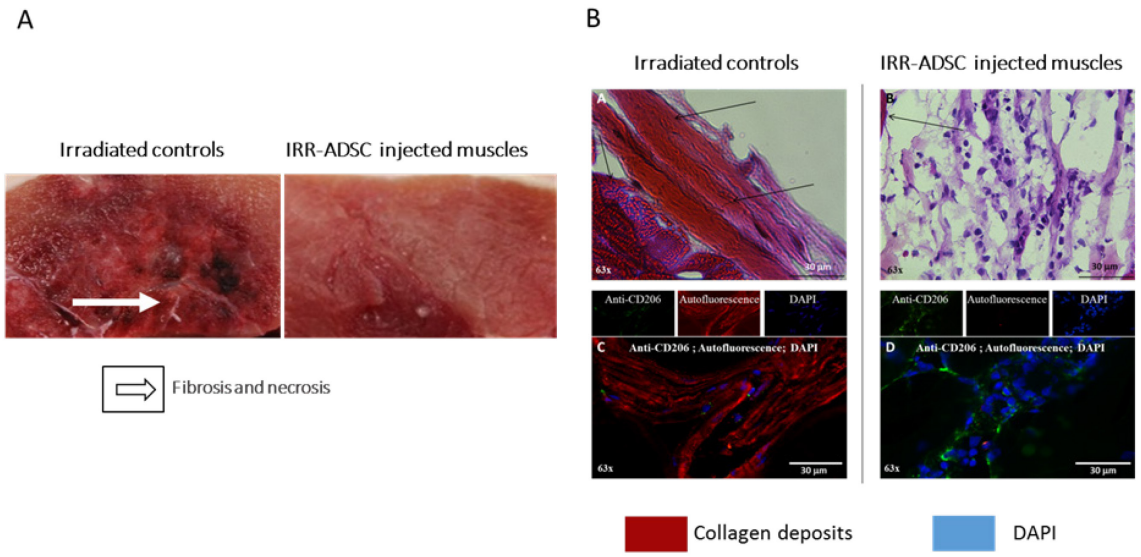


Figure 2-11: Gy Local Irradiated Muscle Tissues Analysis on Day 76 Post Irradiation. (A) Macroscopic observation of necropsies; (B) HPS and collagen (red) muscle nucleus (DAPI) staining.

These results were consistent with a decrease of muscular fibrosis in CRS as well as a modulation of inflammatory response after ADSC intramuscular injections. Indeed, during wound healing M1 polarization of inflammatory response is required to clean the area and initiate tissue repair. Then there is a switch to M2 polarization characterized by the secretion of anti-inflammatory cytokines such as IL10, TGF beta. In this work, these cytokines production was evaluated. IL10 analyzed by western blot was only detected in the irradiated area that received ADSC. Moreover, TGF beta evaluated by in situ hybridization was observed only in irradiated areas that received stromal cells as well. So ADSC seem to promote M2 polarization of the inflammatory response (Riccobono et al. 2018).

Secondly, the use of gene therapy associated or not with ADSC may be an innovative strategy. This allows targeting a specific pathway to optimize the cells secretome and thus to reduce the number of required cells. It is currently evaluated for critical limb ischemia using plasmid encoding for HGF in phase I and II clinical trials for example (Cui et al. 2015). In the Institute, the studies focused on Shh protein because it is a secreted protein implied in angiogenesis, immunomodulation, and cell proliferation (Bambakidis and Onwuzulike 2012). All these properties are very interesting in tissue repair but are also similar to oncogenesis. So, a plasmid encoding for Shh was produced and transiently transfected in ADSC by nucleofection. Thus, there is no integration to avoid tumor processes. We obtained ADSC-Shh (Figure 2-12).

A preliminary study has been performed using the same protocol as previously described, but 5 days before injections ADSC were transfected and 4 subcutaneous injections of only 25 million ADSC-Shh were performed instead of 50 million ADSC. The injections were administered to 2 minipigs in this instance. The evolution of the animals that received ADSC-Shh was very interesting with an induration of the lesion 14 weeks after irradiation and finally wound healing. So, in this preliminary study Shh transfected ADSC improved CRS treatment with twice less cells than ADSC (Riccobono et al. 2014).

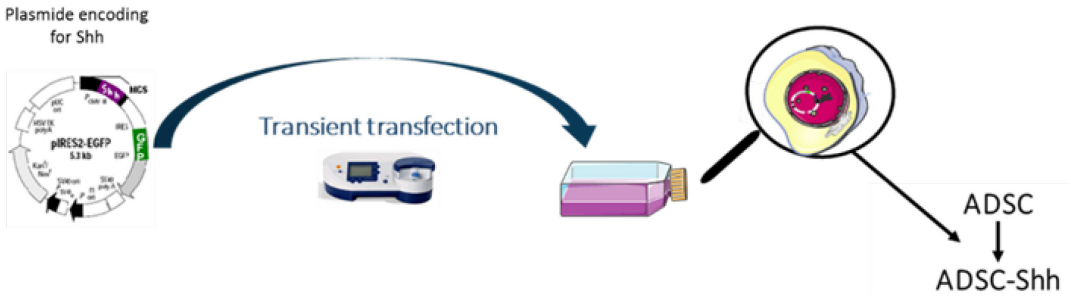


Figure 2-12: ADSC Transfection Protocol.

Finally, the use of stromal cells secretome with more or less gene therapy is the last optimization evaluated in the French Armed Forces Biomedical Research Institute. This technic avoids HLA matching and enables to decrease onset of therapy. Currently, many studies only use a part of the secretome: the exosomes. No results have been reported yet on human but exosomes from ADSC have been shown to increase wound healing in mice skin injury model (Kapur and Katz 2013). An in vitro study was performed at IRBA with the complete secretome. The same transfection protocol as previously described was used to obtain ADSC and ADSC-shh. One week later, fibroblasts were irradiated with 25 Gy. Just after irradiation, culture media were removed and replaced by stem cells culture media to obtain conditioned media with ADSC, ADSC-Shh and a control medium. Different parameters were evaluated. From 2 days to 4 days post irradiation there was a very important and significant difference between the 2 conditioned media ADSC / ADSC-Shh CM and the control concerning fibroblasts senescence (Figure 2-13(A)). Moreover, VEGF secretion by fibroblasts after irradiation was evaluated by RTqPCR and western-blotting and revealed a significant increase of VEGF transcription and production 2 days post irradiation for fibroblast in ADSC-Shh conditioned media (Figure 2-13(B)). So ADSC-Shh conditioned medium also increased pro-angiogenic factors expression in irradiated fibroblasts.

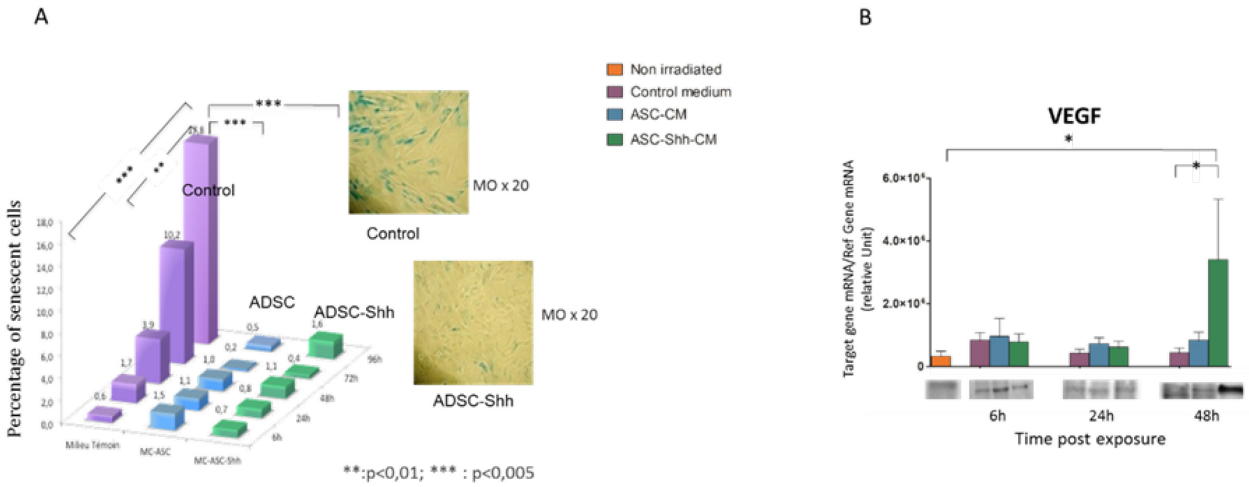


Figure 2-13: Effects of ADSC and ADSC-Shh Conditioned Media on 25 Gy Irradiated Fibroblasts. (A) Analysis of senescence by β-galactosidase staining; (B) Evaluation of VEGF production by RTqPCR and western-blotting.

These 3 optimization strategies for the treatment of cutaneous radiation syndrome were evaluated at IRBA and are very interesting and promising but this is absolutely not an exhaustive list. Many other innovative strategies using the association of stromal cells and biomaterials as well as stromal cells priming are currently assessed. Nevertheless, nowadays all these strategies are currently based on stromal cells or their secretome.

References

Bambakidis, N.C., Onwuzulike, (2012). K. Sonic Hedgehog signaling and potential therapeutic indications. Vitam Horm 88: 379-394.

Bochev, I.G. et al. (2008). Mesenchymal stem cells from human bone marrow or adipose tissue differently modulate mitogen-stimulated B-cell immunoglobulin production in vitro. Cell Biol Int 32(4): 384-393.

Condé-Green, A. et al. (2016). Adipose Stem Cells Isolated from Excised Burned Tissue: Is There Potential for Clinical Use? Plast Reconstr Surg 137(4): 767e-768e.

Cui, S.L. et al. (2015). Clinical safety and preliminary efficacy of plasmid pUDK-HGF expressing Human Hepatocyte Growth Factor (HGF) in patients with critical limb ischemia. Eur J Vasc Endovasc Surg 50(4): 494-501.

Forcheron F et al. (2012). Autologous adipocyte derived stem cells favour healing in a minipig model of cutaneous radiation syndrome." PLoS One 7(2): e31694.

IAEA – International Atomic Energy Agency. (2009). The radiological accident in Nueva Aldea. STI/PUB/1389. Vienna.

Kapur, S. et al. (2013). Review of the adipose derived stem cell secretome. Biochimie 95(12): 2222-2228.

Peter, R.U.(2013). Cutaneous radiation syndrome after accidental skin exposure to ionizing radiation. Hautarzt 64(12): 894-903.

Raposio, E. et al. (2016). Isolation of autologous adipose tissue-derived mesenchymal stem cells for bone repair. Orthop Traumatol Surg Res 102(7): 909-912.

Riccobono, D. et al. (2016). Contribution of INTRAMUSCULAR autologous adipose tissue-derived stem cell injections to treat cutaneous radiation syndrome: preliminary results. Health Phys 111(2): 117-126.

Riccobono, D. et al. (2014). Transient gene therapy to treat cutaneous radiation syndrome: Development in a minipig model. Health Phys 106(6): 713-719.

Riccobono, D. et al. (2018). First insights into the M2 inflammatory response after adipose-tissue-derived stem Cell injections in radiation-injured muscles. Health Phys 115(1): 37-48.

Spencer, N. et al. (2011). Mesenchymal Stromal Cells: Past, Present, and Future. Vet Surg. Feb;40(2):129-39.

Suzuki, E. et al. (2015). Adipose tissue-derived stem cells as a therapeutic tool for cardiovascular disease. World J Cardiol 7(8): 454-465.

2.2.1.2 Low-Level Laser Therapy to Mitigate Radiation Burns: Hype or Reality? D. Riccobono, IRBA, 2018

After irradiation, there is a cascade of physical, chemical and biological events leading to cell death. Ionizing radiation's first effects in cell are water radiolysis and the production of Reactive Oxygen Species (ROS) leading to DNA damage (Figure 2-14(A)). Depending on the efficiency of the repair pathway, all these mechanisms cause cell mutation leading to stochastic effects or cell death leading to determinist tissular pathology. Unlike cell therapy, which operates at the level of tissue lesions, Low-Level Laser Therapy (LLLT-LED) may be used to intervene during initial ionizing radiation effects (Avci, Gupta et al. 2013).

Therapeutic red light is currently used for psoriasis treatment or thermal burn, for example (Chiarotto, Neves et al. 2014). According to its wavelength, light can go through the tissue. Red light and specifically 670 nm wavelength is very interesting because it can go through epidermis and reach the dermis to have biological effect. It activates cytochrome C oxidase in mitochondria leading to an increase of second messengers such as ATP, ROS, nitric oxide, leading to a decrease of cell apoptosis and an improvement in cell migration, adhesion and proliferation (Hayworth, Rojas et al. 2010, Luo, Sun et al. 2013).

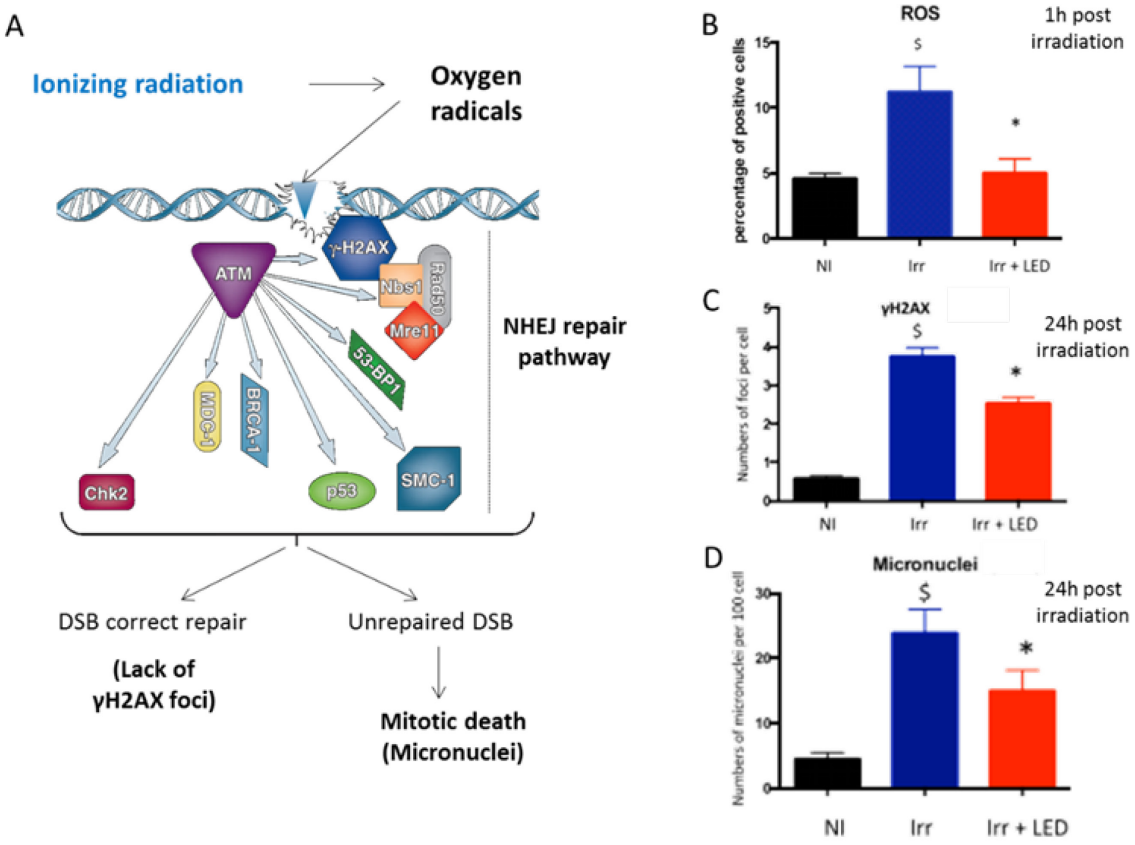


Figure 2-14: Mechanisms Following Irradiation and their Evaluation in a 5 Gy Irradiated Fibroblasts Model Exposed or Not to LLLT (LED). (A) Simplified biological pathway after ionizing radiation exposure; (B) ROS evaluation 1h after irradiation; (C) γH2AX foci evaluation 24h after irradiation; D: micronuclei evaluation 24h after irradiation.

Thus, at the French Armed Forces Biomedical Research Institute a protocol was performed to evaluate the application of LLLT for the treatment of Cutaneous Radiation Syndrome (CRS). Dermal fibroblasts from minipigs dermis were 5Gy irradiated and then exposed or not to LLLT(LED) for a period of 5 min. As irradiation leads to reactive oxygen species and so to DNA breaks, we explored ROS and DNA double stand breaks after LLLT. Concerning ROS creation, we noticed that there was a significant increase of ROS 1h post irradiation for irradiated fibroblasts and significantly less ROS in irradiated + LLLT fibroblasts (Figure 2-14(B)). Concerning DNA repair pathway, phosphorylation of histone H2AX is an interesting marker of DNA double-strand breaks. Moreover, 24h after irradiation it is considered that unrepaired DSB will not be further repaired.

There was also a significant decrease of phospho H2AX foci in irradiated + LLLT fibroblast (Figure 2-14(C)). Finally, unrepaired DSBs lead to mitotic death and the formation of micronuclei. The last results highlight that there was a significant decrease of micro nuclei for irradiated + LLLT fibroblasts compared to irradiated controls (Figure 2-14(D)).

So, to conclude, while further research is necessary, such as ROS enzyme study, components of DNA damage repair evaluation, etc., these preliminary results are very interesting in that they show a decrease of ROS, remaining DSB and micronuclei. Thus, LLLT may represent another innovative strategy for very early management of local acute radiation exposure.

References

Avci, P. et al. (2013). Low-Level Laser (Light) Therapy (LLLT) in skin: Stimulating, healing, restoring. Semin Cutan Med Surg 32(1): 41-52.

Chiarotto, G. et al. (2014). Effects of laser irradiation (670-nm InGaP and 830-nm GaAlAs) on burn of second-degree in rats. Lasers Med Sci 29(5): 1685-1693.

Hayworth, C. et al. (2010). In vivo low-level light therapy increases cytochrome oxidase in skeletal muscle. Photochem Photobiol 86(3): 673-680.

Luo, L. et al. (2013). Effects of low-level laser therapy on ROS homeostasis and expression of IGF-1 and TGF-β1 in skeletal muscle during the repair process. Lasers Med Sci 28(3): 725-734

2.2.1.3 New Models for Local Muscular Irradiation, D. Riccobono, IRBA, 2019

The civilian population but also the armed forces are not protected from being injured by high doses of radiation. These may be in connection with terrorist acts or of accidental origin. In high-dose irradiation situations, the first physiological barrier is subcutaneous musculature after skin. This results in severe injuries, with strong inflammatory and degenerative components whose evolution is highly invalidating (Cutaneous Radiation Syndrome; CRS). However, no satisfactory pharmacological solution is currently available to treat the victims.

It is therefore necessary to develop new therapeutic strategies to improve post irradiation tissue repair. The objective of the project is to evaluate the benefit of a pharmacological protocol, based on the use of recombinant Sonic Hedgehog (Shh) in a model of mouse myoblasts differentiation. Indeed, Shh is well known to be pro-myogenic and the corresponding recombinant peptide has previously been shown to improve muscular repair in vivo in ischemic or inflammatory muscle pathologies in mice (Elia, et al. 2007, Guo, et al. 2015). To evaluate the potential therapeutic effect of mouse recombinant Shh to protect cells after irradiation and to favor their proliferation, survival and differentiation a preliminary study was performed on irradiated myoblasts.

The effects of a high radiation dose (X-rays; 5 Gy) were analyzed in a model of mouse myoblasts (C2C12 cells) in their immature state or during their differentiating process toward mature muscle myofibers, obtained by culture of C2C12 in differentiation medium (DMEM with Horse serum) (Figure 2-15).

Proliferation, metabolism and myogenesis genes expression have been studied. Targeted genes and proteins expression have also been analyzed respectively by real time PCR and immuno-staining. A real and significant negative impact of irradiation has been shown on all parameters between day 0 and day 7 after irradiation (Figure 2-16(A)). Concerning myogenic genes expression, early genes expression (Pax3) were drastically decreased from day 2 to 7 in differentiation condition, reflecting a rapid maturation of C2C12. Irradiation limit this decrease. Conversely, a quick increase of mature cells markers expression was observed when cells differentiate (MyoG, myogenin or ENO3, enolase 3) and irradiation strongly limits this overexpression. Finally, muscle proteins expression has been assessed and a marked decrease of Pax7 (Figure 2-16(B)) and Myf5 expression was observed after irradiation.

Thus, a major scientific interest lies in such a project to improve the understanding of the involved in muscular regeneration in CRS. The next step will be the evaluation of potential therapeutic effect of Shh to protect and improve cells proliferation after irradiation.

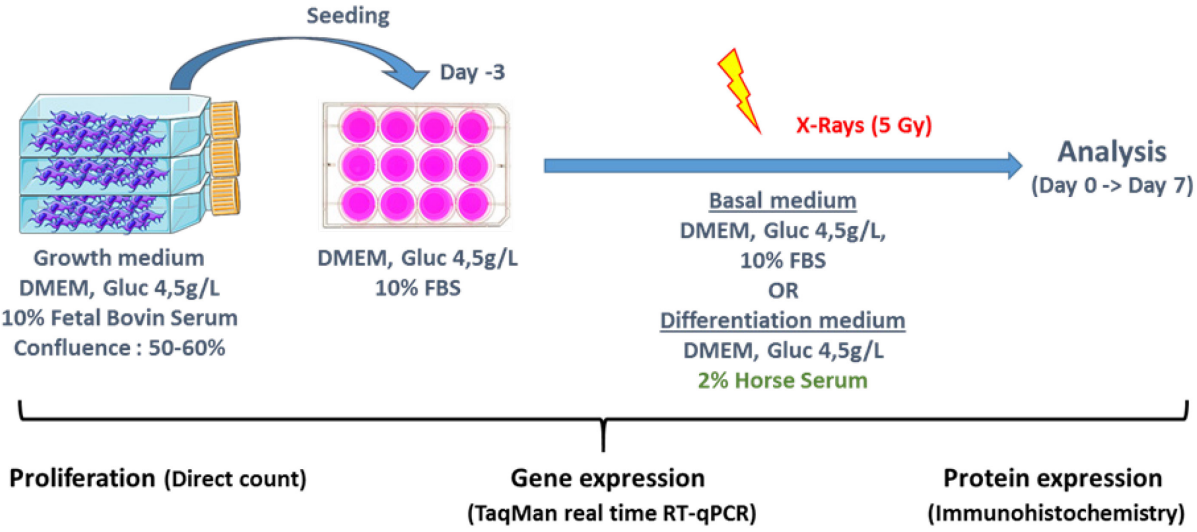


Figure 2-15: Differentiation and Irradiation Protocol of C2 C12 Mice Cells.

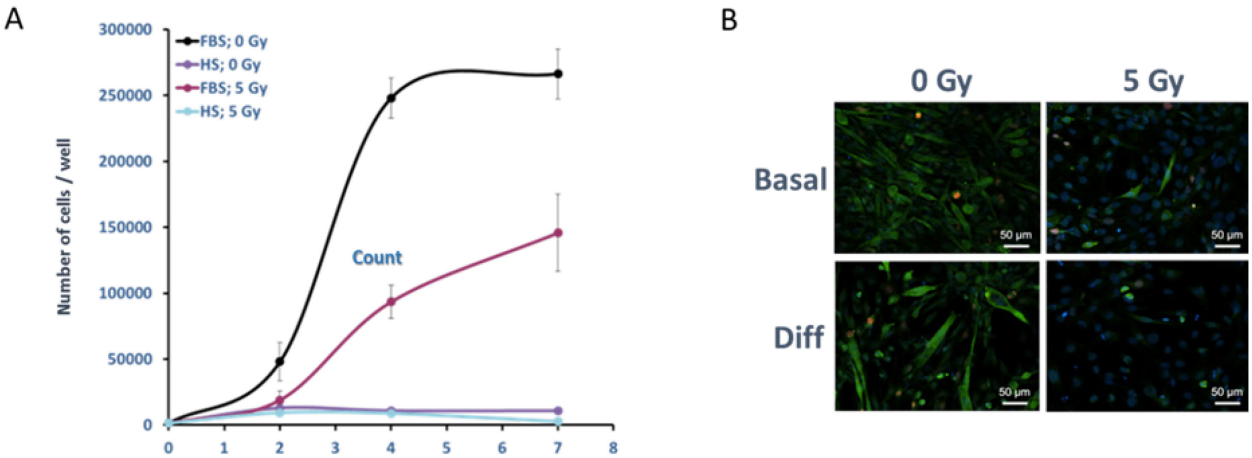


Figure 2-16: Effects of Irradiation on C2C12 with or Without Differentiation Medium. A: Analysis of cell proliferation; B: Pax7 immunostaining 7 days after irradiation.

References

Elia, D. et al. (2007). Sonic hedgehog promotes proliferation and differentiation of adult muscle cells: Involvement of MAPK/ERK and PI3K/Akt pathways. Biochim Biophys Acta 1773(9): 1438-1446.

Guo, W. et al. (2015). Activation of SHH signaling pathway promotes vasculogenesis in post-myocardial ischemic-reperfusion injury. Int J Clin Exp Pathol 8(10): 12464-12472.

2.2.2 Radiation Bioeffects Modulation by a Small Molecule Inhibitor, A. Tichý, CZE, 2017

The increasing risk of acute large-scale radiological/nuclear exposures of the population underlines the need to improve efficient radioprotective tools that could be used for radiation protection purposes in public situations such as a nuclear accident, an act of radiologic terrorism, or military conflict. In addition, radiotherapy is still a key modality for treatment of cancer. Unfortunately, it is associated with a number of negative side effects such as the death of bone marrow hematopoietic stem cells or enterocytes, due to the DNA damage, induced signaling pathways, and inflammatory responses and associated generation of Reactive Oxidative Species (ROS), eventually leading to cell death or senescence of cells in normal healthy tissue (Najafi et al. 2018). A radioprotective effect can be achieved through various mechanisms (enhancement of DNA repair, free-radical scavenging, cell synchronization, modulation of growth factors and cytokines, modulation of redox sensitive genes, inhibition of apoptosis, interaction and chelation of the radionuclides, or via gene therapy or stem cell therapy. When searching for an appropriate target, our effort was based on the fact that Ionizing Radiation (IR) is able to trigger programmed cell death in mammalian cells. While this effect is desirable during radiotherapy of cancer cells, it leads to radiotoxicity in normal healthy tissues.

Two major signaling pathways of apoptosis can be recognized in normal and tumor cells. Extrinsic pathway occurs after ligation of cytokines or factors, such as Tumor Necrosis Factor alpha (TNFalpha) or Fas ligand (FasL), to their specific cytoplasmic membrane receptors. In contrast, the intrinsic one is activated by a wide range of stimuli, including growth factor deprivation, oxidants, DNA damaging agents, or microtubule targeting drugs (Hassan et al. 2014).

We previously identified p53-up-regulated mediator of apoptosis (PUMA) as the key molecule of IR-induced cell death signaling and thus a promising target in radioprotection, but also in the therapy of neurodegenerative and cardiovascular diseases (Tichý et al. 2018). PUMA is often associated with IR-induced apoptosis and it can be up-regulated in both p53-dependent and p53-independent manner. Now, we present the basic characteristics of novel compounds – derivatives of 1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-phenoxypropan-2-ol that was screened and selected as one of the leading structures by Mustata et al. in 2011.

Cytotoxicity screening was performed on a panel of human cell lines. In vitro experiments were carried out in order to assess basic parameters such as half-maximal inhibitory concentration (IC50), Maximum Tolerated Concentration (MTC), and inhibition of IR-induced apoptosis in selected cell lines. Finally, the most promising compounds were subjected to in vivo tests on whole body irradiated mice in order to evaluate their radioprotective potential.

With a deeper molecular understanding of pathways involved in response to ionizing radiation, new targeted therapies could be developed with the advantage of greater specificity towards diverse molecular pathways activated in various distinct cell types (e.g., tumor vs. non-malignant). A multitude of pathways is involved in cell death following irradiation apoptosis induction by intrinsic pathway, but also chronic inflammation and oxidative stress, as well as DNA repair suppression. All of these could be a target of possible radioprotective small molecular inhibitors. Our efforts were driven by the hypothesis that small molecule inhibitors targeting the apoptosis pathway (particularly those interfering with Bcl-2 proteins interaction) should exhibit radioprotective properties. Supported by initial docking study data, we designed a group of potentially radioprotective compounds. These might be applied as inhibitors of pro-apoptotic and anti-apoptotic protein-protein interactions with obvious impact on radiation-induced cell death signaling. This might have great implications not only for radioprotection but also for other areas such as the therapy of neurodegenerative and cardiovascular diseases that rely on the apoptotic pathway (Tichý et al. 2018).

For this study, our team built a unique pipeline consisting of in silico study, in-house synthesis, physicochemical analysis, in vitro cytotoxicity and in vivo toxicity testing followed by radioprotective effect evaluation. As an outcome of this approach we report the synthesis and biological characterization of a novel

series of 1-(4-(2-hydroxyethyl)piperazin-1-yl)-3-phenoxypropan-2-ol derivatives as compounds with radioprotective properties. We modified the aryl moiety of our leading structure to prepare an entire group of compounds. The influence of substituents has been compared with the important parameter of solubility, which is often limiting for further biological tests.

We managed to prepare highly-soluble substances. Beyond that, the presented data show that our compounds had no cytotoxic effect on 10 human cell lines (even at 100 mM). Subsequently, we measured the maximum tolerable concentration for all compounds. Furthermore, we evaluated the protective effect on irradiated MOLT4 cells. Finally, we selected the most suitable compounds based on their toxicological and physicochemical profile. We have performed in vivo study on whole body irradiated mice. Importantly, we observed an interesting structural relationship between the methoxy group in position 2 on the aromatic ring and an improved radioprotective effect of our compounds.

That said, analogue 3e was determined as the most effective radioprotective compound (Figure 2-17). The data from in vitro experiments related to radioprotection were comparable with other compounds, but the in vivo radioprotective effect together with MTC indicate that analogue 3e possesses the highest potential as the radioprotective therapeutic utility.

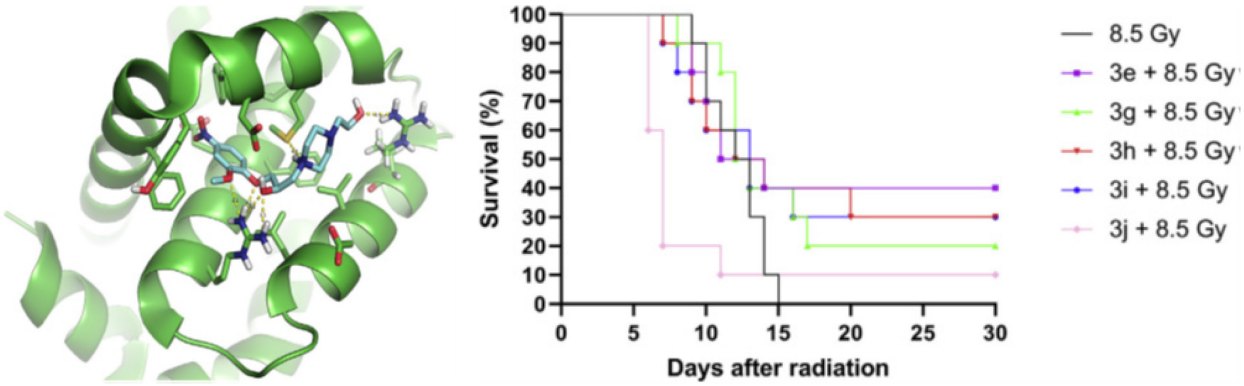


Figure 2-17: (Left) Docking Pose of the Ligand 3e in the Cavity of Bcl-2 Protein, (Right) Survival Curves of Irradiated Mice and Irradiated Mice Injected (i.p.) with Selected Compounds 3e, 3g, 3h, 3i, and 3j for 30 days.

Further research is ongoing in order to reveal a mechanistic explanation of the radioprotective effect, but as our in silico data indicate, the compounds are likely to interfere with Bcl-2 protein-protein interaction, interfering with apoptosis induction. The actual mechanistic explanation will be necessary to design patient tailored radioprotection in cancers with dysfunctional intrinsic apoptotic pathways. It should be also noted that in radiosensitive organs (such as bone marrow) immune system activation and signaling mechanism (involving FasL, TNF-alpha, nitric oxide, and superoxide) contribute to cell death (Burr et al. 2010). Synergic combination of small molecule inhibitor targeting intrinsic apoptotic pathway together with an anti-inflammatory drug could be an effective strategy for radioprotection in mass-casualty scenarios.

References

Burr, K.L., Robinson, J.I., Rastogi, S., Boylan, M.T., Coates, P.J., Lorimore, S.A., Wright, E.G. (2010). Radiation-induced delayed bystander-type effects mediated by hemopoietic cells, Radiat. Res. 173 (6) 760-768.

Hassan, M., Watari, H., AbuAlmaaty, A., Ohba, Y., Sakuragi, N. (2014). Apoptosis and molecular targeting therapy in cancer, BioMed Res. Int. (2014). doi.org/10.1155/2014/150845.

Mustata, G., Li, M., Zevola, N., Bakan, A., Zhang, L., Epperly, M., Greenberger, J.S., Yu, J., Bahar, I. (2011). Development of small-molecule PUMA inhibitors for mitigating radiation-induced cell death, Curr. Top. Med. Chem. 11 (3) 281-290.

Najafi, M., Motevaseli, E., Shirazi, A., Geraily, G., Rezaeyan, A., Norouzi, F., Rezapoor, S., Abdollahi, H. (2018). Mechanisms of inflammatory responses to radiation and normal tissues toxicity: Clinical implications, Int. J. Radiat. Biol. 94 (4) 335-356.

Tichý, A., Marek, J., Havelek, R., Pejchal, J., Seifrtova, M., Zarybnicka, L., Filipova, A., Rezacova, M., Sinkorova, Z. (2018). New light on an old friend: Targeting PUMA in radioprotection and therapy of cardiovascular and neurodegenerative diseases, Curr. Drug Targets 19 (16) 1943-1957.

2.2.3 A Cellular Emergency Approach to ARS Treatment: Ongoing Studies at IRBA, M. Drouet, IRBA, 2018

Radiation-induced Hematopoietic Syndrome (HS) represents the first therapeutic challenge in the case of acute total body exposure to more than 2Gy. The primary causes of HS are radiation-induced suppression of mitosis in hematopoietic stem/progenitor cells and their progeny, resulting in hypocellularity and aplasia of the bone marrow and apoptosis in lymphocytes and other hematopoietic cells (Shao et al. 2014).

References

Shao, L., Luo, Y., Zhou, D. (2014). Hematopoietic stem cell injury induced by ionizing radiation. Antioxid Redox Signal. Mar 20;20(9):1447-62. doi: 10.1089/ars.2013.5635. Epub 2014 Feb 10. PMID: 24124731.

2.2.3.1 Irradiation-Induced Endothelial Dysfunctions and Organ Damages, F.X. Boittin

Irradiation can increase white blood cell adhesion to endothelial cells and also endothelial monolayer permeability, which favors white blood cell migration into tissues, inflammation, oedema development and then organ damages (Williams and McBride, 2011). These effects are mediated through increased expression of adhesion molecules such as ICAM-1, V-CAM-1, P- and E-selectin on the membrane of endothelial cells and through a destabilization of inter-endothelial junctions made of VE-Cadherin (Kishimoto et al. 2018; Kiyohara et al. 2011; Kouam et al. 2019; Ma et al. 2010; Quarmby et al. 1999; Rodriguez-Ruiz et al. 2017; Wang et al. 2019).

Possible therapeutic pathways to reduce endothelial dysfunctions and organ damages occurring after irradiation include protection of endothelial adherens junctions made of VE-Cadherin and reduced expression of adhesion molecule expression. Ongoing studies at IRBA therefore aims to develop efficient countermeasures to prevent radiation effects on endothelial adherens junctions and adhesion molecules.

Soluble Vascular Endothelial Cadherin as a New Biomarker of Irradiation in Highly Irradiated Baboon with Bone Marrow Protection

VE-cadherin is the main component of adherens junctions in the endothelium. This transmembrane protein, which is linked to the actin cytoskeleton, allows cohesion between endothelial cells in the monolayer (up to its adhesive properties). In pathological states involving endothelial dysfunction, VE-Cadherin can be cleaved, releasing the extracellular part of the protein, which is soluble in blood (sVE-Cadherin). As blood concentration of sVE-cadherin may directly reflect endothelial damages (Blaise et al. 2015), plasma levels of sVE-cadherin have been measured in the plasma of baboons after irradiation (Figure 2-18).

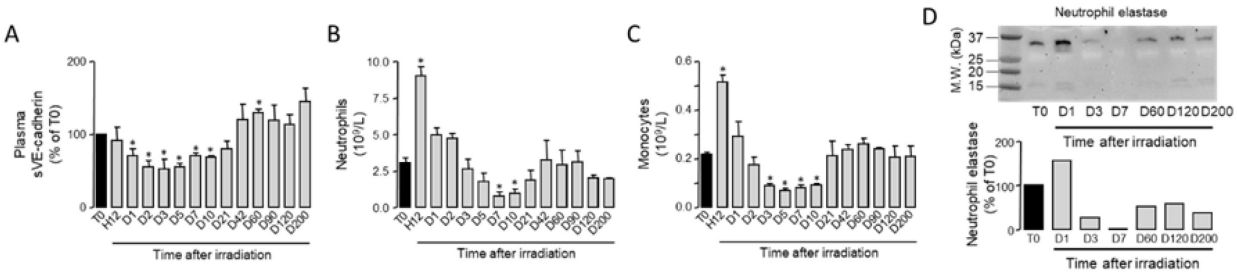


Figure 2-18: Baboons Were Irradiated at 6.25 Gy Using a ^{60}Co Gamma Source with Both Hind Limbs Protected Behind a Lead Wall, Allowing Protection of About 20 % of Total Bone Marrow. A, B, C and D shows respectively the evolution (up to 200 days after irradiation) of sVE-Cadherin plasma levels, blood neutrophil/monocyte counts and neutrophil elastase plasma levels. (Figures from : Hérodin, F., Voir, D., Vilgrain, I., Courçon, M., Drouet, M., Boittin, F.X. Soluble vascular endothelial cadherin as a new biomarker of irradiation in highly irradiated baboons with bone marrow protection. Health Phys. Jun;110(6):598-605 (2016). doi: 10.1097/HP.0000000000000481).

White blood cells counts (neutrophils and monocytes) indicate that the observed early decrease in sVE-Cadherin levels was timely correlated with the disappearance of white blood cells after irradiation (Figure 2-18). Early decrease in sVE-Cadherin after irradiation may be explained by reduced cleavage of VE-Cadherin and/or down-regulation of VE-Cadherin in endothelial cells. During hematopoietic reconstitution, sVE-Cadherin levels were found to increase together with white blood cells counts. After hematopoietic recovery, sVE-Cadherin levels were found to exceed control values, suggesting that **plasma sVE-Cadherin may represent a new biomarker of endothelial damage/dysfunction in the late phase of irradiation.**

A strong correlation was also observed between decreased plasma levels of neutrophil elastase (a protease released by white blood cells) and decreased plasma sVE-Cadherin levels soon after irradiation (Figure 2-18), suggesting that, as is the case in vitro, neutrophil elastase may be involved in VE-Cadherin cleavage in vivo.

Ongoing experiments aims to study the effect of irradiation on VE-Cadherin expression and localization in endothelial monolayers, in order to develop efficient countermeasures to protect endothelial monolayers.

Enhanced Expression of Adhesion Molecules After Irradiation of Endothelial Cells

As shown in Figure 2-19, irradiation-induced plasma membrane ICAM-1 overexpression in human pulmonary microvascular endothelial cells. In accordance with previous studies, pharmacological inhibition of the transcription factor NFκB significantly reduced irradiation-induced ICAM-1 expression, indicating that this transcription factor is indeed involved in the effect of irradiation on the pulmonary microvascular endothelium. However, the effect of irradiation on ICAM-1 expression appears to partly depend on a calcium-dependent transduction pathway, as it was reduced when intracellular calcium was chelated with BAPTA-AM. Calcium entry though cationic plasma membrane channels belonging to the Transient Receptor Potential family (TRP) represent one of the major source of calcium of endothelial cells, but as shown in Figure 2-19, pharmacological blockade of different types of channels (TRPV4, TRPC6 and store-operated channels : ORAI-1) did not affect irradiation-induced ICAM-1 overexpression, indicating that calcium entry though these cationic channels is not involved in the control of ICAM-1 expression in case of irradiation. Altogether, this suggests that calcium release from internal pools may rather be involved and this hypothesis is currently under investigation, as is the case for the initial mechanism of NFkB activation. The role of plasma membrane cationic channels in irradiation-induced endothelial damages (such as necrosis or apoptosis) is also under investigation (Figure 2-19).

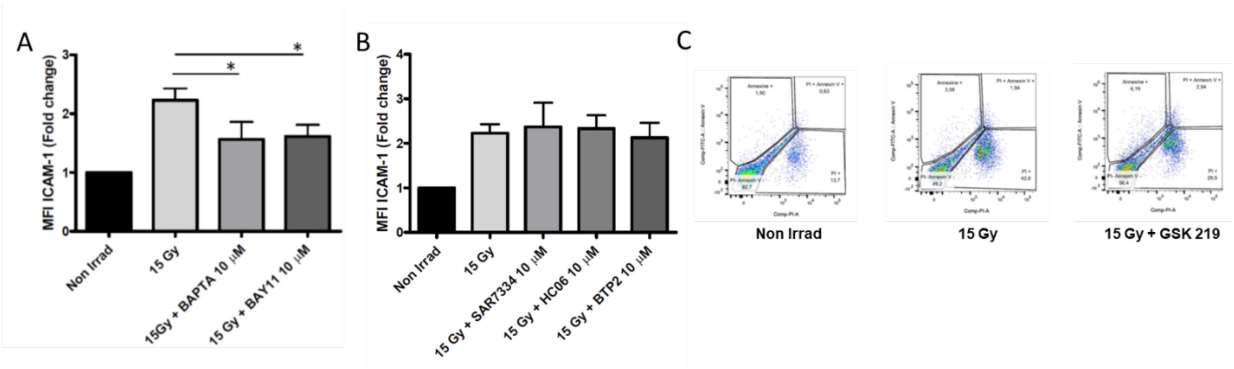


Figure 2-19: (A) High-Dose Irradiation (X-Ray, 15 Gy) Induces Plasma Membrane ICAM-1 Overexpression in Human Pulmonary Microvascular Endothelial Cells, Without Significant Effects on Other Adhesion Molecules (V-CAM-1, E- and P-Selectin (not shown)). Flow cytometry was used to measure adhesion molecule expression (MFI : median fluorescence intensity). The irradiation-induced ICAM-1expression was significantly reduced when intracellular calcium was buffered using the membrane-permeant calcium chelator BAPTA-AM, suggesting that irradiation-induced ICAM-1 expression involves a calcium-dependent transduction pathway. ICAM-1 expression was also reduced by NFκB inhibition using BAY11-7085, indicating that this transcription factor is involved in this effect of irradiation, in accordance with previous studies. (B) Inhibitors of Plasma Membrane Cationic Channels Belonging to the Transient Receptor Potential (TRP) Family (TRPV4 and TRPC6) or Store-Operated Channels Did Not Inhibit the Effect of Irradiation on ICAM-1 Expression, Indicating that Calcium Entry Through these Channels is Not Involved in the Stimulation of ICAM-1 Expression Induced by Irradiation. Altogether, these results suggest that intracellular calcium release may rather be involved in ICAM-1 expression (ongoing experiments). (C) High-Dose Irradiation (X-ray, 15 Gy) Induces Necrosis of Human Pulmonary Microvascular Endothelial Cells (PI+ Cells, Middle Picture). Irradiation-induced necrosis was reduced when cationic plasma membrane channels (TRPV4) were blocked with GSK219 (right picture, ongoing experiments).

References

Blaise, S., Polena, H. and Vilgrain, I. (2015). Soluble vascular endothelial-cadherin and auto-antibodies to human vascular endothelial-cadherin in human diseases: Two new biomarkers of endothelial dysfunction. Vasc. Med. 20, 557-565.

Kishimoto, M., Akashi, M., Kakei, Y., Kusumoto, J., Sakakibara, A., Hasegawa, T., Furudoi, S., Sasaki, R. and Komori, T. (2018). Ionizing Radiation Enhances Paracellular Permeability Through Alteration of Intercellular Junctions in Cultured Human Lymphatic Endothelial Cells. Lymphat. Res. Biol. 16, 390-396.

Kiyohara, H., Ishizaki, Y., Suzuki, Y., Katoh, H., Hamada, N., Ohno, T., Takahashi, T., Kobayashi, Y. and Nakano, T. (2011). Radiation-induced ICAM-1 expression via TGF-beta1 pathway on human umbilical vein endothelial cells; comparison between X-ray and carbon-ion beam irradiation. J. Radiat. Res. 52, 287-292.

Kouam, P.N., Rezniczek, G.A., Adamietz, I.A. and Buhler, H. (2019). Ionizing radiation increases the endothelial permeability and the transendothelial migration of tumor cells through ADAM10-activation and subsequent degradation of VE-cadherin. BMC. Cancer 19, 958.

Ma, Z.C., Hong, Q., Wang, Y.G., Tan, H.L., Xiao, C.R., Liang, Q.D., Cai, S.H. and Gao, Y. (2010). Ferulic acid attenuates adhesion molecule expression in gamma-radiated human umbilical vascular endothelial cells. Biol. Pharm. Bull. 33, 752-758.

Quarmby, S., Kumar, P. and Kumar, S. (1999). Radiation-induced normal tissue injury: role of adhesion molecules in leukocyte-endothelial cell interactions. Int. J. Cancer 82, 385-395.

Rodriguez-Ruiz, M.E., Garasa, S., Rodriguez, I., Solorzano, J.L., Barbes, B., Yanguas, A., Teijeira, A., Etxeberria, I., Aristu, J.J., Halin, C. et al. (2017). Intercellular adhesion molecule-1 and vascular cell adhesion molecule are induced by ionizing radiation on lymphatic endothelium. Int. J. Radiat. Oncol. Biol. Phys. 97, 389-400.

Wang, H., Segaran, R. C., Chan, L. Y., Aladresi, A. A. M., Chinnathambi, A., Alharbi, S. A., Sethi, G. and Tang, F. R. (2019). Gamma radiation-induced disruption of cellular junctions in HUVECs is mediated through affecting MAPK/NF-kappaB inflammatory pathways. Oxid. Med. Cell Longev. 2019, 1486232.

Williams, J.P. and McBride, W.H. (2011). After the bomb drops: A new look at radiation-induced Multiple Organ Dysfunction Syndrome (MODS). Int. J. Radiat. Biol. 87, 851-868.

2.2.3.2 Exosomes Revival, S. Cavallero, S. François

Mesenchymal Stem/stromal Cells (MSC) play multiple roles : limiting inflammation through releasing cytokines, aiding healing by expressing growth factors, differentiate into multiple lineages. MSC also produce extracellular vesicles of varying sizes including exosomes that carry as cargo mRNAs, and proteins, and that horizontal transfer of this cargo induces nonautonomous changes that are therapeutic (Phinney et al. 2017).

Schoefinius et al. suggest the infusion of MSC-derived Extracellular Vesicles (MSC-EV) as efficient and immediate treatment option after irradiation injuries. The transplantation of mouse MSC-EV into lethally irradiated mice resulted in long-term survival but no improvement in short-term reconstitution of the recipients. Comparing the leukocyte and thrombocyte recovery of long-term surviving mMSC recipients to mMSC-derived EV recipients showed a similar kinetic of white blood cell recovery. The recovery of thrombocytes in EV recipients showed an accelerated kinetic compared to mMSC only reaching normal levels within approximately 60 days. Importantly, the radiation rescue was efficient without additional hematopoietic support. The hypothetical mode of action of EV could be that after i.v. injection into lethally irradiated recipients transport several cargos including proteins and various RNAs directly into the BM without notable accumulation in other tissues. EV target bone marrow-resident hematopoietic stem cells and stimulate management of oxidative stress, cell cycle, and probably other regenerative processes. Indeed, EV as an effective first-line treatment to combat radiation-induced hematopoietic failure which might also be helpful in alleviating myelosuppression due to chemotherapy and toxic drug reaction (Schoefinius et al. 2017).

The therapeutic potential of MSC-EVs is a strategy particularly attractive to exploit the clinical benefits of MSC therapy using a cell-based product, limiting immune responses against MSC and the development of ectopic tissue. Moreover, the use of MSC-EVs inserts the possibility of changing the contents of bioactive molecules to achieve highly targeted therapies. Indeed, MSC-EVs have been studied in an increasing number of animal models of organ injury and/or models of inhibiting tumor in the modulation of immune responses. The standardization of isolation and conservation of EVs constitutes major challenges for their therapeutic use. Within the laboratory, the method of precipitation by PEG (Total exosome Isolation, Invitrogen) was chosen to isolate EVs from MSC. This technique provides a simple and reliable method of concentration intact exosomes from cell culture media samples. By tying up water molecules, the reagent forces less-soluble components (i.e., exosomes) out of solution, allowing them be collected after brief, low-speed centrifugation. The first experiments, we have allowed to isolate $1.3.10^9$ exosomes for 1ml of MSC culture and less of 5.10^8 for 1ml of human fibroblats culture. We also tested the effect of radiation on the production of exosomes by MSC. It seems that radiation increases the release of exosomes by MSC, $1.9.10^8$ exosomes in control versus $2,5.10^8$ exosomes after radiation with 16 Gy. Exosomes isolated (Figure 2-20) were then characterized by western blot. The protein CD63, involved in multivesicular biogenesis, is used as a specific maker of exosomes, and calnexin, protein of endoplasmic reticulum, as cellular marker. So, the presence of CD63 and the absence of calnexin in our samples proves that the isolates obtained contain exosomes (Figure 2-21).

In conclusion, the use of MSC-EV in human patients has several potential advantages. First, their use avoids the transfer of cells which may have mutated or damaged DNA. Second, EV are small and circulate readily whereas MSC are too large to circulate easily through the capillaries. Third, the dose of infused MSC decreases rapidly after transplantation, and it may be that the delivery of MSC-EV can achieve a higher "dose" that circulates to a greater extent than the larger cells. Moreover, the functionality of the MSC-EV is strongly influenced by the microenvironment. So, we investigated different MSC culture pre-conditioning in order to obtain EVs with high pro-angiogenic potential and anti-apoptotic potential.

References

Shao, L., Luo, Y., Zhou, D. (2014). Hematopoietic stem cell injury induced by ionizing radiation. Antioxid Redox Signal 20:1447-1462.

Phinney, D.G., Pittenger, M.F. (2017). Concise review: MSC-derived exosomes for cell-free therapy. Stem Cells. Apr; 35(4): 851-858.

Schoefinius, J.S., Brunswig-Spickenheier, B., Speiseder, T., Krebs, S., Just, U., Lange, C. (2017). Mesenchymal stromal cell-derived extracellular vesicles provide long-term survival after total body irradiation without additional hematopoietic stem cell support. Stem Cells 35 :2379-2389.

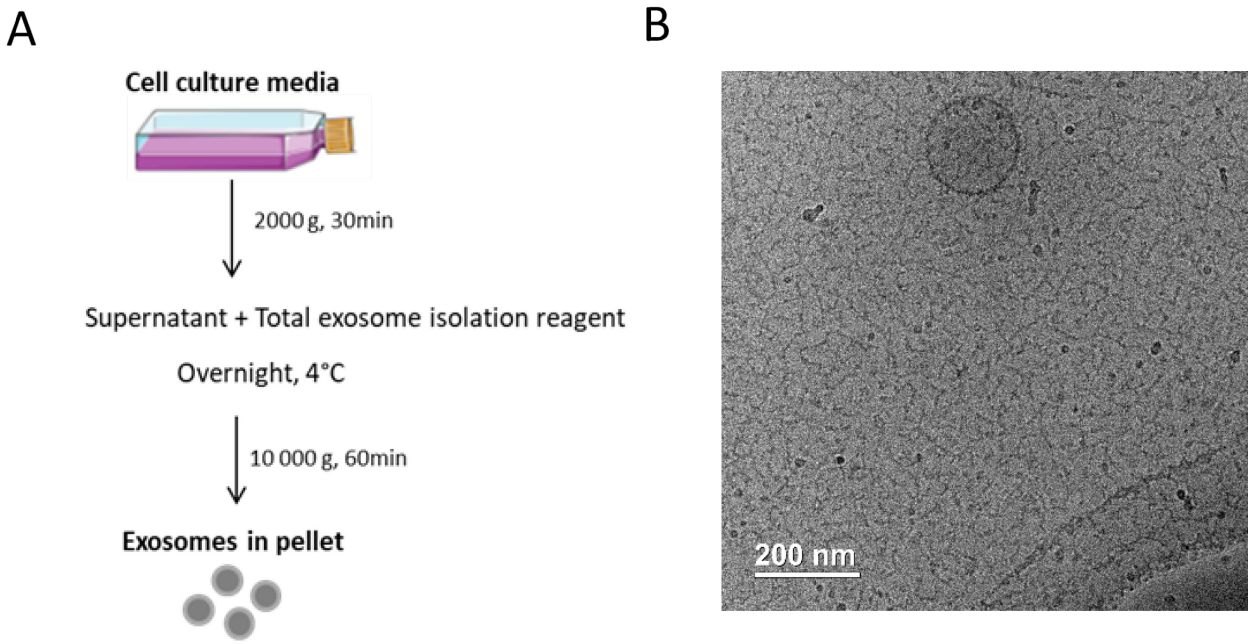


Figure 2-20: (A) Technique Used for Isolated Exosomes of Cell Culture, (B) Visualization of Exosomes Isolated by Cryo-Microscopy.

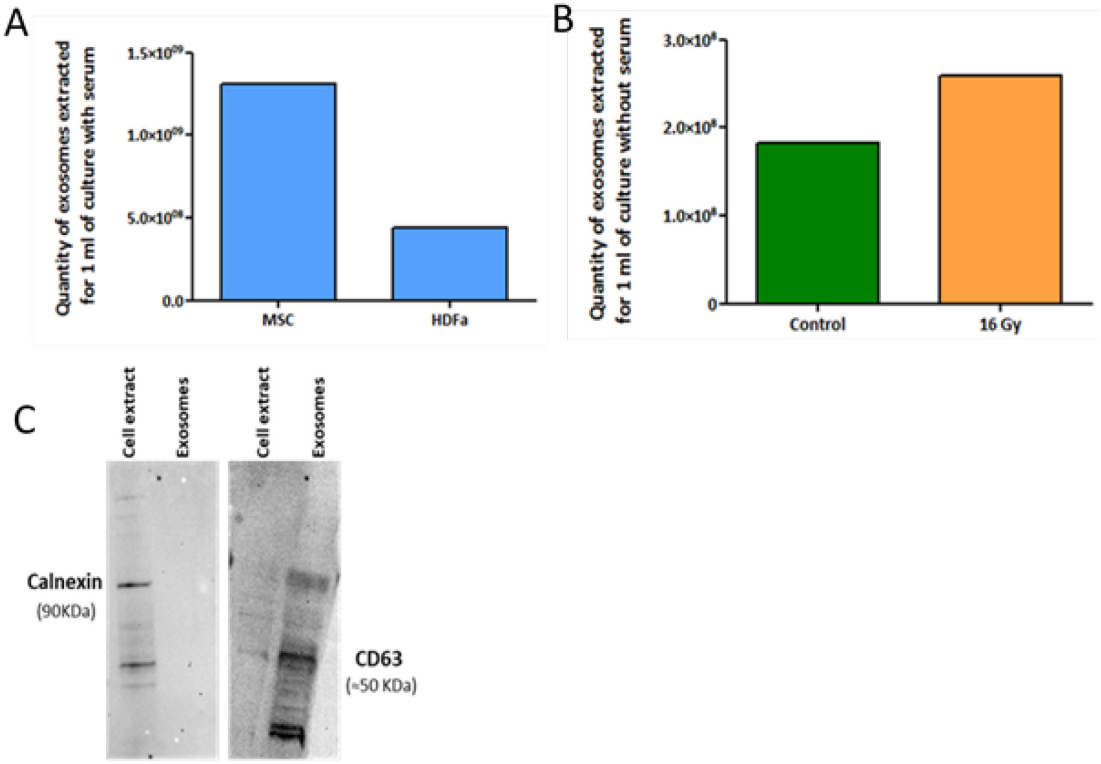


Figure 2-21: (A) Quantity of Exosomes Isolated of 1 ml of MSC or HDFa Culture (B) Quantity of Exosomes Isolated for 1 ml of MSC Culture and 1 ml of MSC Irradiated Culture (C) Characterization of EV Isolated by Western Blot. In exosomes, no expression of calnexin (endoplasmic reticulum marker) and expression of CD63 (a tetraspanin enriched in exosomes). In cell, only expression of calnexin.

2.2.4 Development of Small Molecules for Enhanced Radiation Protection, A. Tichý, UNOB, 2019

Exposure to IR is associated with cell death, genetic mutations, and carcinogenesis. It induces DNA damage in the form of double-strand DNA breaks, which is considered the underlying mechanism of the resulting cell death – apoptosis.

Apoptosis is a very complex process, which is tightly controlled in mammalian cells. Its multi-level regulation has been the subject of medical research for a long time because some pathologies (such as cancer and autoimmune and neurodegenerative disease) are closely associated with dysregulation of programmed cell death. Above that, individual regulatory systems provide suitable targets for novel compounds with desirable pharmacological potential (Favaloro et al. 2012). The testing of thousands of synthetic and natural compounds has consequently led to the discovery of novel radioprotectants or mitigators. Unfortunately, these compounds can often be insoluble or cytotoxic, with low tolerance, or with very low circulating half-life. As a result, their clinical application is rather limited.

The increasing risk of radiation exposure underlines the need for novel radioprotective agents. However, there is very limited current literature concerned with small molecules as potential radioprotective agents. Based on previous studies, we have selected the 1-(2-hydroxyethyl)piperazine moiety as the essential component of the molecule (Mustata et al. 2011; Marek et al. 2020). Notably, some of our structures are already mentioned in the literature but none has been reported in the context of radioprotection. Namely, compound 2 had already been prepared as a leading structure for a novel group of cholinesterase inhibitors. Compound 3 was prepared afterwards as a novel structure according to the procedure for preparation of these inhibitors. Compound 4 was

searched in PubChem and ZINC database PubChem, but no synthesis or biological data were found. The symmetrical molecule 9 consisting of two aniline residues had been prepared by Dains et al. in 1922. None of the other compounds had ever been prepared or tested before (5, 6, 7, 8, 10).

Thus, the aim of this work was to investigate a group of 1-(2-hydroxyethyl)piperazine derivatives as potential affordable radioprotective agents modulation radiation bioeffects. We focused on various modifications of the part of the molecule attached to the basic 1-(2-hydroxyethyl)piperazine moiety (Figure 2-22). We characterized the prepared compounds using Nuclear Magnetic Resonance (NMR) and High Resolution Mass Spectrometry (HRMS), and evaluated their cytotoxicity and radioprotective properties in vitro and in vivo using human cell lines and an animal experimental model (Figure 2-23, Figure 2-24).

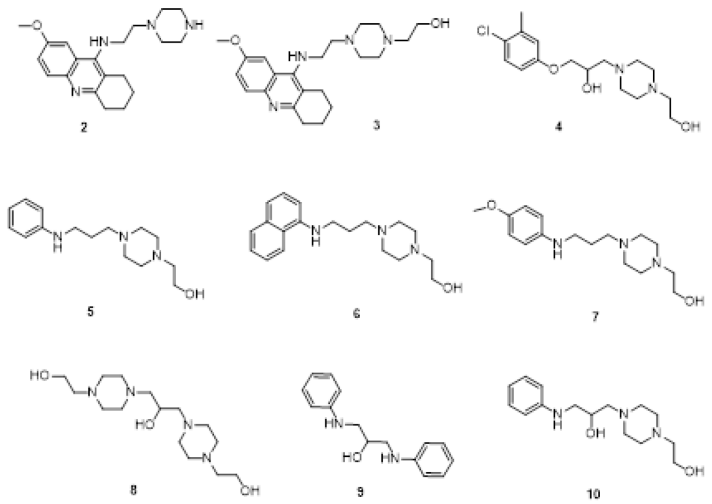


Figure 2-22: Synthesized Compounds as Bases with Potential Radioprotective Properties.

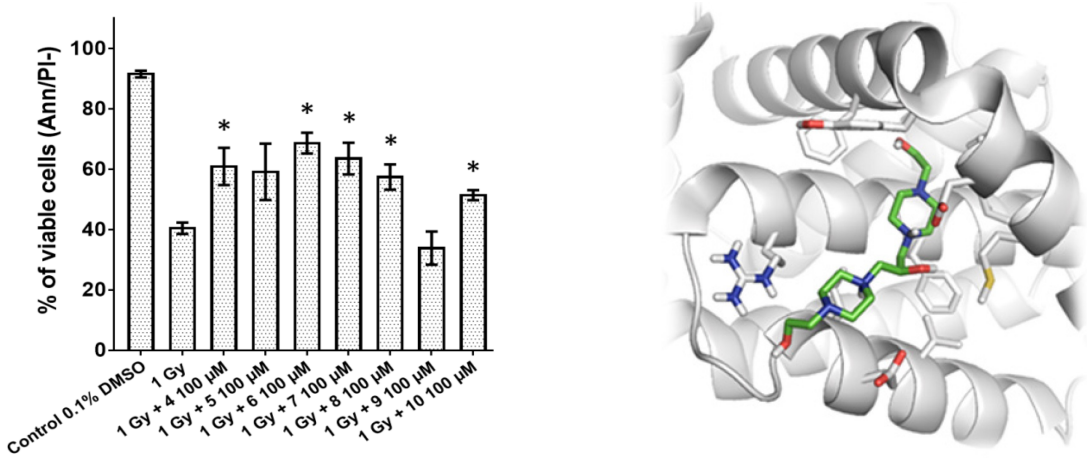


Figure 2-23: The Viability of MOLT-4 Cells after Exposed to IR Alone or in Combination with Inhibitors at 100 µM. (Left) Viability of MOLT-4 cells was determined as the percentage of Annexin-V-Alexa Fluor® 488 and Propidium iodide (PI)-negative cells by flow cytometry 24 h following irradiation with a dose of 1 Gy. The bar graph represents the percentage of viable MOLT-4 cells detected by flow cytometry. Results are shown as the mean ± SD from four experiments. * – significantly different from irradiation alone (t-test, * p < 0.05). (Right) Interaction of compound 8 with Bcl-2 protein. Ionic interaction of protonated piperacilin nitrogen with Asp108, hydrogen bonds Arg143 with hydroxyl oxygen.

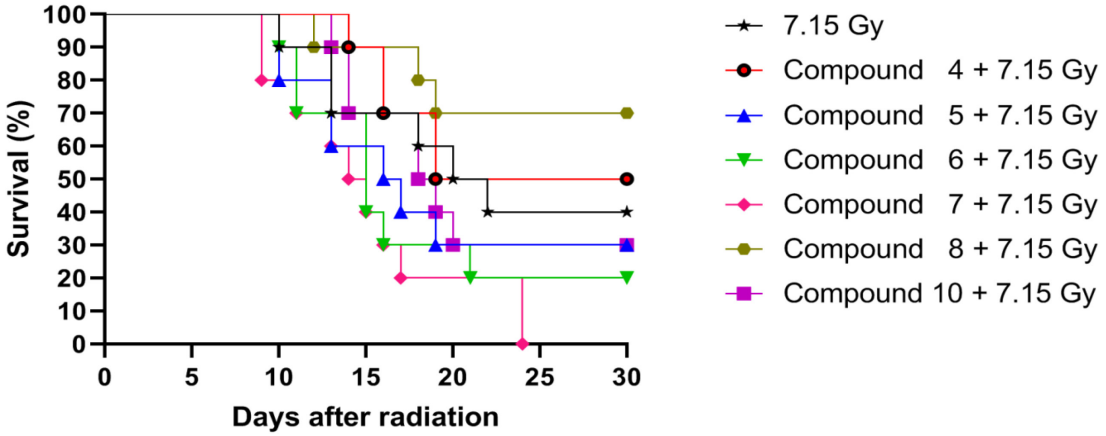


Figure 2-24: Kaplan-Meier Survival Curves of Irradiated Mice (7.15 Gy TBI) Pre-Treated i.p. with Saline Buffer and with Compounds 4, 5, 6, 7, 8, and 10. Each group (n = 10) was observed until termination of the experiment (30 days after irradiation).

Previously, a series of novel 1-(2-hydroxyethyl)piperazine derivatives were designed and synthesized (see Section 2.2.2). Some of the compounds protected human cells against radiation-induced apoptosis and exhibited low cytotoxicity. Compared to the previous series of piperazine derivatives, compound 8 exhibited radioprotective effect on cell survival in vitro and low toxicity in vivo. It also enhanced the survival of mice 30 days after whole body irradiation (although this increase was not statistically significant). Taken together, our in vitro and in vivo data indicate that some of our compounds are valuable for further research as potential radioprotectors.

References

Favaloro, B., Allocati, N., Graziano, V., Di Ilio, C., De Laurenzi, V. (2012). Role of apoptosis in disease. Aging, Albany NY, 4, 330-349.

Marek, J., Tichý, A., Havelek, R., Seifrtova, M., Filipova, A., Andrejsova, L., Kucera, T., Prchal, L., Muckova, L., Rezacova, M., et al. (2020). A novel class of small molecular inhibitors with radioprotective properties. Eur. J. Med. Chem. 187:111606.

Mustata, G., Li, M., Zevola, N., Bakan, A., Zhang, L., Epperly, M., Greenberger, J.S., Yu, J., Bahar, I. (2011). Development of small-molecule PUMA inhibitors for mitigating radiation-induced cell death. Curr. Top. Med. Chem. 11, 281-290.

2.2.5 Extracellular Vesicles as a New Strategy for Hematopoietic Syndrome, D. Riccobono, IRBA, 2019

This presentation is covered by 2.2.6.

2.2.6 Novel Therapeutic Approaches to Radio-Induced Medullary Aplasia: Severe Hematopoietic Syndrome, M. Drouet, IRBA, 2019

High doses of radiation induce the Acute Radiation Syndrome (ARS), which can be subdivided into four syndromes: hematopoietic, gastrointestinal, cutaneous and neurovascular. The Hematopoietic Syndrome (HS) is one of the major components of ARS, clinically that has been graduated by METREPOL in 4 stages (H1-H4) depending on the severity of bone marrow damages. H1 represents mild damage which need no specific therapy, and H2-H4 represents moderate, severe, and irreversible damage, respectively (Fliedner et al, 2001). Multicytokines therapy may be useful to counteract radiation-induced myelosuppression. For grade H3, the

Granulocyte Colony Stimulating factor (G-CSF) infusion to stimulate the residual stem cells after exposure. In case of medullary eradication in grade H4, it is recommended to perform an allogeneic Hematopoietic Stem Cell (HSC) and progenitors transplantation (Drouet et al, 2010).

The HSC environment represents a complex radiosensitive compartment whose repair/manipulation appears crucial for stem cell survival and recovery from Radiation-Induced (RI) myelosuppression. Among the different candidates regulating directly/indirectly hematopoiesis the hedgehog signaling pathway, one of the three mammalian hedgehog proteins, represents a major actor in steady state and stress-induced adult hematopoiesis. Sonic hedgehog morphogene (Shh) may be involved in fetal and adult hematopoiesis, in stimulation of pro-angiogenic and anti-apoptotic activity in adult. Indeed, Shh may represent a key factor to mitigate radiation-induced damage. So, we tested a short-term gene therapy strategy based on intra-osseous injection of Adipocyte-derived Stem Cells (ASCs) transduced with a Shh pIRES2 plasmid. For that, adult male rhesus monkeys were exposed to a whole body (TBI) unilateral front irradiation with a 60Co gamma source to a total midline tissue dose of 8 Gy. Two days after irradiation, Shh-ASCs (range 2.05-3.74 x 10^6 cell/kg, n = 4) or mock-ASCs (range 2-6 x 10^6 cell/kg, n = 4) were injected in a single humerus. Two controls died from radiation toxicity on D19 and D196, whereas all Shh-ASC treated monkeys fully recovered. Thrombocytopenia, neutropenia and anemia duration were reduced in Shh-ASC animals. Areas under the curve of platelets, ANCs and RBC/Hb (Red Blood Cells/Hemoglobin) between D0 and D30 were higher in Shh-ASC injected animals. Moreover, Shh-ASCs matrigel grafted in immunocompromized mice exhibited an increase in RBC colonization when compared with mock-ASCs counterparts. This study suggests that Shh speeds up platelets and RBC reconstitution and protects functional areas of HSC and supportive tissue (Drouet et al, 2014) (Figure 2-25).

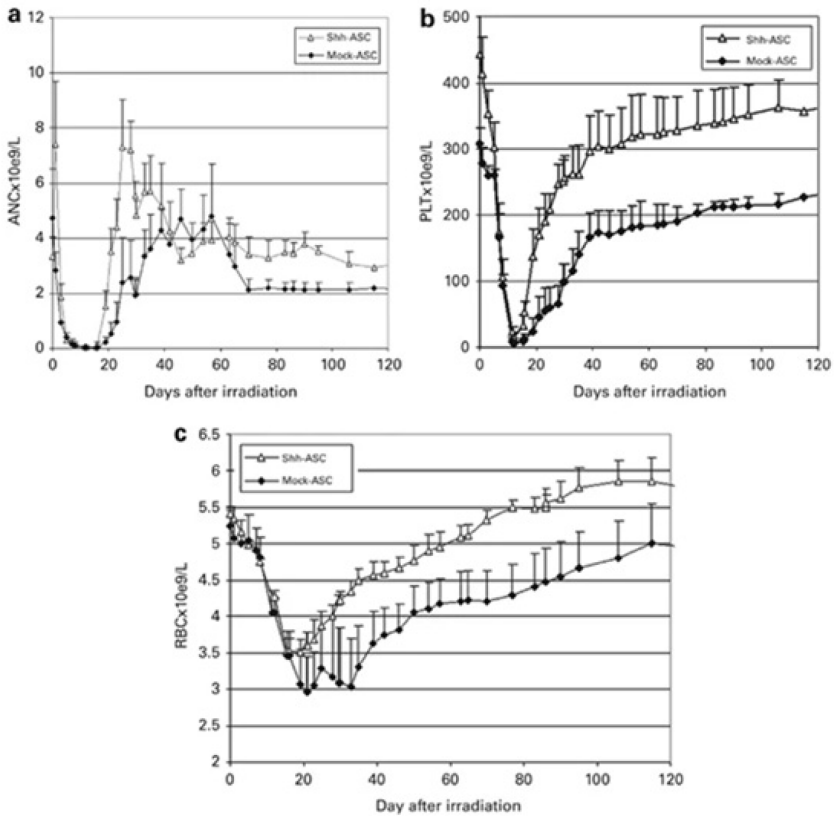


Figure 2-25: Effect of a Single Intra-Osseous Injection of SHH-ASCs on (a) ANC, (b) Platelets and (c) RBC in 8Gy Total Body ^{60}Co Gamma Irradiated Rhesus Monkeys (*n* = 4 for Shh-ASC and Mock-ASC Injected Groups). Porcine ASCs previously nucleofected with piRES2-EGFP-Shh plasmid were injected 2 days after irradiation. Data represent mean value +/- s.d.

However, the clinical use of gene therapy presents certain barriers: the oncogenic potential of the transfected cell and its immunogenicity. Thus, currently, we are working on the development of an acellular therapy allowing functional restoration of bone marrow. Many preclinical animal studies have shown that MSC, mainly by their secretory activity, in particular Extracellular Vesicles (EVs), contribute to the control of inflammation and promote regeneration of tissues by accelerating angiogenesis and re-epithelialization processes (Keshtkar et al. 2018). EVs have been described as mediators between cells that can influence their environment by direct stimulation or by gene and protein transfer, and thus promote the process of tissue regeneration. In sum, MSC-EVs offer a new therapeutic option to limit the evolution of the HS. Indeed, unlike cells, EVs are more easily available in an emergency context and their administration results in low immunogenicity. Composition and functionality of MSC-EVs is strongly influenced by the MSC microenvironment. Thus, within IRBA, our experimental strategy is, firstly, investigated different MSC culture pre-conditioning (cytokines, hypoxia, Shh transfection, etc.) in order to obtain EVs with high pro-angiogenic, anti-apoptotic and anti-inflammatory potential. The EVs will then be isolated from the culture supernatant via the tangential filtration technique and then characterized by the Nanosight. Our first experiments allowed to obtain an average 4 x 10^9 EVs per ml of supernatant. Those have an average size of 120 nm. Secondly, the functionality of the EVs will be tested in vitro. For example, their pro-angiogenic potential will be evaluated via an angiogenesis assay and a study of angiogenic protein expression. Finally, their efficacy in vivo will be evaluated in a murine model of HS. To date, the limiting points of this strategy remains the obtaining of a sufficient amount and storage of EVs to achieve a week of treatment (Figure 2-26).

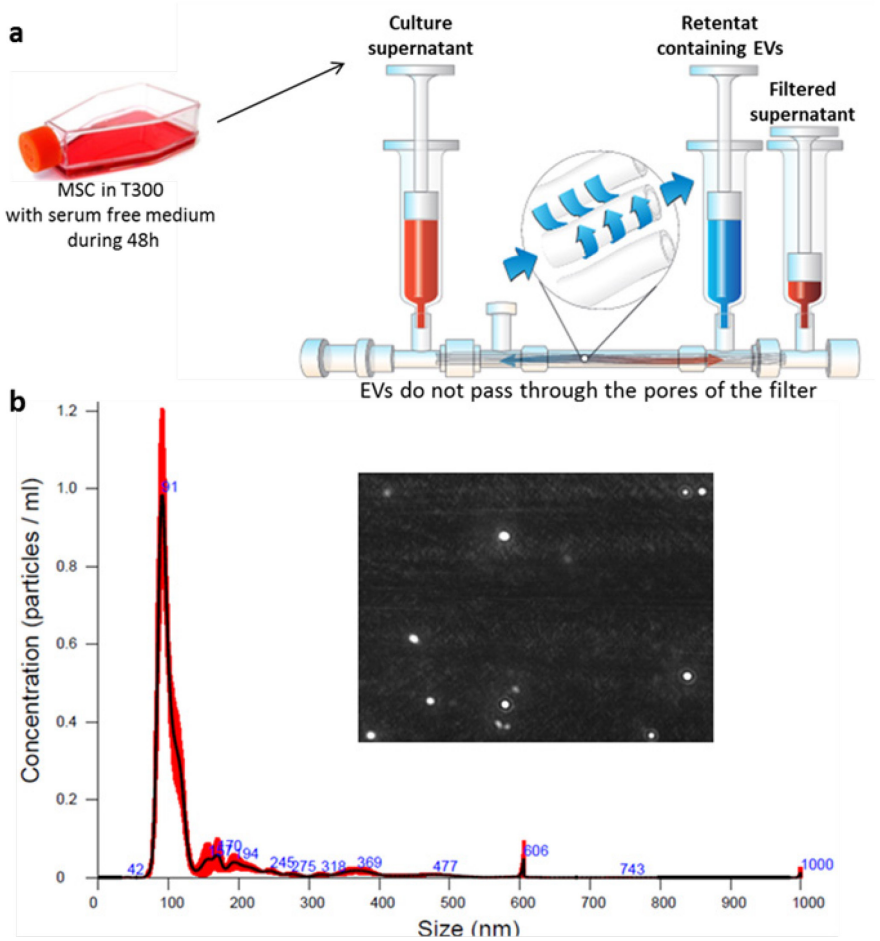


Figure 2-26: Isolation and Characterization of EVs. (a) Tangential filtration technique for EV isolation. (b) Characterization of EVs by Nanosight, to obtain the concentration and size distribution of EVs present in the sample.

In conclusion, our EVs isolation technique is efficient and reproductible. We still have to develop one or two techniques (western blot, electron microscopy) to characterize EVs in order to respond to the MISEV (Minimal information for studies EV) recommendations (Lötvall et al. 2014). We continue to test different MSC culture conditions, including Shh transfection, in order to obtain pro-angiogenic, anti-apoptotic and anti-inflammatory EVs.

References

Drouet, M, Garrigou, P, Peinnequin, A. et al. (2014). Short-term sonic-hedgehog gene therapy to mitigate myelosuppression in highly irradiated monkeys: hype or reality? Bone Marrow Transplant 49, 304-309.

Drouet, M., Hérodin, F. (2010). Radiation victim management and the hematologist in the future: Time to revisit therapeutic guidelines? Int J Radiat Biol 86 :636-648.

Fliedner, T.M., Friesecke, I., Beyrer, K. (2001). (Eds) Medical Management of Radiation Accidents – Manual on the Acute Radiation Syndrome. London: British Institute of Radiology.

Keshtkar, S., Azarpira, N., Ghahremani, M.H. (2018). Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. Stem Cell Res Ther 9, 63.

Lötvall, J., Hill, A.F., Hochberg, F. et al. (2014). Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. J Extracell Vesicles 3: 26913.

2.2.7 Radionuclide Decorporation

2.2.7.1 Aspects of Decorporation – A Review, C. Foster, NAVY, 2018

Nuclear weapons are a core component of NATO's deterrence and defence capability. Although there are only 3 nuclear powers in NATO, it operates as a nuclear alliance with some other NATO nations allowing nuclear weapons to be stationed in, or flown across, their territory. NATO nations therefore need to be prepared to mitigate the health effects of an accident with nuclear weapons, such as that which happened over Palomares, Spain in 1966.

This presentation provided a review of internal contamination by plutonium from a nuclear weapon accident from a UK perspective. It comprised of personal thoughts about plutonium biokinetics, biological monitoring and decorporation typically from unpublished material and/or advice received from Public Health England). Its aim was to stimulate discussion about the limits of knowledge and enquire about other nations plans for managing such an event.

The primary plutonium isotope in nuclear weapons is 239Pu which is present in elemental form. For an accident to occur that could lead to internal contamination it is expected that fire or conventional explosion would lead to the formation of plutonium oxides which could be incorporated via inhalation. These plutonium oxides are thought to represent the greatest radiation hazard even although isotopes of a small number of other radioactive materials could also be present (such as uranium, americium and tritium).

The expectations in the UK Ministry of Defence (MoD) are that the inhaled plutonium oxides would be very insoluble – with about 60% of the initial lung deposit remaining in the lungs one month after intake. The clearance that does occur would be via the muco-ciliary escalator to fecal excretion, with only a tiny amount (several orders of magnitude less) undergoing urinary excretion. The primary health risks would be a stochastic risk of lung cancer, or for very large intakes, the deterministic effects of pneumonitis and pulmonary fibrosis.

Therapeutic whole lung lavage is the treatment of choice for decorporation of large intakes of insoluble plutonium isotopes when the risk/benefit assessment is in favor (Morgan et al, 2010). Intake size can be assessed by the direct technique 'whole body monitoring' which, in the UK, is available at a handful of a specialist laboratories. Decorporation is normally most effective when conducted early. However, when planning whole lung lavage, the optimal time is still undefined partly because there may be a benefit to allowing time for alveolar macrophages to take up plutonium before the lavage process removes them.

An alternative form of decorporation, chelation therapy using DTPA (diethylene-triamine-penta-acetic acid), is recommended for inhaled *soluble* forms of plutonium. It can be delivered by inhalation via a nebulizer or intravenous injection. Although the UK MoD do not expect soluble oxides to be present in great quantities (since many factors in an accident sequence may affect exactly what plutonium compounds form, and in what particle sizes), the need to evaluate whether a soluble fraction has been formed is recognized. Chelation therapy is most effective when conducted early so there is a time pressure for this evaluation. It would be done within the same patients by comparing urine bioassays with whole body monitoring results. However, rapid urine sample preparation and measurement methods are required which trade off minimal detectable amount for speed of processing. Particular care is also needed to avoid contamination of these samples and there can be concerns about validation, especially if the methods are not routinely practiced.

Discussions following the presentation revealed that the RTG participants were unaware of this approach by the UK. The participants present who were familiar with their own nations plans stated that their standard approach would be the early administration of DTPA by inhalation or intravenous injection, or in the case of one nation both simultaneously, without any consideration about particle solubility. The differences in approach identified suggest that further review of the topic would be beneficial with the aim of developing common protocols.

References

Morgan, C. et al. (2010). Therapeutic whole lung lavage for inhaled plutonium oxide revisited. J. Radiol. Prot. 30, 735-746.

2.2.7.2 Calculating Required MCM Resources after Radionuclide Incorporation, A. Rump, BIR, 2018

A large spectrum of scenarios may lead to radiation incidents and the liberation of radioactive material. In the case of a terrorist attack by a "dirty bomb" there is a risk of mechanical and thermal trauma, external irradiation as well as superficial contamination and incorporation of radioactive material. The first treatment priority has to be given to the care of trauma patients with life-threatening injuries as the health effects of radiation occurs with latency. Radionuclide incorporation will lead to a longer lasting irradiation from inside the body associated with a higher risk of stochastic radiation effects (e.g., occurrence of tumors) in the long run. It must be expected that victims having potentially incorporated radionuclides will be much more numerous than trauma patients. The elimination of radionuclides can be enhanced by the administration of decorporation agents like (Ca)DTPA or Prussian Blue, reducing the radiological burden of the body. There is still no consensus whether decorporation treatment should be started immediately based only on a suspicion of radionuclide incorporation ("urgent approach") or if the results of internal dosimetry confirming the necessity of a treatment should be awaited, accepting the delay caused by the measurements and computations ("precautionary approach"). As the therapeutic effectiveness may be substantially decreased if treatment initiation is delayed only by several days, depending on the radionuclide, the physicochemical properties of the compounds involved and the route of absorption, we favor an "urgent approach" from a medical point of view (Figure 2-27).

In doubt, it seems justified to treat victims by precaution as the adverse effects of the medication seems slight. However, in the case of a high number of victims an "urgent treatment approach" may require a large number of daily doses of antidotes and therefore adequate investments in preparedness and antidote stockpiling are necessary.

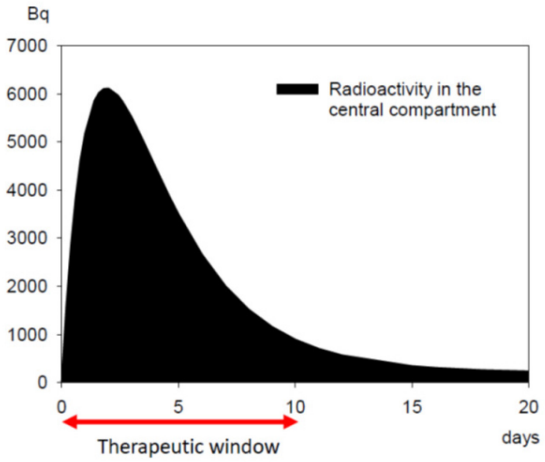


Figure 2-27: Time-Course of Radioactivity in the Central Compartment (Blood, Extracellular Space) Emanating from a Wound Contamination with 37 kBy of Plutonium-239 as a Soluble Compound. Activity falls to low values after about 10 days. The decorporation agent (Ca)DTPA distributes mainly in the extracellular space where it can bind plutonium. Thus, treatment must start within 10 days to be highly effective.

2.2.8 Studies of the Nicotinic Acid Derivatives as Potential Radioprotective and/or Radioremedial Agents, E. Nowosielska, MIHE, 2019

Aneta Cheda, Ewa M. Nowosielska, Marek K. Janiak

Military Institute of Hygiene and Epidemiology, 4 Kozielska St., 01-163 Warsaw, Poland

In today's world, in view of the development of nuclear industry and widespread use of various sources of ionizing radiation, people can be inadvertently exposed to irradiation during accidents in nuclear power plants, a nuclear war, or nuclear/radiological terrorist events. In these instances, some of the casualties may sustain high (i.e., > 1 Gy) doses of radiation. Short-term exposures to radiation at doses in excess of 1 Gy may result in Acute Radiation Syndrome (ARS) commonly associated with suppressed hematopoiesis and, after absorption of doses exceeding 6 Gy, with depopulation of intestinal crypts. Such disorders primarily hinge on the loss of bone marrow and peripheral blood cells as well as on the damage and dysfunction of vascular endothelium which is related to primary and secondary inflammation and thrombosis.

Ever since the harmful effects of ionizing radiation were recognized, a search has been ongoing for effective radioprotectors, i.e., compounds designed to reduce radiation-induced damage in normal tissues, on the molecular, cellular and/or tissue level, that could be applied as prophylactic (protectors - given before the exposure to irradiation) and/or therapeutic (mitigators and remedial agents - given during or after the exposure to irradiation, respectively) agents. Thus far, the only registered radioprotective compound is amifostine (Ethyol®), which is almost exclusively used to reduce side effects of radiotherapy of the head and neck cancers. Recently though, the U.S. Food and Drug Administration (FDA) has approved three radiomitigators to increase survival of patients with the hematopoietic presentation of ARS all of which are cytokines: Granulocyte-Colony Stimulating Factor (G-CSF), polyethylene glycolylated G-CSF, and recombinant granulocyte–macrophage colony stimulating factor (GM-CSF). However, the search for new and more efficient agents continues and many substances of synthetic and natural origin are being investigated for their potential radioprotective, radiomitigative, and/or radioremedial properties.

Some pyridine compounds, exert both anti-thrombotic and anti-inflammatory properties owing to their capacity to stimulate secretion of prostacyclin (PGI_2) by the vascular endothelial cells and down-regulate the

levels of pro-inflammatory cytokines in peripheral blood. Thus, in the present studies, we aimed to assess potential radiopreventive and radioremedial activities of the selected pyridine compounds, such as nicotinic acid (NAc - niacin, vitamin B3), nicotinamide (NA - NAc metabolite), 1-methylnicotinamide (MNA - primary nicotinamide metabolite), or l-methyl-3-acetylpyridine (1,3-MAP - synthetic analogue of NAD). The examined compounds were given to BALB/c mice in drinking water at 100 mg/kg b.m. daily, starting 7 days before, on the day of, or 7 days after the exposure to ionizing radiation and continued until death of the animals or the end of observation. The mice were exposed to whole body irradiation (WBI) at 5.0-7.5 from γ source.

The experiments demonstrated:

1) Significantly prolonged survival of mice irradiated at:
   - $LD_{30/30}$ (6.5 Gy), $LD_{50/30}$ (7.0 Gy) or $LD_{80/30}$ (7.5 Gy) when the MNA administration started as late as 7 days post irradiation,
   - $LD_{80/30}$ (7.5 Gy) when the 1,3-MAP administration started as late as 7 days post irradiation,
   - $LD_{30/30}$ (6.5 Gy) or $LD_{80/30}$ (7.5 Gy) when the NAc administration started on the day of irradiation;
2) Markedly reduced numbers of circulating leukocytes, blood platelets, bone marrow cells, and spleen cells in all the groups of the animals. The examined compounds did not lead to the increase in the number of the examined cells in the irradiated animals;
3) Significantly increased production of pro-inflammatory cytokines, i.e., IL-1β, IL-6, IL-8, and TNF-a in the blood serum after WBI of mice at 5 Gy. The examined compounds decreased the concentrations of the cytokines as compared to the irradiated control, however, the detected values were higher than in the unirradiated counterparts;
4) Increased production of PGI_2 in the blood serum after WBI of mice at 5 Gy. The examined compounds only slightly modified concentration of PGI_2.

Overall, our results show that MNA may represent a prototype of a radioremedial agent capable of mitigating the severity and/or progression of radiation-induced injuries when applied several hours or days after exposure to high doses of ionizing radiation. Also, the obtained data suggest that the enhanced survival of the animals was more likely related to modulation of inflammatory and/or thrombotic vascular reactions rather than to stimulation of hematopoiesis.

This study was funded by the Grant No. DEC-2011/01/B/NZ7/05406 of the Polish National Science Center.

2.3 MODELING AND OTHERS

2.3.1 Updates on DTRA's Human Survivability Research and Development Program, D. Stricklin, USA, 2017

D. Stricklin, J. Bellman, D. Oldson, T. Dant, K. Kramer, K. Millage, and P. Blake

This presentation provided an update on the Defense Threat Reduction Agency's (DTRA) Nuclear Detonation (NUDET) Human Survivability Research and Development (HSRD) program which aims to improve human injury models for NUDET environments. DTRA serves as a DoD combat support agency, that safeguards USA and its allies from Weapons of Mass Destruction (WMD) by providing capabilities to reduce, eliminate, and counter the threat and mitigate its effects. DTRA also maintains DoD's WMD assessment codes.

The NUDET HSRD program supported by DTRA involves the development of health effects models to better understand the impact to affected populations after nuclear detonation scenarios. The modeling uses physiological and mechanistic approaches to understand how animal data relate to humans, to estimate the

impact from combined injuries, and to predict the time-course of injury. The granularity of models employed in the HSRD work are tailored to meet the specific requirements. For example, high level empirical models may be sufficient to predict the number of casualties from radiation or burn, whereas mechanistic models are required to understand the synergistic effects of combined injury or to perform cross-species extrapolations. Once fully developed, the individual models are integrated into the standalone software, Health Effects of Nuclear and Radiological Environments (HENRE). To date, models that can predict the time dependent mortality, symptom severity, and performance decrement from prompt neutron and gamma exposure and from protracted exposure to fallout have been integrated into HENRE.

Recent projects in the HSRD program have focused on evaluating the impact of neutrons in urban nuclear detonation scenarios, the impact of medical treatment on casualty estimation, examining the impact of age on acute radiation health effects and casualty estimations, and combined injury mechanistic models involving the small intestine and sepsis.

To address the concern that neutron exposures might impact the health effects of populations surviving a nuclear detonation, radiation transport simulations studies were conducted to examine the amount and energy of neutrons in the survivable zone of urban scenario. The studies provided preliminary insight on the potential doses and energies of neutrons that might be encountered in these scenarios.

Current casualty estimates focus on mortality from untreated injuries. To better understand the impact of effective care of injured populations, the modification of mortality estimates by standard of care treatment was explored. As an initial step, the use of a Dose Modification Factor (DMF) for the LD_{50} was used to examine the impact of treatment on casualty estimates. Similarly, DMFs for different ages at exposure were derived from animal experiments to adjust for the variable sensitivity to acute radiation at different ages. These DMFs allow for more accurate assessment of the impact of radiation among actual populations containing children and elderly persons.

The mechanistic modeling process used to address combined injury exposures was reviewed. The steps include development of a conceptual model, simplifications, establishment of parameter values, model validation, and final high level correlations. An overview of the mechanistic interactions encountered in radiation and thermal combined injuries were provided. A small intestine model was developed to account for the impact of gastrointestinal effects on intermediate mortality (2 – 14 days) after acute radiation exposure. The aim of the mechanistic model was to predict fluid loss, risk of hemorrhage, loss of GI integrity and risk of bacterial translocation. The model outputs were correlated with citrulline levels and the duration of crypt cell counts under a critical threshold.

A preliminary model for sepsis was described using Procalcitonin (PCT) as biomarker of infection and risk of sepsis. PCT is up-regulated during acute inflammatory response, particularly in response to infection, and has been established as one of the most promising biomarkers of burn-induced sepsis. PCT levels return to normal after successful treatment of infection. Some correlation has been observed with PCT concentration and the percent total body surface area burned (%TBSA).

Finally, to better inform patient streams and medical resource needs, an effort to link environments, health effects, and patient codes in medical planning tools is underway. Future work aims to explore the impact of population co-morbidities, trauma combined injury, cutaneous radiation injury, advanced treatment, partial body exposure, and the Relative Biological Effectiveness (RBE) of neutrons on casualty estimations. Computational aspects such as sensitivity and uncertainty analyses and population distributions will be addressed as well.

2.3.2 Introducing HENRE (Health Effects from Nuclear and Radiological Environments), D. Stricklin, ARA, 2018

D.L. Stricklin, J. Bellman, D. Oldson, T. Dant, K. Kramer, K. Millage, P. Blake

To begin this talk, Dr. Stricklin provided an introduction to the Defense Threat Reduction Agency (DTRA), highlighting their mission to enable the U.S. and the U.S. Department of Defense (DoD) to prepare for and combat weapons of mass destruction and improvised threats and to ensure nuclear deterrence. The agency's evolution over 75 years to incorporate evolving mission space and complexity of threats was summarized. Currently, DTRA serves as a DoD combat support agency, that safeguards USA and its allies from Weapons of Mass Destruction (WMD) by providing capabilities to reduce, eliminate, and counter the threat and mitigate its effects. DTRA also maintains DoD's WMD assessment codes.

The DTRA program relevant to the working group has been the Nuclear Detonation (NUDET) Human Survivability Research and Development program which aims to improve human injury models for NUDET environments. The program supports the development of a spectrum of models (environment, human response, injury criteria, physiological, and medical planning models) and their integration into operational tools.

Among the models developed for DTRA, the health effects models of the Health Effects of Nuclear and Radiological Environments (HENRE) software were highlighted. The health effects models are physiological and mechanistic models that enable extrapolation of animal data to humans and provide a means to account for mechanistic interactions that can lead to synergistic effects with combined injuries. This modeling approach also provides time dependent, clinically relevant information for medical planning, which in turn enables the estimation of the time-course of clinical symptoms, patient flow, and resource requirements. Ongoing model development for the HENRE algorithms include multi-scale approaches to understand the effects of dose protraction, combined injury, Relative Biological Effectiveness (RBE) of neutrons, demographic impacts, and treatment and recovery. Each of the models are incorporated into the standalone software, HENRE, currently at version 2.3. These models have also been integrated into the DTRA hazard prediction platforms and are currently being linked to DoD medical planning tools.

The health effects models incorporated into HENRE 2.3 include estimations from prompt gamma and neutron exposures and protracted gamma exposures. The models predict time dependent probability of mortality, symptom severity, and performance decrement from those exposures. Acute radiation syndrome models include prediction of bone marrow depletion (MarCell), GI effects, hematopoietic effects, and the modification of ARS by treatment (standard care). The models can account for combined radiation and burn injury on early mortality through a circulatory shock model, hematopoietic effects, and the small intestine model. A demographic model can account for the age of the person or ages among the populations exposed. A sepsis model for burn and a hemorrhage model-based shock has been incorporated. Current activities to support future development include examining neutron exposures in urban nuclear detonation scenarios and relevant RBEs, modification of mortality prediction based in treatment with G-CSF, evaluation of the impact of age on acute radiation health effects and the impact on casualty estimations, additional combined injury models, and development of patient condition codes for medical planning.

The presentation was concluded with a demonstration of the HENRE 2.3 software highlighting all of the input and output features along with a few simulations.

2.3.3 New Emergency Services Protocols for Managing CBRN Incidents in the UK, C. Foster, NAVYINM, 2019

The UK is implementing a modified response to chemical, biological, radiological and nuclear and hazardous material incidents that combines an initial operational response with a revision of the existing specialist operational response for ambulant casualties. The process is based on scientific evidence, initially from trials

performed as part of a program of work known as Optimization through Research of CHemical Incident Decontamination Systems ('ORCHIDS'). It focuses on the needs of casualties rather than the availability of specialist resources such as personal protective equipment, detection and monitoring instruments, and bespoke showering (mass-casualty decontamination) facilities.

Two main features of the revised process are:

1) The introduction of an emergency disrobe and dry decontamination step prior to the arrival of specialist resources; and
2) A revised protocol for mass-casualty (wet) decontamination that has the potential to double the throughput of casualties and improve the removal of contaminants from the skin surface.

This presentation reported on a recently published review article about practical aspects of the new operational responses (Chilcott et al. 2019). Optimized methods for performing dry and wet decontamination of CBRN contaminants were described with additional comments and references about the key principles for dealing with radiological contaminants. Although dry decontamination was considered the default CBRN incident response option, the review specifically recommended wet decontamination for solid forms of radiological contaminants. Other key principles discussed included the UK General Medical Council 'Good Medical Practice' published duty on doctors not to deny treatment to patients because of risk posed by their condition; and the Joint Emergency Services Interoperability Program statement: 'At the scene the clinical need of those affected should be balanced against any radiological hazard present. Priority 1 patients with life-threatening injuries should not have their treatment and transfer delayed for decontamination' (JESIP 2016).

References

Chilcott, R.P., Larner, J., Matar, H. (2019). UK's initial operational response and specialist operational response to CBRN and HazMat incidents: A primer on decontamination protocols for healthcare professionals. Emergency Medicine Journal; 36:117-123.

Joint Emergency Services Interoperability Program (2016). Responding to a CBRN(E) Event: Joint Operating Principles for the Emergency Services. First Edition September 2016.

Chapter 3 – LOW-LEVEL RADIATION BIOEFFECTS

3.1 EFFECT OF WHOLE BODY IRRADIATIONS WITH LOW DOSES OF X-RAYS ON DIABETIC VASCULAR ENDOTHELIUM

E. Nowosielska, MIHE, 2018

Ewa M. Nowosielska[1], Aneta Cheda[1], Marta Wincenciak[1], Jolanta Wrembel-Wargocka[1], Elżbieta Buczek[2], Aleksandra Gregorius[2], Bartosz Proniewski[2], Marta Smęda[2], Kamil Przyborowski[2], Stefan Chłopicki[2], Marek K. Janiak[1]

[1] Military Institute of Hygiene and Epidemiology, 4 Kozielska St., 01-163 Warsaw, Poland

[2] Jagiellonian Centre for Experimental Therapeutics, 14 Bobrzynskiego St., 30-348 Krakow, Poland

Atherosclerosis and diabetes mellitus are preceded by and associated with the development of endothelial dysfunction coinciding with vascular inflammation and thrombosis. In diabetes, one of the pivotal mechanisms underlying micro- and macrovascular changes is the hyperglycemia-induced overproduction of reactive oxygen species in endothelial cells. It has been demonstrated that exposures to low doses of low-LET irradiations during various stages of atherosclerosis protect against the progression of the disease in genetically-modified mice, attenuate the development of various diabetic complications such as nephropathy, vascular and cardiac inflammation, and impaired proliferation of neurons and wound healing. Since, in contrast to high-dose exposures, irradiations at low doses of X- or γ-rays have been shown to stimulate anti-oxidant functions in various cells and tissues, it can be hypothesized that such beneficial effects of the low-level exposures result from the radiation-induced suppression of the oxidative damage to the endothelial lining of micro- and macro-vasculature.

We presumed that endothelial effects of low doses of low-LET radiation would afford protection against atherosclerotic complications of diabetes. Hence, the aim of the present investigation was to evaluate the effects of low doses of X-rays on the structure and function of vascular endothelium in mice before and after the development of hyperglycemia – a cause of the vascular complications in diabetes.

In this project, the experiments were carried out on diabetic db/db, non-diabetic db/+, and wild-type (control) C57Bl/6 mice. The animals were exposed to five Whole Body Irradiations (WBI) at 0.002, 0.02, or 0.1 Gy of X-ray per day for five consecutive days. The irradiations started from the age of 6 (early-stage diabetes) or 16 (fully-developed diabetes) weeks. Based on the time of the development of endothelial dysfunction in the db/db mice, 8 and 14 weeks after WBI the selected parameters associated with endothelial dysfunction were assessed.

The obtained results showed that db/db mice develop hyperglycemia associated with alterations in the blood lipids, pro-inflammatory cytokines, and markers of endothelial dysfunction and that the tested low-level X-ray exposures applied at the early-stage diabetes do not seem to induce significant and consistent changes in the measured parameters in these mice. The obtained results showed that the low-level X-ray exposures applied during the fully-developed diabetes do not significantly affect most of the measured parameters in the db/db mice. The only exception was the significantly improved endothelial-dependent vasodilation in response to acetylcholine in the 30-week-old mice pre-irradiated for 5 days at 0.01 Gy/day. To confirm or exclude a possible curative effect of LDR on endothelial dysfunction in diabetes, further studies need to be conducted using considerably larger experimental groups (at least 25 animals per group).

This study was supported by the EU FP7 DoReMi (grant agreement 249689 – ELDoREndo project) on "Low Dose Research Towards Multidisciplinary Integration."

3.2 EFFECT OF INTERNAL CONTAMINATION WITH TRITIATED WATER ON THE INNATE ANTI-TUMOR AND ANTI-INFLAMMATORY REACTIONS IN RADIORESISTANT AND RADIOSENSITIVE MICE

Ewa M. Nowosielska, Aneta Cheda, Marek K. Janiak

Military Institute of Hygiene and Epidemiology, 4 Kozielska St., 01-163 Warsaw, Poland

Today people can be exposed to occupational, medical, and accidental irradiation from external sources or from external or internal radioactive contamination. One of the significant sources of internal contamination is tritium, a low-energy β-radiation-emitting isotope of hydrogen, that binds with hydroxyl radicals to form the easily internalized tritiated water (HTO). Tritium is used by a number of industries, for research and diagnostic purposes, and is also a common by-product of nuclear reactors. The development of nuclear plants and the increased emissions of tritium to the atmosphere enhance the risk of internal contamination of workers and members of the public with tritium reviving concern about its biomedical effects, especially in view of its possibly higher relative biological effectiveness than that of X- or γ-rays.

Radiation doses absorbed by tissues after internal contamination with T are within the low dose range, i.e., do not exceed 0.1 Gy when absorbed in a short time or 0.1 mGy/min. dose rate during a protracted irradiation. Such exposures do not cause direct health disorders, such as acute radiation syndrome, but can affect cancer incidence in the exposed population. Initiation and progression of cancer depend to a large extent on the function of the immune system which can exert both anti- and pro-neoplastic as well as anti- and pro-inflammatory responses.

Recent epidemiological and experimental data – including our own – indicate that external low-level irradiations with single or fractionated X- or γ-rays inhibit progression of both primary and secondary tumors and that these effects are related to up-regulated functions of NK lymphocytes and activated macrophages. Thus, the aim of the present study was to evaluate whether single internal contamination of mice with HTO affects the development of pulmonary tumor colonies and anti- or pro-neoplastic functions of macrophages and NK lymphocytes, circulating cytokine's levels, and numbers of bone marrow, spleen, and peripheral blood cells in the relatively radiosensitive BALB/c and the relatively radioresistant C57BL/6 mice whose pro-inflammatory and macrophage-type responses are differently expressed. Both strains of mice were intraperitoneally injected with HTO so that the total absorbed doses of radiation per mouse were 0.01 Gy (dose level encountered in occupational exposures), 0.1 Gy (upper limit of the acute low dose range) or 1.0 Gy (higher reference dose). Due to the biological half-time of HTO in mice, assessment of the selected parameters started on day 7 after administration of HTO (i.e., on the day when practically all of the HTO-derived radioactivity was absorbed by a mouse body).

The obtained results demonstrated that:

a) Internal contamination with HTO of radiosensitive BALB/c mice and radioresistant C57BL/6 mice leading to the whole body absorption of low doses of β-radiation stimulates cytotoxic activity of NK cells and macrophages, but the kinetics, duration and/or magnitude of the stimulation differs in the two strains: in BALB/c mice it occurs mainly at the absorbed dose of 0.1 Gy, while in C57BL/6 mice – mainly at 0.01 Gy absorbed dose;

b) The enhanced cytotoxic activity of splenic NK cells is associated with the increased production of IFN-γ, whereas the cytocidal activity of activated macrophages is associated with the increased production of nitric oxide by these cells;

c) Contamination with HTO leading to the absorption of 0.01, 0.1, or 1.0 Gy of β-radiation did not affect the development of the induced tumor colonies in the lungs of both radiosensitive BALB/c and radioresistant C57BL/6 mice;

d) Internal contamination with HTO did not affect the serum levels of pro- (IL-1β, IL-2, IL-6, TNF-α) and anti-inflammatory (IL-1Ra, IL-4, IL-10) cytokines in both strains of mice;

e) Exposure of mice of both strains to low and intermediate doses from the tritium-emitted β-particles did not result in any significant changes in the numbers of bone marrow, spleen, and peripheral blood cells.

Overall, the obtained data enrich our knowledge on biomedical effects of internalization of tritium by a living organism, indicating that internal tritium contamination of both radiosensitive and radioresistant mice leading to low and intermediate absorbed β-radiation doses is not immunosuppressive but may enhance some but not all components of anti-cancer immunity.

This study was funded by the Grant No. DEC-2011/01/D/NZ7/05389 of the Polish National Science Centre.

3.3 PRECLINICAL EVALUATION OF WHOLE BODY IRRADIATIONS AT LOW DOSES OF X-RAYS COMBINED WITH INHIBITION OF IMMUNE CHECKPOINTS AND A HEAT SHOCK PROTEIN AS A NOVEL THERAPY FOR LUNG CANCER

Ewa M. Nowosielska1, Aneta Cheda[1], Mateusz Pociegiel[2], Lukasz Cheda[3], Paweł Szymański[1,4], Antoni Wiedlocha[1,5,6], and Marek K. Janiak[1]

[1] Military Institute of Hygiene and Epidemiology, 4 Kozielska St., 01-163 Warsaw, Poland

[2] National Centre for Nuclear Research Radioisotope Centre POLATOM, 7A Soltana St.,05-400 Otwock, Poland

[3] Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, 101 Żwirki i Wigury St., 02-089 Warsaw, Poland

[4] Department of Pharmaceutical Chemistry, Drug Analyses and Radiopharmacy, Faculty of Pharmacy, Medical University of Lodz, 1 Muszyńskiego St., 90-151 Lodz, Poland

[5] Department of Molecular Cell Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Montebello, 0379 Oslo, Norway

[6] Centre for Cancer Reprograming, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Montebello, 0379 Oslo, Norway

Non-Small Cell Lung Cancer (NSCLC) continues to be the leading cause of cancer death worldwide including Poland. In contrast to a considerable progress made over the recent years in the diagnosis and treatment of malignant neoplasms, the prognosis for lung cancer patients, which are usually diagnosed at an advanced stage of the disease, remains poor. No wonder therefore that the new, more effective therapies of lung carcinoma are being actively investigated. As evidenced by the results of preclinical and clinical investigations, Whole Body Irradiations (WBI) with x-rays at less than 0.1-0.2 Gy per fraction can induce remissions of various neoplasms without inciting adverse side effects of conventional chemo-and radiotherapy.

The immune system is the organism's most important guardian against neoplasia. Although a significant progress has been made in designing and implementing new therapies aimed at stimulation of anti-cancer immunity in oncological patients, the major obstacle to the effective control of cancer progression is the development of immunosuppression within the tumor site. A critically important role in immunosuppression is played by the Immune Checkpoints (ICs). It seems that Immune Checkpoints Inhibitors (ICSs) might have significant utility in treating cancer. In fact, immunotherapy with ICIs has become the most important novel

treatment modality for lung cancer. In addition, blockade of intracellular molecules that promote cancer progression has been tested in preclinical models as a potential anti-cancer treatment. Among the molecular targets of such treatment are Heat Shock Proteins (HSPs), such as HSP90, which facilitate the function of oncoproteins and have been considered as co-factors for the development and progression of the malignant phenotype. Hence, the aim of the present investigation was to evaluate the therapeutic efficacy of WBI of the lung tumor-bearing mice combined with the blockade of the function of one or two ICs and/or the HSP90 chaperone.

Syngeneic Lewis Lung Carcinoma (LLC1) cells – the only reproducible experimental model for the human NSCLC to date – were used for the induction of tumor nodules in mice. We injected LLC1 cells into C57BL/6 mice via three different routes:

a) Intravenously (i.v.), to assess the development of the induced tumor colonies in the lungs;

b) Orthotopically (o.t.), to evaluate the growth of a primary tumor in the injected lung and its possible metastases; and

c) Subcutaneously (s.c.), to follow the development of a primary tumor and the occurrence of its possible metastases.

The animals were exposed to fractionated x-rays (5 days per week for one week) at total doses of 0.1 and 1.0 Gy. Antibodies directed against the CTLA-4 and PD-1 ICs and synthetic inhibitor of HSP90 protein (NVP-AUY922) were injected i.p. three times a week for one week. Then, the following parameters were estimated: morphometric assessment of the tumor growth, number of tumor colonies in lungs, clonogenic potential in vitro of the tumor cells obtained from the mice injected with the LLC1 cells, the levels of the selected pro- and anti-inflammatory cytokines involved in immune surveillance. Also, given the importance of immune cells in the tumor microenvironment, the tumor-associated immune cells such as TAMs, TILs, or NK cells in the pulmonary and subcutaneously growing tumor s.

The present results confirm and expand our previously reported observations of the anti-neoplastic activity of multiple WBIs of mice at low doses of x-rays. We demonstrated that such WBIs at five daily doses of 0.02 and 0.2 Gy suppress the growth of LLC1 cells in vivo and in vitro and that the effects are accompanied by the relevant changes in the numbers of both pro- and anti-neoplastic immune cells in the developed tumors as well as in the serum levels of the selected pro- and anti-inflammatory cytokines in the irradiated mice. We indicated, for the first time, that a combination of WBIs with blockade of the CTLA-4 and PD-1 ICs, especially when the two had been inactivated together, also potently inhibited the growth of lung cancer cells in vivo and their clonogenic potential in vitro. Such a combined treatment seemed to promote the cellular and cytokine alterations induced by the sole use of WBIs. Generally, inactivation of the function of HSP90 applied concurrently with WBI appeared to be less effective in these regards. Notably, the anti-neoplastic effects in vivo of WBIs used solely and in combination with the blocking agents were comparably expressed in mice with tumors induced by i.v., o.t., or s.c. injection of the LLC1 cells. Alas, the observed shifts in the composition of the immune cells and secreted cytokines addition of any or all of the tested blockers to WBI did not seem to markedly augment the already well pronounced anti-neoplastic effect of the latter. Moreover, application of most of the inhibitors without the concomitant WBI appeared to be almost totally ineffective in inhibiting the development of the transplanted tumors in vivo; only simultaneous inactivation of CTLA-4 and PD-1 suppressed the potential of LLC1 cells to produce neoplastic colonies in vitro and to grow into subcutaneous tumors in vivo. The obtained results supplemented with further mechanistic explanations provided by future investigations will help design the effective strategies of treatment of lung and other cancers based on inactivation of the immune checkpoint and/or heat shock molecules combined with low dose radiotherapy.

This research was funded by the grant No. 510/2017/DA from the Polish Ministry of National Defence.

Chapter 4 – RTG HFM-291, PLATFORM FOR INTERNATIONAL COLLABORATIONS

4.1 ESTABLISHING GENE EXPRESSION MEASUREMENTS IN SALIVA, A CZECH REPUBLIC-GERMAN COLLABORATION, P. OSTHEIM, BIR, 2018

Human saliva is mainly composed of fluid and compounds produced by the major salivary glands, including the parotid, submandibular and sublingual glands, as well as the minor salivary glands. The salivary glands secrete fluid transported from serum as well as tissues surrounding the glands. Other human saliva constituents are from the oral mucosa, periodontium, and the oral microbiome (Caporossi et al. 2009).

During the last two decades, saliva has become of increased interest as an easily accessible and non-invasive source of human biomarkers. Besides DNA, proteins and various metabolites, RNA has also been shown as a promising marker in other tissues and body fluids, providing complex gene expression information. Because saliva is derived from several tissue sources and that it also contains large amounts of total RNA make it one of the most attractive diagnostic, prognostic, and monitoring tools for both systemic and oral diseases (Maron et al. 2010, Watanabe et al. 2017, Lacombe et al. 2017). Hereby, saliva has been shown to contain RNA biomarkers (mRNA and smallRNA species) for prediction and diagnosis of several diseases (Kaczor-Urbanowicz et al. 2017), especially of the oral cavity such as oral cancer (Ghizoni et al. 2019, Li et al. 2004) or disorders of the salivary glands (Chen et al. 2015, Michael et al. 2010).

Before using precious human samples collected from e.g., head and neck tumor patients in Czech Republic, we performed systematic analysis and identified two problematic issues not coherently described before: 1) Most of the isolated RNA originates from the oral microbiome; and 2) The amount of isolated human RNA is comparatively low.

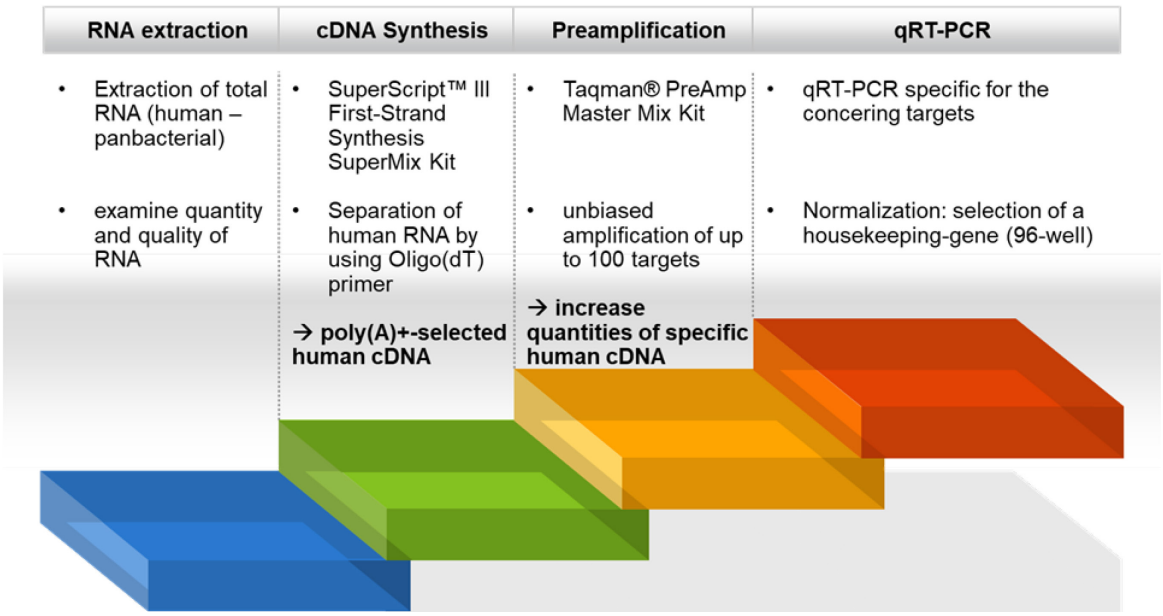


Figure 4-1: Modification of the Conventional Workflow by (1) Altered cDNA Synthesis (Employing Poly-A Tail Primer and Selection of Human cDNA Only) and (2) A Pre-Amplification Step.

The degree of bacterial contamination showed ratios up to 1:900,000, so that only about one out of 900,000 RNA copies was of human origin. As a possible solution, we modified the methodology to select only human RNA during cDNA synthesis by aiming at the poly(A)+-tail (Figure 4-1). We found pre-amplification of human RNA before qRT-PCR was required. Further, the pre-amplification step had to be controlled for each gene and the recommended Ct values below 35 for unamplified cDNA were insufficient. This had to be augmented by the criteria of showing a linear pre-amplification. After implementing these changes, we successfully performed qRT-PCR for detection of mRNA expression for specific genes associated with radiation response.

We report improved methods to overcome challenges of salivary RNA, namely the overwhelming bacterial contamination and low abundance of human RNA, in order to perform unbiased gene expression analysis on human RNA.

References

Caporossi, L., Santoro, A., Papaleo, B. (2010). Saliva as an analytical matrix: State of the art and application for biomonitoring. Biomarkers, Sep, 15(6):475-87. doi:10.3109/1354750X.2010.481364.

Maron, J.L. et al. Neonatal salivary analysis reveals global developmental gene expression changes in the premature infant. Clin. Chem. (2010). doi:10.1373/clinchem.2009.136234.

Watanabe, K., Akutsu, T., Takamura, A., Sakurada, K. (2017). Practical evaluation of an RNA-based saliva identification method. Sci. Justice, Nov, 5 7(6):404-408. doi:10.1016/j.scijus.2017.07.001

Lacombe, J. et al. (2017). Analysis of saliva gene expression during head and neck cancer radiotherapy: A pilot study. Radiat. Res, Jul, 188(1):75-81. doi:10.1667/rr14707.1.

Kaczor-Urbanowicz, K.E. et al. (2017). Saliva diagnostics – Current views and directions. Experimental Biology and Medicine, Mar, 242(5):459-472. doi:10.1177/1535370216681550.

Ghizoni, J.S., Nichele, R., de Oliveira, M.T., Pamato, S., Pereira, J.R. (2019). The utilization of saliva as an early diagnostic tool for oral cancer: MicroRNA as a biomarker. Clinical and Translational Oncology, Jun, 22(6):804-812. doi:10.1007/s12094-019-02210-y.

Li, Y. et al. (2004). Salivary transcriptome diagnostics for oral cancer detection. Clin. Cancer Res, Dec 15, 10(24):8442-50. doi:10.1158/1078-0432.CCR-04-1167.

Chen, W., Cao, H., Lin, J., Olsen, N., Zheng, S. G. (2015). Biomarkers for Primary Sjögren's Syndrome. Genomics, Proteomics and Bioinformatics, Aug, 13(4): 219-223. doi:10.1016/j.gpb.2015.06.002.

Michael, A. et al. (2010). Exosomes from human saliva as a source of microRNA biomarkers. Oral Dis. Jan, 16(1):34-8. doi:10.1111/j.1601-0825.2009.01604.x.

4.2 ESTABLISHING GENE EXPRESSION FOR H-ARS PREDICTION, A FRENCH-CZECH REPUBLIC-GERMAN COLLABORATION, M. MAJEWSKI, BIR, 2018

In 2012, Francis Hérodin's group working at the former Centre de Recherches du Service de Santé des Armées (CRSSA, Grenoble, France) which is now the Institut de Recherche Biomédicale des Armées (IRBA, Brétigny-sur-Orge, France) conducted a molecular biological study on 18 baboons. Baboons were irradiated using a Cobalt-60 source (IRDI 4000; Alsthom, Levallois, France). By shielding different body parts, several

patterns of partial body exposure (partial body irradiation, PBI) and total body (Total Body Irradiation, TBI) exposures (e.g., 2.5 and 5 Gy, respectively) were attained (Figure 4-2). Two baboons were exposed per pattern, which summed up to 18 baboons receiving PBI or TBI corresponding to an equivalent TBI dose of 2.5 or 5 Gy. These sublethal doses induced primarily the Hematologic Acute Radiation Syndrome (H-ARS), which was the focus of this research, in the absence of a gastrointestinal ARS (GI-ARS).

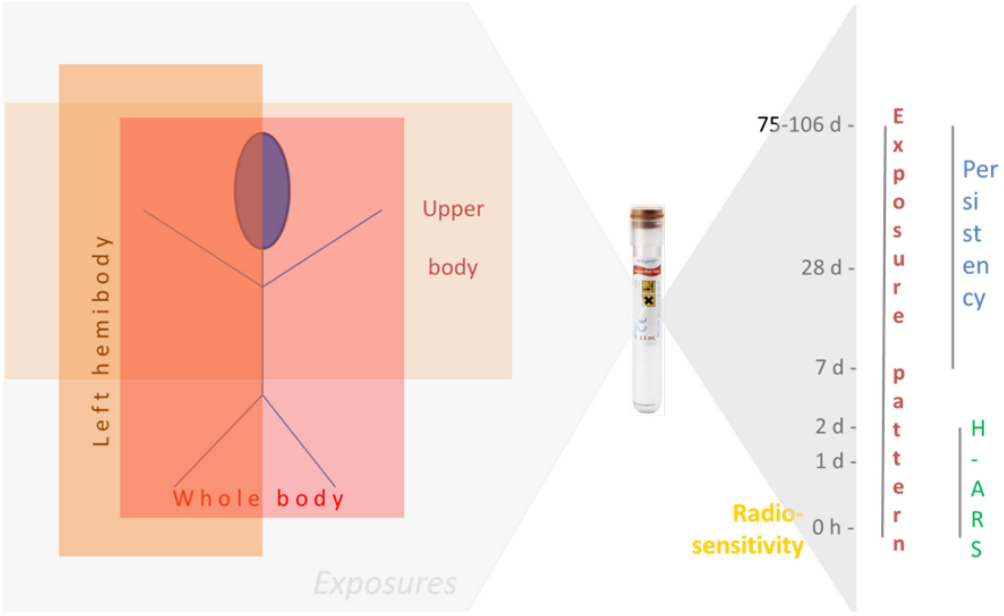


Figure 4-2: Radiation Exposure to the Whole Body (e.g., 2.5 Gy or 5 Gy), the Left Hemibody (5 and 10 Gy) and the Upper Body (e.g., One or Two Legs Shielded or Exposures of Head and Arms Only) Are Shown on the Left Side. Total body exposure, but head and neck shielded is not presented. Blood samples were taken at the indicated time points to the right as well as the associated radiobiological topics examined. The 0 h value reflects the unexposed or pre-exposure blood samples used as a reference in all analysis.

Blood samples were taken before (pre-exposure samples), 1, 2, 7, 28 and 75 – 106 days after irradiation (Figure 4-3). This allowed examinations of radiobiological topics related to radiosensitivity (pre-exposure samples only), H-ARS prediction (1 and 2 days after exposure) and examinations related to persistent gene expression changes (7, 28 and 75 – 106 days after exposure) and discrimination of exposure pattern examined over the whole period of time (Figure 4-2).Via RTG, Germany, known for its methodological expertise in gene expression analysis, was generously offered to participate in this study. This study is unique not only because of the precious animal model, but also because blood samples were collected at several time points, thus, allowing for a molecular biological follow-up over time.

With the arrival of blood tubes containing peripheral whole blood and starting the baboon-project in January 2013, five major radiobiological areas could be systematically examined over the next eight years, following a previously agreed analysis plan (Figure 4-2).

These areas include:

1) Radiosensitivity: Does the transcriptional status of cells before irradiation dictate the later developing degree of radiation damage, namely the H-ARS severity?
2) Effect prediction: Predicting the later developing H-ARS and pancytopenia based on early gene expression changes. This approach on effect prediction was favored in contrast to the more established field of dose estimation for reasons presented herein.

3) Persistency of gene expression over time: When working on diagnostic tools, persistent instead of alternating gene expression changes over time would reduce complexity and increase robustness of the diagnostic tool.
4) Discriminating total from partial body exposure pattern: Pairs of baboons were irradiated realizing nine different whole or partial body exposure patterns. Is that reflected by gene expression changes?
5) Methodological considerations: Our research always followed a two-stage study design using a part of the samples for screening and the remaining samples for validation utilizing different technologies. That bears advantages and some difficulties, which will be addressed herein.

In this presentation we aim to summarize key results on these five topics, explain underlying concepts/rationales, share experiences, offer a matured diagnostic tool for H-ARS prediction freely applicable in other laboratories (Figure 4-3), describe pitfalls and misinterpretations, provide solutions and compare these findings with research, which followed and which deepened our understanding of underlying radiobiological processes/methods used.

Diagnostic tool		gene combination, fold-deregulated relative to mean normal range			
		FDXR and DDB2		WNT3 and POU2AF1	
H-ARS severity degree	Indications	onset (h)	fold-chance	onset (h)	fold-chance
0	unexposed, no hospitalization required	2-4	Ø	4-8	Ø
0-1	no or low radiation exposure, no acute effects expected, no hospitalization required, eventually late health effect, further screening required	2-4	↑	4-8	Ø
2-3 (4)	clinically significant exposure, hospitalization and early therapy onset required	2-4	↑	4-8	↓

Figure 4-3: Description of the Diagnostic Tool Based on Radiation-Induced Gene Expression Changes and Predicted Clinical Outcomes of the Hematological Acute Radiation Syndrome (H-ARS Severity Degree).

Knowledge in science is a flow and we feel privileged contributing to it. What we show now is a moment and we must accept that it will be altered with the next experiments – that is the nature of science and applies to this review as well.

4.3 DISCRIMINATING TBI FROM PBI USING GENE EXPRESSION IN IRRADIATED BABOONS, A FRENCH-GERMAN COLLABORATION, M. ABEND, BIR, 2019

Scenario dependent it is likely that either Total Body Irradiation (TBI) or different Partial Body Irradiation (PBI) pattern will occur. For instance, the atomic bomb detonation over Hiroshima and Nagasaki caused divergent partial body exposure pattern (Cullings et al. 2006, Sakata et al. 2012, Preston et al. 2004), but total

body exposures are reported in the context of the Chernobyl nuclear power plant accident (UNSCEAR 2010) and detailed reports of radiation victims stored in SEARCH (System for Evaluation and Archiving of Radiation accidents based on Case Histories) reflect that. However, estimating the absorbed dose and conclude about clinical outcomes becomes difficult in the case of PBI versus TBI exposures. When dealing with stochastic effects (random mutations and other cell changes leading for example to cancerogenesis) the International Commission on Radiological Protection (ICRP) suggested tissue weighting factors so that by multiplication of the equivalent dose with the locally irradiated tissue an effective dose could be calculated (ICRP 2007).

Through the effective dose local exposures can be compared with each other and made comparable with a whole body exposure, but with the focus on a late clinical outcome such as cancerogenesis. Weighting factors such as that are missing for deterministic effects (e.g., the Acute Radiation Syndrome, ARS) where cell death mechanisms predominate and not mutations. Hence, the conversion of a local exposure into a whole body equivalent becomes challenging. Also, the same absorbed dose given whole body versus partial body will result in differences regarding e.g., survival. Irrespective of the dose estimation problematic the knowledge of the exposure pattern will provide inputs to better predict the clinical outcome.

With this intention we examined the post-transcriptome (miRNA) for gene expression changes which can be measured early and in high-throughput in easy accessible biosamples such as the peripheral blood of radiation exposed animals (Port et al. 2016) In collaboration with the French Army Biomedical Research Institute, we assessed blood samples obtained from 17 irradiated baboons taken before (day 0) and at 1, 2, 7, 28 and 75 – 106 days after exposure. Animals received an upper body, left hemibody or total body exposure of 2.5 or 5 Gy. On the blood samples we screened for 667 miRNAs using a qRT-PCR platform.

We could identify altogether 55 genes over all time points after irradiation combined providing hints to the exposure pattern. Candidate genes such as miR-17, miR-128 or miR-15b significantly discriminated TBI from different PBI exposure pattern and 5-10-fold changes in gene expression between the groups were observed. Twenty-two miRNAs (including miR-17) did also reveal significant linear associations of gene expression changes with the percentage of the exposed body area ($p<0.0001$, Figure 4-4). All these changes were primarily observed at day seven after exposure and almost no genes were detected either before or after 7 days. A significant association in the reduction of lymphocyte counts in TBI compared to PBI exposed animals corresponded with the number of candidate genes. This might suggest that our target genes predominantly originated from irradiated lymphocytes. Hence, gene expression changes in the peripheral blood provide indications of the exposure pattern and even the exposed body area but that is in particular observed and restricted to a certain post exposure time.

References

Cullings, H.M., Fujita, S., Funamoto, S., et al. (2006). Dose Estimation for Atomic Bomb Survivor Studies: Its Evolution and Present Status. Radiat Res. Jul, 166(1 Pt 2):219-54. doi: 10.1667/RR3546.1.

International Commission on Radiological Protection. (2007). The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. Ann ICRP. doi: 10.1016/j.icrp.2004.12.002.

Port, M., Herodin, F., Valente, M. et al. (2016). First Generation Gene Expression Signature for Early Prediction of Late Occurring Hematological Acute Radiation Syndrome in Baboons. Radiat Res 186, 39-54. doi: 10.1667/RR14318.1.

Preston, D.L., Pierce, D.A., Shimizu, Y., et al. (2004). Effect of recent changes in atomic bomb survivor dosimetry on cancer mortality risk estimates. Radiat Res 162, 377-389.

Sakata, R., Grant, E.J., Ozasa, K. (2012). Long-term follow-up of atomic bomb survivors. Maturitas Jun, 72(2):99-103.

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (2010). Sources and Effects of Ionizing Radiation: UNSCEAR 2008 Report.

4.4 PROJECT PLAN ON GENE EXPRESSION MEASUREMENTS IN MACMUL, A US-GERMAN COLLABORATION, M. ABEND, BIR, 2019

In 2019 AFRRI (Dr. V. Singh) and BIR (Drs. P. Ostheim and M. Abend) agreed to a joint collaboration and developed an analysis plan.

The work should be shared as follows:

V. Singh-group, AFRRI, Bethesda, Maryland, USA

- Providing PaxGene tubes filled with 1x1 ml peripheral whole blood per rhesus macaque before irradiation and ideally at 1, 2, 3, 35 and 60 days after irradiation (sampling until day of death; in 3 exceptional cases additionally on one of days 14/15/18).
- Providing clinical data of the animals, e.g., day of death after irradiation, blood cell counts and symptoms during the whole follow-up (up to 60 days) after irradiation.
- M. Abend-group, InstRadBioBw, Munich, Germany.
- Developing a two-stage study design for prediction of ARS° (effect) based on gene expression changes.
- Validating and developing the already evolved models based on gene expression analysis for prediction of BCC-depletions (effect) in close communication and agreement with the other participants.
- Examining whether some gene expression changes will persist even > 7 days after exposure.
- Developing a two-stage study design for searching on radiosensitivity (gene expression before irradiation vs. survival at 60 days), gender dependent in close communication and agreement with the other participants.
- Isolating mRNA and smallRNA and doing QC.
- Selecting RNA samples for both stages of the study design.
- Sending RNA samples to IMGM to perform the whole genome screening (NGS).
- Performing qRT-PCR for final validation of mRNA and smallRNA species from screening stage (validation phase).
- Data analysis of data from both stages.
- Writing a draft manuscript and discussing it in close agreement with all participants.

Material provided for analysis

- For each rhesus macaque whole blood samples (altogether 338 samples) were drawn at different points in time after irradiation (Table 4-1). Per time point V. Singh (AFFRI) provided 1 PaxGene tube for RNA analysis (equivalent to 1x1ml peripheral blood).

Table 4-1: Summary of Effects, Treatment, Available Blood Samples Taken at Certain Days Before and After Exposure "O" ≙ sample for screening, "x" ≙ Sample for Validation.

				treatment		PAXGene tubes - days after exposure								samples used for		
NHP ID convert	gender	dose	H-category	GT3	vehicle	7 days before exp.	1 day before exp.	1	2	3	14/15 /18	35	60	stage I screening	stage II validation	task
1	m	5.8 Gy	H4	x		x		x	x	x					all days	
2	m	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
3	m	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
4	m	5.8 Gy	H4	x		x		x	x	x					all days	
5	f	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
6	m	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
7	m	5.8 Gy	H4	x		x		x	x	x					all days	
8	m	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
9	m	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
10	m	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
11	m	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
12	f	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
13	f	5.8 Gy	H4	x		x		x	x	x		x			all days	
14	f	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
15	f	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
16	f	5.8 Gy	H3	x		x		x	x	x		x	x		all days	
17	m	5.8 Gy	H4		x	O		x	x	x				O	1, 2, 3	III
18	m	5.8 Gy	H4		x	x		x	x	x					all days	
19	m	5.8 Gy	H4		x	x		x	x	x					all days	
20	m	5.8 Gy	H3		x	x		x	x	x		x	x		all days	
21	m	5.8 Gy	H3		x	O		x	O	x		x	x	O	1, 3, 35, 60	III
22	m	5.8 Gy	H3		x	x		O	O	O		O	O	O	-7	I, II
23	m	5.8 Gy	H3		x	x		x	x	x		x	x		all days	
24	m	5.8 Gy	H3		x	x		x	x	x		x	x		all days	
25	m	5.8 Gy	H3		x	x		O	x	O		O	O	O	-7,2	I, II
26	f	5.8 Gy	H3		x	O		x	O	x		x	x	O	1, 3, 35, 60	I, III
27	m	5.8 Gy	H3		x	x		x	x	x		x	x		all days	
28	f	5.8 Gy	H3		x	x		O	O	x		x	x	O	-7,3, 35, 60	I
29	f	5.8 Gy	H4		x	O		O	O	O				O		I, III
30	f	5.8 Gy	H4		x	O		O	O	O				O		I, III
31	f	5.8 Gy	H3		x	O		x	O	x		x	x	O	1, 3, 35, 60	I, II, III
32	f	6.5 Gy	H4	x		x		x	x	x					all days	
33	m	6.5 Gy	H3	x		x		x	x	x		x	x		all days	
34	f	6.5 Gy	H3	x		x		x	x	x		x	x		all days	
35	m	6.5 Gy	H3	x		x		x	x	x		x	x		all days	
36	f	6.5 Gy	H4	x		x		x	x	x					all days	
37	f	6.5 Gy	H3	x		x		x	x	x		x	x		all days	
38	f	6.5 Gy	H4	x		x		x	x	x					all days	
39	m	6.5 Gy	H3	x		x		x	x	x		x	x		all days	
40	f	6.5 Gy	H4		x	O		x	x	x				O	1, 2, 3	I, II, III
41	f	6.5 Gy	H3		x	O		x	x	x		x	x	O	1, 2, 3, 35, 60	I, II, III
42	f	6.5 Gy	H3		x	x		O	x	O		O	O	O	-7,2	I, II
43	f	6.5 Gy	H3		x	x		O	x	O		O	O	O	-7,2	I, II
44	m	6.5 Gy	H3		x	x		x	x	x		x	x		all days	
45	f	6.5 Gy	H3		x	x		x	x	x		x	x		all days	
46	f	6.5 Gy	H3		x	O		x	x	O		O	O	O	1, 2	I, II, III
47	f	6.5 Gy	H4		x	O		O	O	O				O		I, III
48	m	7.2 Gy	H3		x		x	x	x	x		x	x		all days	
49	m	7.2 Gy	H4		x		O	x	x	x	x			O	1, 2, 3, 18	III
50	m	7.2 Gy	H4		x		x	O	O	O	O			O	-1	I
51	m	7.2 Gy	H3		x		O	x	O	x		x	x	O	1, 3, 35, 60	I, II, III
52	m	7.2 Gy	H3		x		x	x	x	x		x	x		all days	
53	m	7.2 Gy	H3		x		O	x	x	x		x	x	O	1, 2, 3, 35, 60	I, II, III
54	m	7.2 Gy	H3		x		x	x	x	x		x	x		all days	
55	m	7.2 Gy	H4		x		x	x	x	x	x				all days	
56	m	7.2 Gy	H3		x		O	x	x	x		x	x	O	1, 2, 3, 35, 60	I, II, III
57	m	7.2 Gy	H3		x		x	x	x	x		x	x		all days	
58	m	7.2 Gy	H4		x		O	x	x	x				O	1, 2, 3, 35, 60	III
59	m	7.2 Gy	H3		x		x	O	x	O		O	O	O	-1,2	I, II
60	m	7.2 Gy	H4		x		x	x	x	x					all days	
61	m	7.2 Gy	H4		x		x	O	O	O				O	-1	I
62	m	7.2 Gy	H4		x		O	O	O	O				O		I, III
63	m	7.2 Gy	H4		x		x	x	x	x					all days	

Exposures – background

- According to the original task 64 rhesus macaques were irradiated with three different doses of Cobalt-60 whole body irradiation. One rhesus macaque was excluded from the study due to a viral infection so that the total number of irradiated rhesus macaques included in this study is 63. In particular, there were 3 exposure patterns/groups with doses of 5.8, 6.5 and 7.2 Gy (dose rate 0.6 Gy/min).
- The groups irradiated with 5.8 (n = 31) and 6.5 Gy (n = 16) were further subdivided in 2 parts: half of the group was treated with GT3, whereas the other half did not receive any drug treatment except the chemistry in which the drugs are diluted, called vehicle samples.
- The group of NHP irradiated with 7.2 Gy (n = 16) did not receive any drug treatment (only the vehicle substance).
- These exposures correspond to:
  - LD 29/60 (9 from 31 monkeys) at 5.8 Gy;
  - LD 31/60 (5 from 16 monkeys) at 6.5 Gy; and
- LD 50/60 (8 from 16 monkeys) in 7.2 Gy. These numbers are almost comparable for both subgroups of GT3 treated and untreated monkeys gender dependent. The exposures are expected to primarily inducing a hematopoietic syndrome and a few minor degree gastrointestinal symptoms. In the literature, GT3 is expected to cause hematopoietic recovery after its administration to irradiated NHPs by decreased neutropenia and thrombocytopenia compared to vehicle-treated irradiated controls.
- Clinical data such as BCC were provided by V. Singh (AFFRI) and depicted for each animal (tables not shown). Therapeutic interventions are almost negligible (not shown).

The topics to be examined included the following tasks:

1) We will check for changes on the mRNA level and small RNA level and correlate ARS severity (deduced from BCC counts changes after exposure) with radiation-induced gene expression changes for the time points available. Therefore, we use gene expression as a bioindicator of effect, not dose. From the clinical point of view, it is even more attractive to be able predicting ARS severity during the first days after exposure which have to be treated by the clinician (categories with therapeutic implications). We expect gene expression to change within the first 3 days after irradiation, before the manifestation of acute radiation syndromes. Based on gene expression changes measured in the peripheral blood we aim to validate our mathematical models for prediction of the later occurring H-ARS and its different degrees of severity. This will be performed by examining transcriptional (mRNA) and posttranscriptional changes (smallRNA) using bioinformatic tools and logistic regression analysis. For this purpose, we will at first focus on genes constantly up- or down-regulated within the first 3 days after irradiation. Hereby, we can also check if there is a difference between vehicle-treated NHP and a specific drug treatment (GT3).
2) Epigenetic research indicates that exposures will be memorized by cells and even transferred to the next cell generation by e.g., DNA-methylation. Therefore, we intend to examine gene expression at later time points after irradiation as well, starting at 35 days after exposure and later (up to 60 days). Again, we intend to check for changes on the mRNA level and the smallRNA level too. We can also check on the impact of specific drug treatment (GT3) and how that translates into late gene expression changes examined in the survivors (60 days after exposure) for both genders.
3) We are interested to search for radiosensitivity differences among monkeys on the transcriptome and post-transcriptome level. In this experiment we have 3 major groups of animals: treated with 5.8 Gy, 6.5 Gy and 7.2 Gy whole body exposures. Some of the animals died within 3 – 60 days, others survived and were sacrificed at day 60 after exposure. Blood samples taken before irradiation are

available for males and females. With a two-stage study design we would screen for transcriptional (mRNA) and posttranscriptional changes (smallRNA) in a first stage using part of the samples and would validate the candidate genes during second stage using the remaining samples and changing the methodology (for details see next chapter). With the samples provided we can also search for gender specific differences and similarities in radiosensitivity.

4) Our recent research at the InstRadBioBw in part requires an inter- and intra-species spanning validation of certain candidate genes and this will be accomplished within this project as well.

4.5 WORK OF CBRNMED WG AND NATO EX CLEAN CARE 2020 EXERCISE PLANNING, S. BLAND, ROYAL NAVY, 2019

Report on behalf of Surgeon Commander Dr. Steve Bland, Chair of CBRNMed WG by Michael Abend.

The chair of CBRNMed WG, Dr. Steve Bland, expressed his appreciation for the RTG works group during the 2012 – 2016 period and congratulated the RTG for winning the prestigious STO Scientific Award.

Dr. Bland provided an overview on the work of CBRNMed WG, the integration in existing panels and the hierarchy of STANAG documents. Developing STANAG documents related to CBRN medical issues represents the main focus of this group.

In order to examine the conversion of these documents into real-life scenarios, over the years CBRNMed WG organized a series of exercises called “CLEAN CARE”. Dr. Bland provided an overview on the forthcoming NATO CLEAN CARE 2020 exercise supposed to take place in Salisbury 11 – 20 September 2020. RTG discussed and proposed a participation of a joint multinational RTG Task Force including medical expertise from France, Italy and Germany.

Unfortunately, this exercise had to be postponed due to the corona pandemic. It is referred to as Ex CLEAN CARE at certain places in this manuscript.

4.6 GENE EXPRESSION MEASUREMENTS IN HDFA CELLS, A GERMAN-ITALIAN COLLABORATION, V. FRANCHINI, ITA, 2019

Electromagnetic Field (EMF) exposure in humans will certainly involve the skin. As an alternative to in vivo measurements it would be desirable to establish in vitro skin models to reproducibly perform replicate EMF experiments on the skin.

Human Dermal fibroblasts (HDFa) can be easily isolated from the skin and cultured (Janson et al. 2012). They are supposed to represent a meaningful although simplified skin model and are thought useful as a surrogate for dermal exposure (Janson et al. 2012). Using primary HDFa cells is of advantage since they represent normal and not immortalized cells. Primary cells including HDFa cells incubated in vitro divide only for a certain period of time due to e.g., suboptimal in vitro conditions and the number of passages is restricted. Also, the cell culture time per passage number is limited. These aspect not only limit the in vitro experiments on the time scale, but they also add undesired variance to the in vitro model which might mask effects caused by exposures with e.g., EMF. Another difficulty when working with primary cells refers to the restricted availability of cells from the same donor so that in vitro experiments using the same donor are limited. This adds inter-individual variance to the experiments which, again, might mask slight effects caused by the exposure. On the contrary it is important performing experiments to address inter-individual variance, but the numbers of replicate measurements on the same individual are restricted when using primary cells. Also, HDFa cells can be cultivated in special media supplemented with growth factors

such as the 106 medium including human epidermal growth factor (10 ng/ml) and basic fibroblast growth factor (3 ng/ml) and 2% fetal bovine serum (from LSGS kit) or in DMEM supplemented with e.g., 10% fetal calf serum. Hence, different sources of variance have to be considered and cannot be avoided when performing in vitro experiments. It would be desirable to know and identify the cell status of HDFa cells before starting with the exposure so that reproducible experiments under the same cell status conditions could be performed. We aimed to contribute to this challenge and examined primary HDFa cells in two different media, namely DMEM supplemented with 10% fetal calf serum and 106 medium supplemented with growth factors (Low Serum Growth Supplement (LSGS) kit from Gibco).

We seeded HDFa cells from two different donors with 0,5x106 cells/flask over 24 hours. During the next 6 days growth curves, vitality, morphology as well as gene expression of genes coding for cell proliferation (PCNA, CDKN2A, CDKN1A, SFN), differentiation (PDGFRA, TGM2, ACTA2, PDPN, NTN1, MGP, PPP1R14) and SFN target genes (TP63, MMP1, MMP3) were examined in both media and passage numbers 3-4, 5-6 and >6 in three independent experiments (Figure 4-4). At passages 3-4 a doubling time <48 hours could be observed in both media which indicated proliferating cells. Cells incubated in DMEM over passages 4-5 and >6 remained to proliferate while cell numbers in 106 medium persisted around the seeded numbers. Most differentiation marker remained at or close to control values, but e.g., TGM2 revealed a 10 – 50 fold down-regulation in both media and all passage numbers (Figure 4-4). This down-regulation of TGM2, morphology and cell counts were more indicative of a papillary fibroblast type under these conditions. A 3 – 10 fold down-regulation of SFN (known negative regulator of mitotic translation and cell differentiation) coincided with proliferating HDFa cells over all examined conditions. Downstream SFN target genes in proliferating cells appeared 2-10-fold up-regulated (TP63) and up to 100-fold down-regulated (MMP1/MMP3), which is suggestive for a cell status characterized by proliferation (down-regulated SFN), increased stemnesses (up-regulated TP63) and increased wound healing capacity (down-regulated MMP1, MMP3). Resting cells in 106 medium at >5 passages (SFN control values) were associated with gene expression control values of TP63 and MMP1/MMP3 which is suggestive for reduced stemnesses and reduced wound healing capacity.

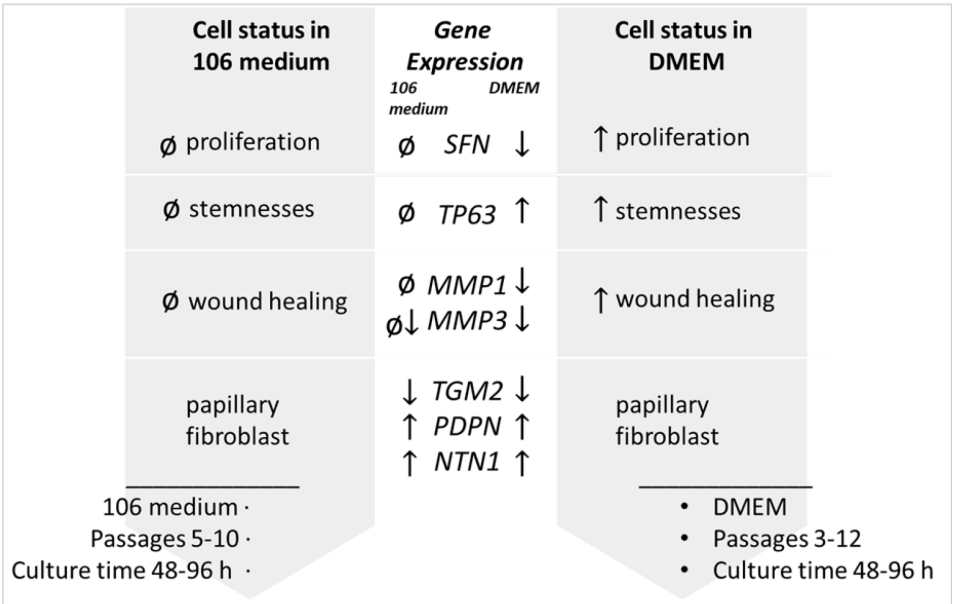


Figure 4-4: A Summary of Gene Expression Results and Associations to Cell Status Conditions Such as Proliferation, Stemnesses, Wound Healing and the Papillary Fibroblast Type. These measurements were found to be constant over a certain passage number and culture time in primary HDFa cells either cultivated in 106 medium (left) or DMEM (right).

In conclusion, a set of gene expression marker related to proliferation (SFN), differentiation (TGM2), stemnesses (TP63) and wound healing (MMP1/MMP3) allow a cell culture characterization so that cells can be exposed under two different cell status conditions, thus, enabling reproducible EMF experiments on primary HDFa cells under comparable and measurable cell status conditions in different donors.

References

Janson, D.G., Saintigny, G., van Adrichem, A., Mahe, C., El Ghalbzouri, A. (2012). Different gene expression patterns in human papillary and reticular fibroblasts. J Invest Dermatol 132: 2565-2572.

Chapter 5 – DELIVERABLES, MEDICAL RADIATION PREPAREDNESS

5.1 FIRST NATO STTARS WORKSHOP IN BRETIGNY, PARIS, FRANCE, 2019

5.1.1 Team and Sub-Meetings

The NATO workshop entitled "Software Tools for Triage of the Acute Radiation Syndrome: a practical workshop (StTARS)" was organized as one of the key-deliverables of our RTG. To ensure that, a Task Force comprising 5 RTG members from three Nations was build and these members met seven times:

1) **December 2017,** Paris: Working on a EUROPEAN consensus; FRA, GER (email distribution).
2) **February 2018,** Washington DC: working on a EUROPEAN and AMERICAN consensus; USA, GER.
3) **October 2018**, Paris: Framework decision (location, host, fee); FRA, GER.
4) **November 2018**, Paris: Working on details (title, 2-pager, advertising etc.); FRA, GBR, GER CZE, USA.
5) **March 2019**, Paris: Working on details; FRA, GBR, GER.
6) **May 2019**, Munich (after ConRad): Prefinal draft of StTARS; FRA, GBR, GER.
7) **September 2019**, Gosport (after third RTG meeting), fine-tuning; FRA, GBR, GER.

Topics changed for each meeting as outlined above. An overview regarding all RTG meetings and short summaries of results and agreements are presented in Annexes C – F. Several "tools" required for such a workshop were developed and will be presented below.

5.1.2 Two-Pager and Advertisement Strategy

The two-pager (Figure 5-1) was distributed via all RTG members and their Institutions and networks. The WHO Newsletter (2019), IAEA and several conferences such as ConRad 2019 (international radiobiological conference in Germany) or IMRIS 2018 (international radiobiological conference in France) or NATO groups such as the CBRNMed Wg were other places for advertisement.

A shortened version for advertisement purposes was shown at several other conferences (Figure 5-2).

5.1.3 Logo and Registration Platform

This workshop required a name and a logo (Figure 5-3). This was a smooth and creative process performed jointly within the RTG group.

For a professional and smooth registration, we decided in favor of a commercial supplier (Converia). This platform had already been used for the ConRad Conference in recent years with very good experiences regarding easiness, service and robustness. Alternatively, we could have used the NATO platform, but over several months this platform in 2019 was malfunctioning. This would have caused severe impact on our conference.

For Converia we developed an introductory screen showing the place where the workshop should take place (Figure 5-4).

Dear Colleagues,
It is a pleasure to announce our first NATO workshop on:

'Software Tools for Triage of the Acute Radiation Syndrome: a practical workshop (STARS)'

which will take place in Brétigny/Paris from 9-11 October 2019.

THE WORKSHOP

Within this workshop we will describe the purpose and function of *software tools* developed by scientific groups within NATO. These tools either allow an integrated estimation of dose (BAT, WinFRAT), or the prediction of ARS severity based on changes in blood cell counts (H-module) in the first days after an exposure to ionizing radiation. You will be introduced into these tools primarily by those who developed them, so that you will receive first hand tuition about their strengths and limitations.

As a short chapter you will also gain a deeper insight into *diagnostic and therapeutic strategies that are currently under development* - such as the use of mesenchymal stem cells to countermeasure radiation-induced local injury. These will also be presented by experts in their field.

Finally, you *will practice your diagnostic skills* by predicting clinically relevant degrees of the ARS using a *database which includes real case histories* – such as those arising from the Chernobyl incident and other accidental industrial exposures.

At the beginning of the course you will receive copies of the software tools and the case history database to download onto a personal laptop. The tools and dataset can then *be used for teaching within your own nations*.

ORGANIZATIONAL DETAILS

- The workshop will take place in Brétigny sur Orge close to Paris from 9th to 11th of October 2019
- You can register at www.radiation-medicine.de starting at January 2019
- Cancellations can be accepted up till 29th April 2019 by written notification (including email)
- The conference fee is 200 €
- The workshop is for civilian or military personnel with a medical background or dealing with medical decision making in the field of radiological or nuclear threats
- The number of participants is restricted (30-40)
- Registration does not imply participation, but in June 2019 you will receive a conformational letter of your participation
- With the conformational letter we require your passport as a pdf via email (see below) due to security reasons.

We are looking forward seeing you in Brétigny/Paris in 2019.

Sincerely,

Colonel Prof. Dr. med. MSc Michael Abend
Chair, NATO workshop 2019
Bundeswehr Institute of Radiobiology
affiliated to the University of Ulm
Munich, Germany

Phone: +49 89 992 692 2280
Email: michaelabend@bundeswehr.org

Figure 5-1: Two-Pager Developed to Advertise the First NATO StTARS Workshop.

Figure 5-2: Slide Presented for Advertising the NATO StTARS Workshop.

Figure 5-3: Logo of the NATO StTARS Workshop 2019.

Figure 5-4: Introductory Screen of the NATO StTARS Workshop at the Converia Registration Platform.

The introductory screen provided an overview on the registration procedure and detailed information regarding general information (welcome address), registration, travel and transport, workshop secretary, workshop location, accommodation, scientific program, etc. was given (Figure 5-5). Details of the different screens are shown in Annex H.

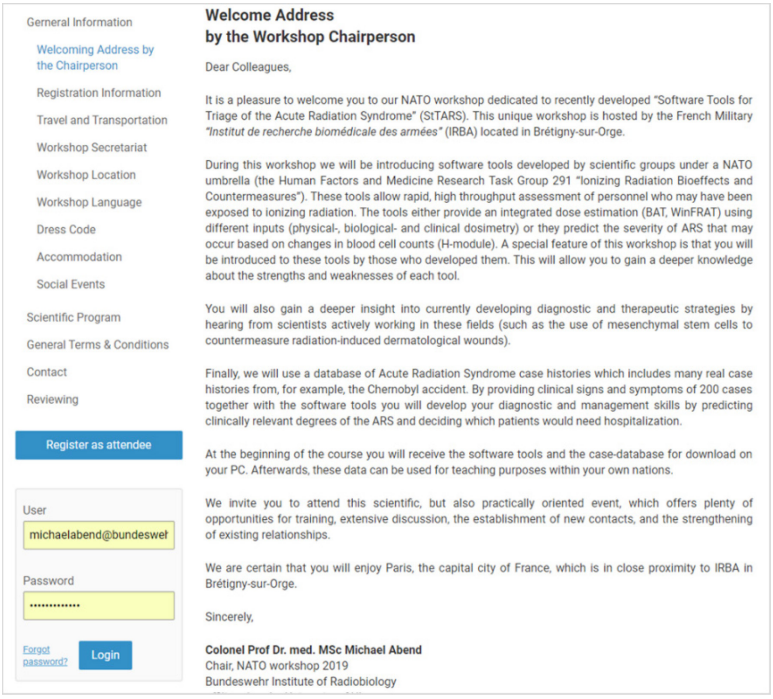


Figure 5-5: Overview and Guide Through the Registration Process. Separate screens are presented in Annex H.

5.1.4 Course Material

This workshop provided software tools for the participants. We, therefore, opted for a pencil with USB stick (Figure 5-6).

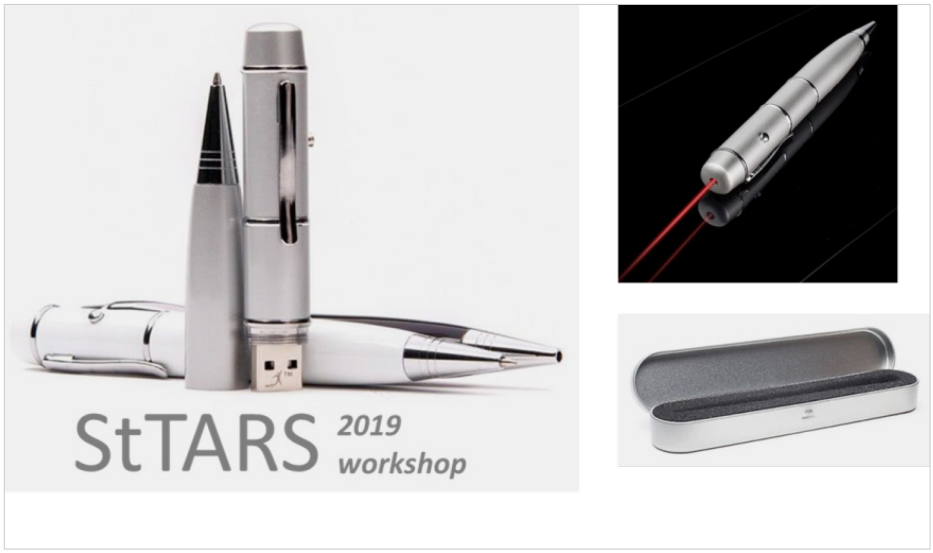


Figure 5-6: First Version USB Stick for NATO StTARS Participants Comprising NATO Software Tools.

With the arrival of the pencils, we had to deal with an unexpected quality issue: about 50% of the pencils spontaneously fell apart in two pieces. Alternative USB pencils were ordered immediately (Figure 5-7) and arrived in time before the start of the first workshop.

Figure 5-7: Second Version USB Stick for NATO StTARS Participants Comprising NATO Software Tools.

We also developed a map to store all hardcopies of the presentations, the program, etc. for the participants (Figure 5-8).

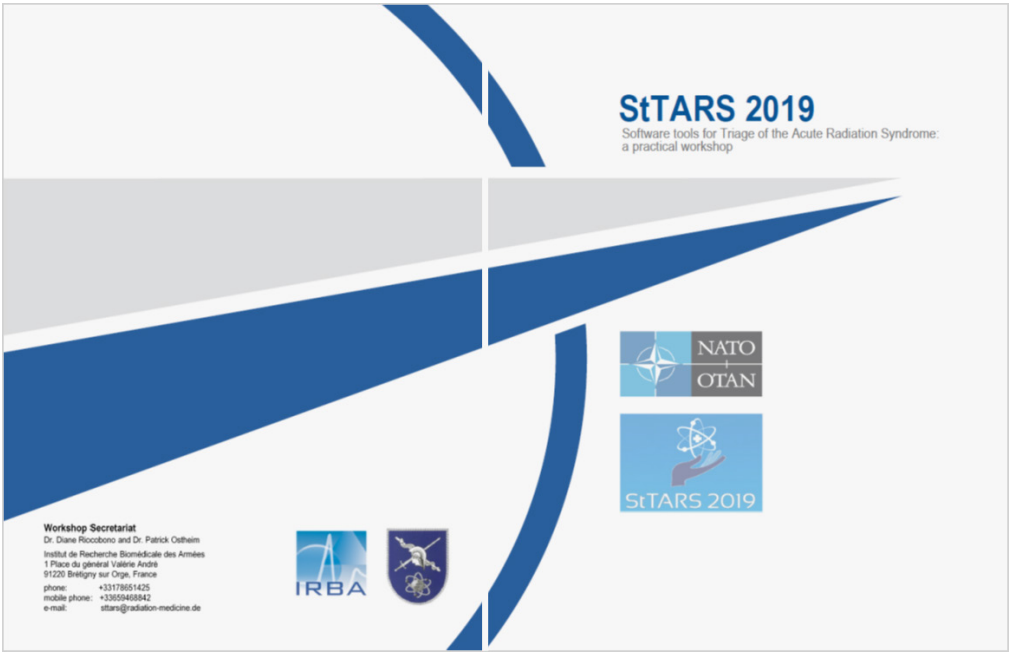


Figure 5-8: Cover of the Map to Store Hardcopies Relevant for the NATO StTARS Workshop.

To ensure corporate identity and recognition of our intellectual property we developed a slide design and stored presentations as pdf on the USB stick (Figure 5-9).

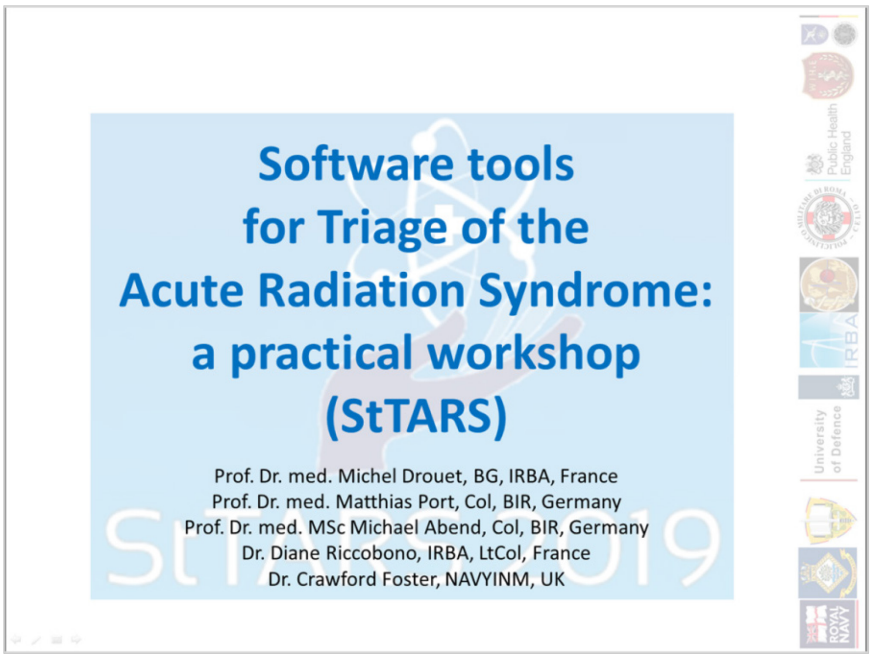
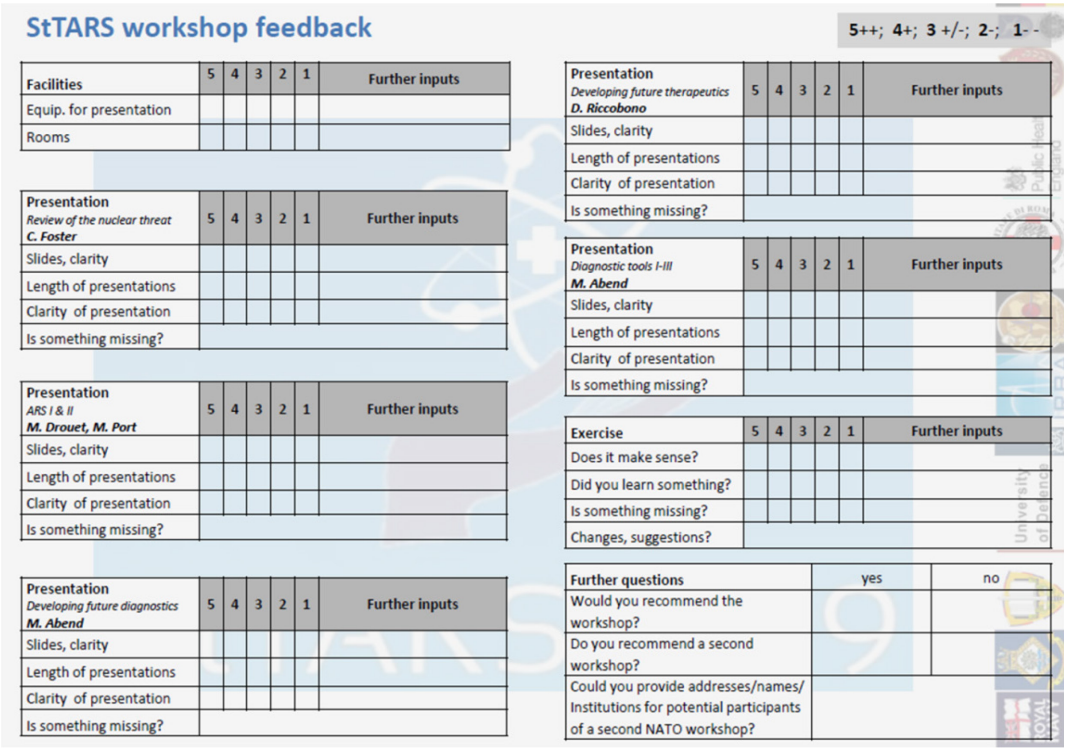


Figure 5-9: Slide Design of the NATO StTARS Workshop Presentations.

Some participants requested a certificate or educational credit points. We decided in favor of the European Credit Transfer and accumulation System (ECTS). ECTS is designed to enable academic recognition for periods of study, to facilitate student mobility and credit accumulation and transfer. Based on the length of the workshop this converted in 1 ECTS point which was granted along the certificate (Figure 5-10). The ECTS point can be converted into other credit systems where required.

Figure 5-10: Certificate for Participation on the NATO StTARS Workshop 2019.

To ensure an improvement of the NATO workshop we asked participants for feedback and developed a feedback sheet (Figure 5-11).



StTARS workshop feedback

5++; 4+; 3 +/-; 2-; 1- -

Facilities	5	4	3	2	1	Further inputs
Equip. for presentation						
Rooms						

Presentation *Review of the nuclear threat* ***C. Foster***	5	4	3	2	1	Further inputs
Slides, clarity						
Length of presentations						
Clarity of presentation						
Is something missing?						

Presentation *ARS I & II* ***M. Drouet, M. Port***	5	4	3	2	1	Further inputs
Slides, clarity						
Length of presentations						
Clarity of presentation						
Is something missing?						

Presentation *Developing future diagnostics* ***M. Abend***	5	4	3	2	1	Further inputs
Slides, clarity						
Length of presentations						
Clarity of presentation						
Is something missing?						

Presentation *Developing future therapeutics* ***D. Riccobono***	5	4	3	2	1	Further inputs
Slides, clarity						
Length of presentations						
Clarity of presentation						
Is something missing?						

Presentation *Diagnostic tools I-III* ***M. Abend***	5	4	3	2	1	Further inputs
Slides, clarity						
Length of presentations						
Clarity of presentation						
Is something missing?						

Exercise	5	4	3	2	1	Further inputs
Does it make sense?						
Did you learn something?						
Is something missing?						
Changes, suggestions?						

Further questions	yes	no
Would you recommend the workshop?		
Do you recommend a second workshop?		
Could you provide addresses/names/ Institutions for potential participants of a second NATO workshop?		

Figure 5-11: Feedback Sheet for Evaluation of the NATO StTARS Workshop by the Participants.

5.1.5 The Workshop, General

Altogether 30 individuals with different background (e.g., nuclear medicine, physics, radiobiology, internal medicine, health physics, nurse and radiation protection), representing 8 different nations (Czech Republic, France, Germany, Lithuania, Netherlands, Sweden, Switzerland, USA) participated in this workshop. Most of them worked either in Military Institutions, Radiation Protection Facilities, Scientific Institutions, NASA or represented educational Institutions of international reputation (Radiation Injury Treatment Network (RITN), Radiation Emergency Assistance Center/Training Site (REAC/TS)) dealing with diagnosis and treatment of acute radiation health effects.

The workshop started at Wednesday (09th October 2019) and ended on Friday (11th October 2019, Figure 5-12).

Figure 5-12: Agenda of the NATO StTARS Workshop 2019.

The first 1.5 days were used to introduce into three main topics, namely:

- Threat due to radiological and nuclear scenarios;
- Diagnosis and therapy of acute health effects after radiation exposure; and
- Introduction into NATO software tools such as BAT, WinFRAT and H-module.

Additionally, experts in these fields provided an inside in actual research regarding diagnosis and therapy. Another half day was used to familiarize participants in the use of the software tools. Also, teams were formed (2 – 3 participants worked together) to facilitate discussions within the teams and teams required time to find their way sharing the workload. For this part of the workshop participants were asked to predict the severity of acute health effects from 3 patients using clinical signs and symptoms which were provided as an excel sheet. At the end of the day a solution was provided and discussed.

At the last day participants received 191 case (clinical signs and symptoms in an excel sheet) and were asked to do a prediction of acute health effects within 3 hours by using the tools they prefer using for this kind of work. After lunch, their performance was presented in a last presentation which finished the course.

5.1.6 The Workshop, Results

Altogether nine teams with 2 – 3 participants were formed (Table 5-1).

Table 5-1: Overview on Participating Teams, Their Background, Tools They Used and the Number of Cases, Which Were Classified Within Three Hours.

#	background	tools used	data set provided # cases
1	Nuclear medicine, Physics, Trauma-surgery	H-Module, WinFRAT, BAT	50
2	Bone marrow transplant, Internal medicine, ER-med - Emergency Prep	H-Module	61
3	Health Physics, Radiobiologist, Space Radiation Research	H-Module, WinFRAT, Hemodose, mobile FRAT	191
4	Radiobiologist, Health Physicist	H-Module, WinFRAT, Hemodose, mobile FRAT	92
5	Physician, Biophysics, ER Nurse	WinFRAT, H-Module, prodromi list	116
6	Physician, Physician	H-Module, WinFRAT, Prodromi list	65
7	Physician, Physician	H-Module, WinFRAT	191
8	Biologist, Biologist, Biologist	H-Module, WinFRAT	191
9	Biologist, Toxikologist, Anesthesiologist	H-Module, WinFRAT	91

Participants always favored to do the work as a team instead of doing the exercise alone, because of the valuable and enlightening discussions they experienced within the teams. Also, the workload with 2 – 3 participants per team could be easily divided. One third of the teams managed to classify all 191 cases and the remaining teams classified between 50 – 116 cases. That was an active decision by the teams, and it was partly caused by extensive discussions within the teams which they found important in order to deepen the understanding and to better extract advantages and disadvantages of the software tools they were working with.

On average 93.3% and 94.1% of cases were correctly predicted as clinically relevant ARS requiring hospitalization (Table 5-2). Only one group showed an about 10% performance increment that might have been caused by the language barrier and other groups performed with 98.5% correct predictions. These groups followed an efficient strategy and used blood cell count changes (in particular decreased lymphocyte counts) as a first parameter to efficiently separate unexposed from radiation exposed individuals.

Table 5-2: Summary on the Correct Predictions of Clinically Relevant ARS Severity and Hospitalization Requirement.

#	correct predictions of....	
	ARS	hospitalization
1	96	94
2	91,8	91,8
3	94,2	95,3
4	77,1	80,4
5	98,3	98,3
6	93,8	98,5
7	95,8	95,8
8	96,9	97,4
9	95,6	95,6
Mean.	**93.3**	**94,1**

In 2015, our RTG performed the first exercise using the 191 cases. At that time participants (experts in the field of Medical Management of RN threats) were not introduced into the setting and each team performed based on a different background. However, after teaching (either NATO StTARS workshop 2019 participants or Students of a Masterclass in Radiobiology in 2016-2019) on average two-times more cases could be processed per hour than without teaching (Figure 5-13, right). This again emphasizes the value of

teaching. For instance, teacher guided participants how to deal with the wealth of data they received per case (e.g., delete those information/variables which are not required to gain an overview). They were also introduced into a strategy by identifying the RC category first and from there to deduce about a developing clinically relevant ARS (RC 2-4) and hospitalization requirements. Interesting, the on average two-fold increased number of processed cases per hour showed a variance, which was even higher after teaching, although the correct decisions appeared comparable among all taught teams (Figure 5-13). As outlined above, this was partly caused by the team's intention to prefer discussions over high speed (personal communication during the StTARS workshop 2019). On the other hand, it is caused by certain diagnostic "shortcuts" employed by participants (e.g., lymphocyte counts used as a diagnostic to identify unexposed/low exposed cases). Given that 134 from 191 cases fell into this category of unexposed/low exposed cases, a throughput of 118 cases/h or about 2 cases per minute by teams employing this approach appears logical.

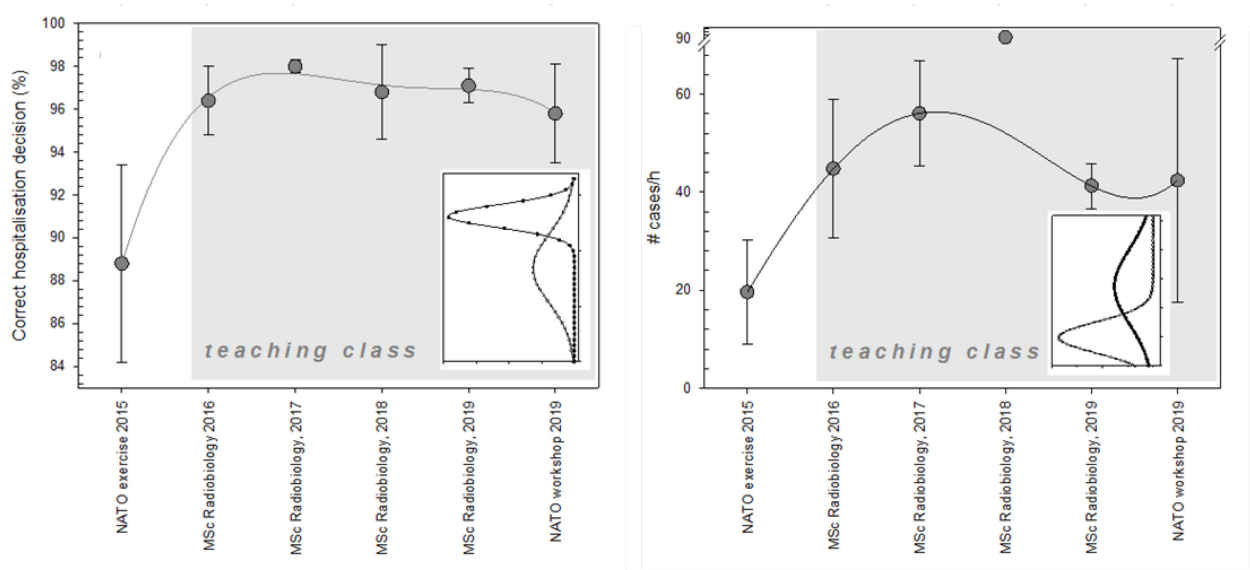


Figure 5-13: Comparison in Performance Among Experts in Medical Management of an RN Event Without Training (NATO Exercise 2015) versus Radiobiology Master Class Students and NATO StTARS Workshop Participants Who Received a Training (Teaching Class) Ahead of the Exercise.

Also, with teaching a significant about 10% increase in the correct hospitalization decisions could be measured so that even non-experts in the field performed as good (and better) than medical management experts who were not trained before (Figure 5-13, left).

Taken together, prodromes and changes in blood cell counts were successfully used to support early urgent clinical decisions such as hospitalization, ARS prognosis and treatment recommendations in up to 98%. Teaching classes such as the NATO StTARS workshop significantly improve the outcome predictions and enable even participants without a medical background to perform comparable to best medical clinical teams.

The preferred choice of software tools in 2019 was WinFRAT for dose estimation and H-module for clinical outcome prediction. Merging H-module into WinFRAT or creating a new platform with prodromes and changes in blood cell counts used as input data and generating clinical outcome predictions automatically via artificial intelligence that represents future approaches for increasing the throughput.

5.1.7 The Workshop, Feedback

Participant's feedback can be summarized as follows:

1) Oral presentations for introduction into the different areas of Medical Management on average ranged between 4.1 – 4.6 from 5 points, where 5 points represent the highest value. Hence, the satisfaction rate ranged between 82 – 92 %.
2) The exercise itself received 4.7 points converting into a 94% satisfaction rate.
3) All participants recommended a second workshop.

These results forced us to consider a second NATO StTARS workshop and Dr. Carol Iddins (Director from REAC/TS) who participated in this workshop offered to use their facility for this task. RTG agreed to follow this attractive offer.

5.2 SECOND NATO StTARS WORKSHOP IN OAKRIDGE, TENNESSEE, USA, 2021

Because of the coronavirus pandemic, the second NATO StTARS workshop planned in 2020 in Oakridge REACT/TS had to be postponed. It has now been agreed that it will presumably take place between 16 – 19 November 2021 at REAC/TS. Same teams and slightly modified material (slides) will be provided. This report cannot describe the workshop retrospectively, since this report has to be delivered before November 2021, but the RTG's life-cycle will end in October 2021. Hence, the following chapters will reflect the status of the workshop based on that what has been organized until October 2021.

During the last meeting of the RTG group in September 2021 it was decided to again postpone the 2020 meeting caused by the insecure COVID-19 situation. Nevertheless, many activities already performed and decided in 2021 will be useful (e.g., a workshop will take place at REAC/TS and presenters will stay the same) for 2022 and are shown below.

5.2.1 Team and Sub-Meetings

It is agreed that the same team (teacher) will perform for the second NATO StTARS workshop as well. However, the resurgence of AFRRI's scientific expertise (caused by change in the position of AFRRIs director) makes it now possible for Dr. Blakely to introduce participants to the software tools that he has developed. This move fulfils the strategy of the workshop, namely, to have software developers introduce the participants to the software tools so that participants better understand pros and cons.

The envisioned team and subjects are as follows:

- Radiological and nuclear scenarios — Dr. Foster (GBR)
- ARS, definition, pathomechanism, diagnosis, treatment — Dr. Drouet (FRA) and Dr. Port (DEU)
- New ARS treatment strategies — Dr. Riccobono (FRA)
- Diagnosis, introduction into software tools — Dr. Blakely (USA) and Dr. Abend (DEU)
- Exercise and presentation of results — Dr. Abend (DEU).

With the first NATO StTARS workshop, a robust template already exists. Therefore, fine-tuning of the second NATO StTARS workshop can and will be accomplished via email and virtual meetings where required.

5.2.2 Two-Pager and Advertisement Strategy

The Institutional brand logos and the augmented team (teacher) as well as the new location and date were added to the already existing two-pager (Figure 5-14).

Figure 5-14: Two-Pager Developed to Advertise the First NATO StTARS Workshop.

The two-pager was distributed via all RTG members and their Institutions and networks. The WHO Newsletter (2021), IAEA and several conferences such as ConRad 2021 (international radiobiological conference in Germany) or NATO groups such as the CBRNMed Wg were other places for advertisement. REAC/TS represent the central Institution for education in medical management after radiation exposure. Via their own channels, many specialists working in this field already pre-registered. Due to the nature as a workshop the number of participants will be restricted to 50 on-side. The workshop will be held as a hybrid platform. This enables another 50 professionals to participate. As a learning experience from our recently executed Medical Management course for radiobiologist in the context of the Masterstudy radiobiology at Technical University Munich (TUM), online participants as well as on-side participants will do the exercise in small groups (2 – 3 participants). This ensures a better harmonization of basic principles taught during the workshop.

A shortened version for advertisement purposes was shown at several other conferences (Figure 5-15).

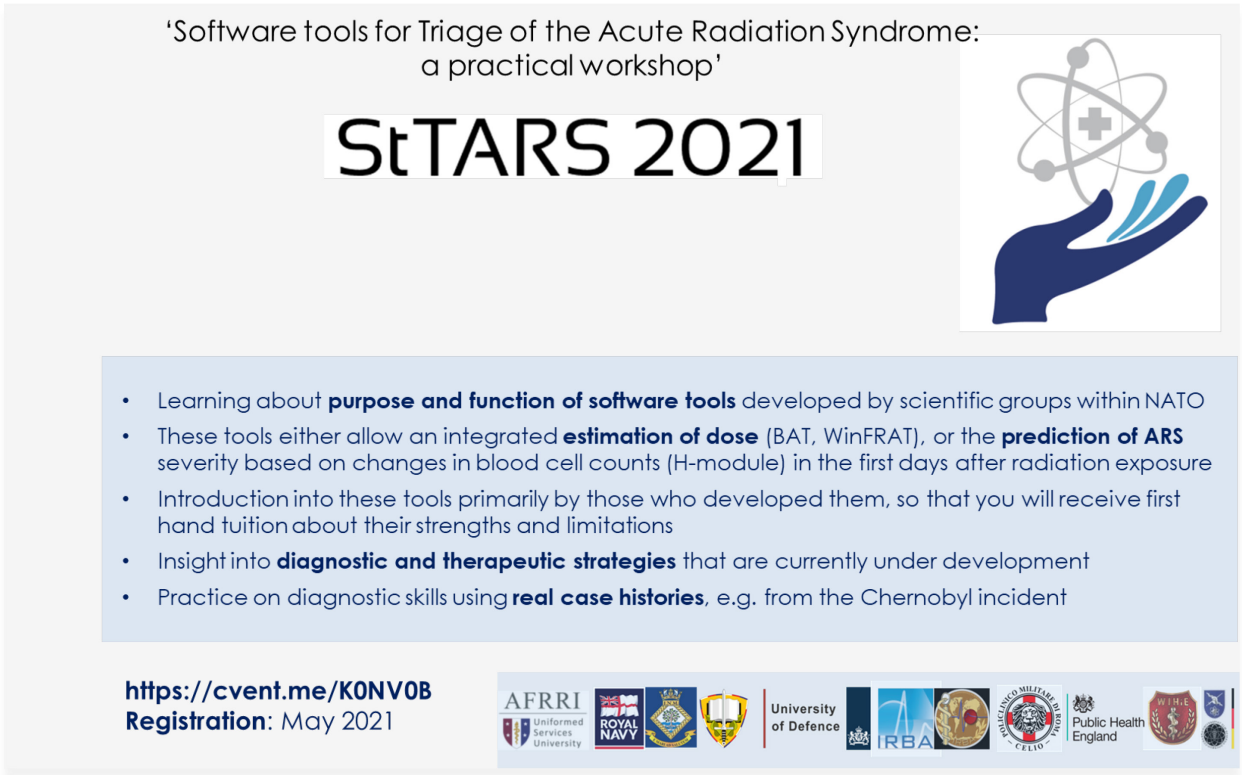


Figure 5-15: Slide Presented for Advertising the NATO StTARS Workshop at Different Occasions.

5.2.3 Logo and Registration Platform

The same logo will be used for the second NATO StTARS workshop. The registration platform this time is Cvent. REAC/TS is processing all their courses via this platform.

5.2.4 Course Material

(For figures, See Section 5.1.4).

This workshop introduces software tools that will be provided to the participants along with a USB stick as part of a pencil, similar to the one shown in Section 5.1.4.

We will again develop a map to store all hardcopies of the presentations, the program, etc. for the participants.

To ensure corporate identity and recognition of our intellectual property we developed a slide design and stored presentations as pdf on the USB stick.

Some participants requested a certificate or educational credit points (ECTS). Based on the length of the workshop this will convert in 1 ECTS point which will be granted along the certificate. The ECTS point can be converted into other credit systems where required.

To ensure an improvement of the NATO workshop we asked participants for feedback. The previous feedback sheet proved sufficient and will be used again.

5.2.5 The Workshop, General

The agenda, the content, and the structure of the course is very similar to the first NATO StTARS workshop (Figure 5-16).

'Software Tools for Triage of the Acute Radiation Syndrome: a practical workshop (StTARS)'

Oak Ridge, TN

16 November – 19 November 2021

Tuesday, 16th November 2021

0730 – 0800 Sign In

0800 – 0930 Introduction and organizational remarks

0930 – 1000 Coffee Break

1000 - 1200 Medical Management of the ARS I (Dose-response relationship, RN threat, dose concept, LD50/60, ARS)

1200 – 1300 Sponsored lunch (lobby)

1300 – 1430 Medical Management of the ARS II (Dose-response relationship, RN threat, dose concept, LD50/60, ARS)

1430 – 1530 Development of future diagnostics (gene expression etc.)

1530 - 1600 Coffee break

1600 – 1700 Development of future therapeutics (Mesenchymal Stem Cells etc.)

1800 – 2030 Sponsored Dinner (Calhoun's Barbeque, Oak Ridge)

Wednesday, 17th November 2021

0830 – 1030 Diagnostic tools I

1030 - 1100 Coffee Break

1100 – 1200 TBD

1200 – 1300 Sponsored lunch (lobby)

1230 – 1400 Diagnostic tools II

1400 – 1430 Coffee break

1430 – 1630 Exercise preparation, 5 cases

Thursday, 18th November 2021

0830 – 1130 Triage exercise, 191 cases

1130 – Free Afternoon

Friday, 19th November 2021

0830 - 1100 Results, discussion & closing remarks

Figure 5-16: Agenda of the NATO StTARS Workshop 2021.

However, this time two half-days will be included to ensure more time for the analysis of participants' results and more time for presentation of results. Both aspects appeared too dense in the course of the first NATO StTARS workshop. Due to time restrictions, results could be not presented in the granularity required and participants recommended to change that.

5.2.6 The Workshop, Results

We cannot report on the results of this workshop, because we released the report to STO before the end of our RTG life-cycle (October 2021). However, based on previous experiences and given the skill and the profession of the team we expect similar results to the first NATO StTARS workshop.

5.2.7 The Workshop, Feedback

Here, we have to wait for responses.

5.3 CRRIS (COMPARING RADIATION EXPOSURE RISKS WITH DAILY LIFE RISKS), A NATO APP FOR IMPROVING RISK COMMUNICATION IN A RN EVENT

5.3.1 Risk Communication Guidance, a Literature Review with Focus on Our Envisioned CompRadRisk NATO Tool, C. Foster, GBR, 2017

This presentation described the results of an initial review of risk communication following a radiation event. It was conducted to help inform and guide the future work of the RTG. It was based on a review of English language official publications, textbooks, and selected journal articles.

A review of AMedP7.1 Medical Management of CBRN Casualties (Edit A Version1 Ratification Draft) revealed the extent of the gap in current published NATO guidance on managing the psychological effects in radiological and nuclear casualties. Only the most basic classification of potential psychological casualties from any CBRN incident is described. However, the review identified that this is not due to lack of knowledge. Since the 1980s, risk communication has emerged as a distinct science and there is a plethora of published information about it relating to environmental health hazards, public health emergencies, terrorist incidents, CBRN incidents and, specifically, nuclear/radiological incidents. A common structure within articles describing the psychological effects of radiological hazards includes: a review of case studies (such as nuclear power plant accidents), the characteristics of radiation and how through risk perception it presents a unique threat, the spectrum of psychological responses that may arise, and ways to reduce the psychological impact through building resilience and risk communication.

Tables of radiation dose comparison, such as that presented online by Public Health England (PHE 2011), are often used to illustrate doses that may occur from background radiation or medical exposures. Tables of risk comparison go a step further by trying to equate radiation doses with other risks to health. By comparing risks of radiation related cancer deaths with mortality in other industries they are one way that the system of radiological protection has been evaluated. When the risk of death from other activities, or other events from other activities are added (such as winning a lottery after buying a ticket), a 'table of everyday risks' is formed. This provides a resource that may appear helpful for risk communication. However, there are pitfalls for the unwary when using such risk comparisons. Recipients may not face the average risk or may perceive the risk in a different way – particularly when decisions about the acceptability of risk are based on factors other than the size of the risk. Those comparisons that are poorly done can, 'confuse, mislead and antagonize recipients.' Unless done in a scientifically sound way, risk comparisons are unlikely to be useful and relevant and hence should be avoided. (Fischhoff, 2009; Covello, 1991).

Poor numeracy skills are a clear barrier to risk communication but can be mitigated by graphical techniques such as the use of stick figures and visual risk ladders, or the use of reference points to contextualize numerical information, such as the capacity of a local football stadium or town (BMA Board of Science, 2012).

The Center for Disease Control (CDC) model of the psychological phases of a radiation disaster shows a lengthy tail of reduced public confidence which relates to psychological ill health. The Sandman formula of risk perception defines perceived risk as being the sum of a measurable hazard and a sense of outrage or injustice. Many of the 12 principal components of outrage can be associated with radiological incidents and these factors, together with media amplification, lack of scientific consensus, social effects, and several others can explain the tail in the model. Several psychological effects can be expected after a radiological incident such as acute stress reactions, adjustment disorder, anxiety, depression, post-traumatic stress disorder and substance abuse. Psycho-social reactions would also be expected to have adverse effects on physical health and there is the possibility of a subsequent syndrome like illness developing with 'multiple unexplained physical symptoms.'

The CDC has also produced several excellent resources to direct efforts to mitigate the risks of psychological effects after a radiological incident through prompt effective risk communication. These include an online training program on 'Psychological First Aid in Radiation Disasters.' Their booklet 'Crisis and Emergency Risk Communication' also provides an evidence-based framework for risk communication in any public health emergency including a nuclear or radiological emergency. Throughout it, six principles of effective crisis and risk communication are emphasized:

1) Be First: Crises are time-sensitive. Communicating information quickly is almost always important. For members of the public, the first source of information often becomes the preferred source.
2) Be Right: Accuracy establishes credibility. Information can include what is known, what is not known, and what is being done to fill in the gaps.
3) Be Credible: Honesty and truthfulness should not be compromised during crises.
4) Express Empathy: Crises create harm, and the suffering should be acknowledged in words. Addressing what people are feeling, and the challenges they face, builds trust and rapport.
5) Promote Action: Giving people meaningful things to do calms anxiety, helps restore order, and promotes a restored sense of control.
6) Show Respect: Respectful communication is particularly important when people feel vulnerable. Respectful communication promotes cooperation and rapport.

Other notable resources identified during the review included advice from the Centre for the Study of Traumatic Stress entitled 'Psychological and Behavioural Issues Healthcare Providers Need to Know when Treating Patients Following a Radiation Event,' and two papers that explain practical tools and techniques for effective risk communication including message mapping and sample questions (Covello, 2011; Hyer et al. 2017).

The presentation concluded with thoughts about the complexity of risk communication and how in a nuclear or radiological emergency it needs to embrace much more than simply presenting tables of comparable risks. Sufficient material is already available to improve published NATO guidance and the example of an undated internal report from NATO Committee uncovered during the review (Wessely et al.) was used as an example of how other specialist expertise could potentially be sought to aid this work.

References

BMA Board of Science (2012). Risk: What's Your Perspective? A Guide for Healthcare Professionals.

CDC. Centers for Disease Control and Prevention. Training and continuing education online, psychological first aid in radiation disasters. Web based course no: WB1645. https://www2a.cdc.gov/tceonline/registration/detailpage.asp?res_id=2490 (Accessed Dec 2017).

Covello, V. (1991). Risk comparisons and risk communication: Issues and problems in comparing health and environmental risks. In: R.E. Kasperson and P.J.M. Stallen (eds.), Communicating Risks to the Public, 79-124. Kluwer Academic Publishers.

Covello, V. (2011). Risk communication, radiation, and radiological emergencies: strategies, tools and techniques. Health Phys. 101(5):511-530.

Fischhoff, B. (2009). Risk perception and communication. In: Oxford Textbook of Public Health (5th ed.) Vol 2 Sec 8.8, Oxford University Press. DOI: 10.1093/med/9780199218707.003.0057.

Hyer, R.N. et al. (2017). Breaking bad news in the high-concern, low trust setting: How to get your story heard. Health Phys. 112(2): 111-115.

National Research Council (2006). Scientific review of the proposed risk assessment bulletin from the Office of Management and Budget. National Academy Press, Washington, DC.

PHE Guidance. Ionising radiation: Dose comparisons (18 March 2011). https://www.gov.uk/government/publications/ionising-radiation-dose-comparisons/ionising-radiation-dose-comparisons

Sandman, P. Outrage management (low hazard high outrage). The Peter M. Sandman Risk Communication Website (http://www.psandman.com/index-OM.htm).

Wessely, S., Fischhoff, B., Krasnov, V. et al. (Date unavailable). Guidelines for communicating the risk of chemical, biological or nuclear terrorism: how to inform the public, improve resilience and not generate panic. Prepared for the NATO Committee on Psychological and Social Consequences of Chemical, Biological or Nuclear Terrorism.

5.3.2 Literature Overview on Existing Apps and Discussion Regarding the NATO App on Risk Comparisons, D. Stricklin, M. Abend, 2018

D.L. Stricklin, N. Flaig, M. Abend

Dr. Michael Abend started the session by reviewing the initial discussion regarding the development of an application for the comparative assessment of radiation risk, CompRadRisk, CRRis. The group was asked to refine the purpose of the effort by defining what the purpose of the App would be for: conversion of radiation dose into daily life equivalent risks for the purpose of risk communication. The end-users were defined as possibly soldiers after occupational radiation exposure, military decision makers, patients undergoing medical diagnostic exposures, civilians living in contaminated areas, and "Citizen Scientists." However, the App would not be intended for dose estimation, cancer risk estimation, or the conversion of a scenario into a dose estimation.

Dr. Daniela Stricklin followed the introduction with a literature review of existing radiation risk communication tools and Apps and a brief summary of the overview is provided below. For a detailed account of this review, please refer the subsequently published paper (Abend, Stricklin et al. 2020).

The topics researched in the literature review included radiation risk communication, radiation risk perception, radiation equivalency, radiation exposure dose comparison, and applications for measuring radiation (tools). Databases used in the search were: PubMed, BioMed Central, BioOne Complete, DOAJ, Google Scholar, ScienceDirect, ARRS, Ovid, GooglePlay, and the Apple Store. The results returned 38 relevant articles out of >10,000 results.

The literature regarding risk communication indicated that it is a combination of "tools" and "processes" that integrate risk communication factors into the overall risk management of an issue. The most effective messages are those that balance what the audience wants to know with what you need them to know. Successful communication finds the right message, emphasize benefits (if applicable), and put risks in context, and the communicator should understand the audience. A number of guides are available for emergency responders to provide insight on how best to communicate during a crisis and what to avoid. Regarding risk perception, surveys indicate that there is a significant gap in patient and medical professional awareness about ionization radiation risk. The presentation by RTG member, Crawford Foster, on dose comparisons for radiation risk was referenced. We further noted several sources of peer-reviewed or journalistic making additional statements that may enforce the reasoning that there is not a simple way to make radiation dose comparisons.

The search for existing relevant Apps revealed four examples for risk assessment, one for emergency information, more than 15 associated with radiation detection, and two that could provide radiation readings. The highlights are provided here.

Radiation Risk:

Radio-Risk Software (Carpeggiani, Paterni et al. 2012)

DoseMonitor™, http://www.dosemonitor.com/ (2010)

RadiationPassport, http://www.tidalpool.ca/radiationpassport/ (2018)

RadRat, (Berrington de Gonzalez, Apostoaei et al. 2012)

X-rayRisk, http://www.xrayrisk.com/ (2018)

Radiation Communication:

REMM: *Guidance for Healthcare Providers about Radiological and Nuclear Emergencies.* http://www.remm.nlm.gov

Radiation Detectors:

GammaPix: Gamma Radiation Detector, http://gammapix.com

RadioactivityCounter, www.hotray-info.de/html/radioa_ios.html.

Overall, the search identified some documented radiation dose comparisons to non-radiation activities. However, none of the comparisons align amongst sources and there are varying thresholds for different genders, organs, and ages. Attempts have been made to bridge the gap in comparing radiation risk/exposures with daily life risk. There are tools that deal with risk communication; however, there are still differences in exposure estimates that remain unresolved. Individual risk estimates are made possible through several tools; however, most require exposure doses to be manually entered and the amounts may greatly vary. Comparison of radiation exposure with daily life risk is available from several sources, but a tool that uses comparisons needs the examples to be "closer to home." The extent of a "mitigation" feature in current radiation risk tools are graphs and pictures that show comparisons and cumulative personal exposure. It is difficult to create individual tumor risk estimation based on dose after low level radiation. No single tool brings together cumulative/historical individual exposure, dose and risk estimation, live readings, and general information/guidance. While there are tools currently available, these lack mitigation and ease-of-comparison features.

Dr. Abend revisited how the group should proceed, noting that research indicates information on ionizing doses can be made easily available, but that all-encompassing tools are not yet on the market. He noted that non-radiation dose equivalencies need work, and that dose measurements are not exact report to report. The group as a whole was in favor of continuing the development of the CRRis App.

References

Abend, M., Stricklin, D., Flaig, N., Badie, C., Drouet, M., Foster, C., Janiak, M.K., Kuipers, T., Lista, F., Nowosielska, E.M., Riccobono, D., de Sanctis, S., Franchini, V., Tichý A., Port M. (2020). Bringing Radiation Exposures and Associated Health Risks into Perspective-Development of an App. Health Phys 119(1): 59-63.

Berrington de Gonzalez, A., Iulian Apostoaei, A., Veiga, L.H., Rajaraman, P., Thomas, B.A., Hoffman, F.O., Gilbert, E., Land, C. (2012). RadRAT: a radiation risk assessment tool for lifetime cancer risk projection. J Radiol Prot 32(3): 205-222.

Carpeggiani, C., Paterni, M., Caramella, D., Vano, E., Semelka R.C., Picano, E. (2012). A novel tool for user-friendly estimation of natural, diagnostic and professional radiation risk: Radio-Risk software. Eur J Radiol 81(11): 3563-3567.

5.3.3 Update on CRRIS, Next Steps, M. Abend, 2019

This report on the current status of CRRis started with a reference to previous discussion within the annual RTG session and the sub-meeting of the CRRis TF. In brief:

1) At the 2017 meeting:
   a) C. Foster provided an overview on NATO documents revealing a gap regarding this topic.
   b) Doubts regarding the development of a tool dealing with risk communication due to the lack of an appropriate expertise of the group and unsolved issues related to an individual risk estimate were discussed.
   c) A discussion on how to build a (crude) tool/App which would provide the user (either civilian or military) with a rough idea of the exposure risk or magnitude by doing comparison with daily life risk (e.g., cigarette smoking) of equal magnitude as the radiation exposure evolved.
   d) The inclusion of a "mitigation" feature to the radiation risk tool was also discussed.
   e) A task group was created comprising the following members: M. Abend, M. Port, Crawford Foster, Daniela Stricklin, W.F. Blakely. W.F. Blakely due to the AFRRI director's decision could not participate in the following meetings until 2020 when the directory at AFRRI changed.
2) Introducing CRRis at other societies in 2017/2018:
   a) At NERIS an extensive session on risk communication happened, but without clear output/content, emphasizing the need for a tool closing the gap between existing and assumed radiation risks.
   b) Visiting the Center for the Study of Traumatic Stress (Uniformed Services University of the Health Science, Bethesda, USA) was supportive and the concept shown was judged as a "…first step to start risk communication…" After drafting a version CSTS USU promised support in wording, etc.
   c) According to the German Radiation Protection Board no individual tumor risk estimations and only absorbed dose (no equivalent or effective dose) should be provided.

3) Purpose of CRRis, discussion in 2018:

What is it for (purpose)?

a) Convert dose into daily life risk equivalent (e.g., driving car, smoking).

b) First step to communicate radiation risk.

c) Primarily LLR, but also high doses (ARS)?!

d) Customer:

i) Soldier after occupational radiation exposure.

ii) Military decision maker.

iii) For all after medical diagnostic/therapeutic? Exposures.

iv) Civilians living in contaminated areas.

v) Interface to "Citizen Science."

What is it not for?

a) No dose estimation.

b) No tumor risk estimation.

c) No conversion of a scenario into a dose estimate?

4) D. Stricklin and Nicole Flaig provided an overview on existing software tools regarding risk communication (see Section 5.3.2).

5) Based on the overview it was summarized what exist and discussed how to proceed:

a) Research indicates information on ionizing doses can be made easily available.

b) All-encompassing tools are not yet on the market.

c) Non-radiation dose equivalencies need work.

d) Dose measurements are not exact report to report.

6) In 2019 CRRis was presented at ConRad and a manuscript was drafted and published. The major feature is the conversion of radiation exposure into different exposures of comparable health risk as outlined in the Figure 5-17.

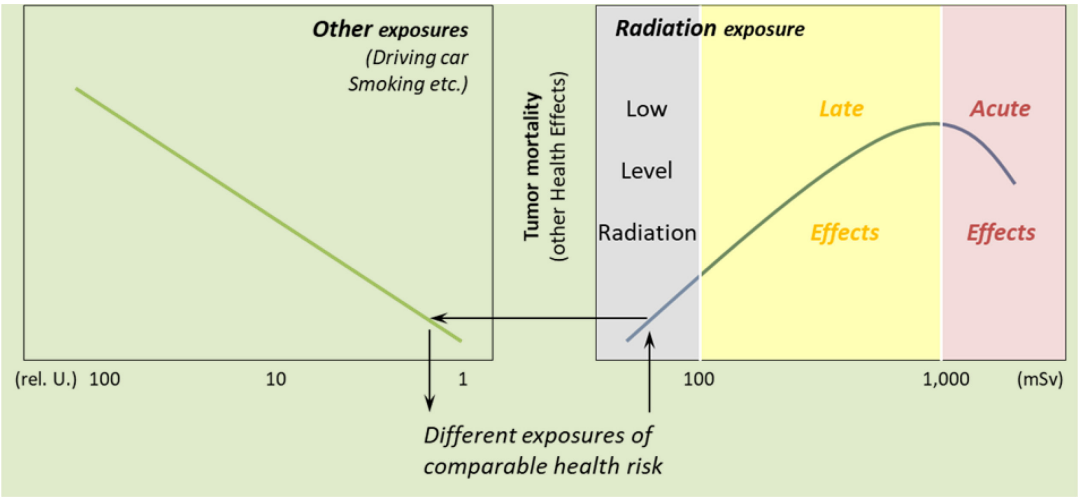


Figure 5-17: Concept for Conversion of Radiation Exposure into Different Exposures (e.g., Driving Car, Smoking) of Comparable Health Risk.

Chapter 6 – ARTICLES PUBLISHED BY RTG MEMBERS DEALING WITH THE RTGS

[1] Abend, M., Amundson, S.A., Badie, C., Brzoska, K., Hargitai, R., Kriehuber, R., Schüle, S., Kis, E., Ghandhi, S.A., Lumniczky, K., Morton, S.R., O'Brien, G., Oskamp, D., Ostheim, P., Siebenwirth, C., Shuryak, I., Szatmári, T., Unverricht-Yeboah, M., Ainsbury, E., Bassinet, C., Kulka, U., Oestreicher, U., Ristic, Y., Trompier, F., Wojcik, A., Waldner, L., Port, M. (2021). Inter-laboratory comparison of gene expression biodosimetry for protracted radiation exposures as part of the RENEB and EURADOS WG10 2019 exercise. Sci Rep, May 7, 11(1):9756. doi: 10.1038/s41598-021-88403-4. PMID: 33963206; PMCID: PMC8105310.

[2] Abend, M., Blakey, W.F., Ostheim, P., Schule, S., Port, M. Early molecular markers for retrospective biodosimetry and prediction of acute health effects. J. Radiol. Prot. (accepted, 16 July 2021 online ahead of press and in press).

[3] Abend, M., Nisbet, A., Gering, F., Averin, V., Andersson, K., Schneider, T., Mothersill, C., Zeeb, H., Scholz-Kreisel, P., Yamashita, S., Pölz-Viol, C., Port M. (2020). "Living in Contaminated Areas" – Consideration of Different Perspectives. Health Phys, Jul, 119(1):2-11. doi: 10.1097/HP.0000000000001218. Erratum in: Health Phys, Sep, 119(3):365. PMID: 32205714.

[4] Abend, M., Pfeiffer, R.M., Port, M., Hatch, M., Bogdanova, T., Tronko, M.D., Mabuchi, K., Azizova, T., Unger, K., Braselmann, H., Ostheim, P., Brenner, A.V. (2021). Utility of gene expression studies in relation to radiation exposure and clinical outcomes: thyroid cancer in the Ukrainian-American cohort and late health effects in a MAYAK worker cohort. Int J Radiat Biol, 97(1):12-18. doi: 10.1080/09553002.2020.1748739. Epub 2020 Apr 20. PMID: 32310011.

[5] Abend, M., Port, M. (2019). Contribution of biodosimetry to different medical issues. Radiat Prot Dosimetry, Dec 31, 186(1):123-125. doi: 10.1093/rpd/ncy278. PMID: 30576526.

[6] Abend, M., Stricklin, D., Flaig, N., Badie, C., Drouet, M., Foster, C., Janiak, M.K., Kuipers, T., Lista, F., Nowosielska, E.M., Riccobono, D., de Sanctis, S., Franchini, V., Tichý, A., Port, M. (2020). Bringing radiation exposures and associated health risks into perspective-development of an app. Health Phys, Jul, 119(1):59-63. doi: 10.1097/HP.0000000000001246.

[7] Agbenyegah, S., Abend, M., Atkinson, M.J., Combs, S.E., Trott, K.R., Port, M., Majewski, M. (2018). Impact of inter-individual variance in the expression of a radiation-responsive gene panel used for triage. Radiat Res, Sep, 190(3):226-235. doi: 10.1667/RR15013.1. Epub 2018 Jun 20. PMID: 29923790.

[8] Ainsbury, E., Badie, C., Barnard, S., Manning, G., Moquet, J., Abend, M., Antunes, A.C., Barrios, L., Bassinet, C., Beinke, C., Bortolin, E., Bossin, L., Bricknell, C., Brzoska, K., Buraczewska, I., Castaño C.H., Čemusová, Z., Christiansson, M., Cordero, S.M., Cosler, G., Monaca, S.D., Desangles, F., Discher, M., Dominguez, I., Doucha-Senf, S., Eakins, J., Fattibene, P., Filippi, S., Frenzel, M., Georgieva, D., Gregoire, E., Guogyte, K., Hadjidekova, V., Hadjiiska, L., Hristova, R., Karakosta, M., Kis, E., Kriehuber, R., Lee, J., Lloyd, D., Lumniczky, K., Lyng, F., Macaeva, E., Majewski, M., Vanda Martins, S., McKeever, S.W., Meade, A., Medipally, D., Meschini, R., M'kacher, R., Gil, O.M., Montero, A., Moreno, M., Noditi, M., Oestreicher, U., Oskamp, D., Palitti, F., Palma, V., Pantelias, G., Pateux, J., Patrono, C., Pepe, G., Port, M., Prieto, M.J., Quattrini, M.C., Quintens, R., Ricoul, M., Roy, L., Sabatier, L., Sebastià, N., Sholom, S., Sommer, S., Staynova A., Strunz, S., Terzoudi, G., Testa A., Trompier, F., Valente, M., Hoey, O.V., Veronese, I., Wojcik A., Woda, C. (2017). Integration of new biological and physical retrospective dosimetry methods into EU

emergency response plans – Joint RENEB and EURADOS inter-laboratory comparisons. Int J Radiat Biol, Jan, 93(1):99-109. doi: 10.1080/09553002.2016.1206233. Epub 2016 Jul 20. Erratum in: Int J Radiat Biol. 2017 Jan, 93(1):x. PMID: 27437830.

[9] Andrejsová, L. Šinkorová, Z., Šinkora, J., Tichý, A., Filipová, A., Němcová, M., Šinkora, M. (2019). In vivo biodosimetry of porcine t-lymphocyte subsets and Nk Cells. Radiation Protection Dosimetry 186 (2-3): 181-85. doi: 10.1093/rpd/ncz199.

[10] Arndt, A., Steinestel, K., Rump, A., Sroya, M., Bogdanova, T., Kovgan, L., Port, M., Abend, M., Eder S. (2018). Anaplastic lymphoma kinase (ALK) gene rearrangements in radiation- related human papillary thyroid carcinoma after the Chernobyl accident. J Pathol Clin Res, Jul, 4(3):175-183. doi: 10.1002/cjp2.102. Epub 2018 May 26. PMID: 29633575; PMCID: PMC6065115.

[11] Becker, B.V., Richter, C., Ullmann, R., Beinke, C., Majewski, M., Exner, V., Weisel, G., Abend, M., Port M. (2017). Exploring the Link between Radiation Exposure and Multifocal Basal Cell Carcinomas in a Former Chernobyl Clean-up Worker by Combining Different Molecular Biological Techniques. Radiat Res, Nov, 188(5):571-578. doi: 10.1667/RR14819.1. Epub 2017 Sep 27. PMID: 28952879.

[12] Beinke, C., Port, M., Ullmann, R., Gilbertz, K., Majewski, M., Abend, M. (2018). Analysis of Gene Expression Changes in PHA-M Stimulated Lymphocytes – Unraveling PHA Activity as Prerequisite for Dicentric Chromosome Analysis. Radiat Res, Jun, 189(6):579-596. doi: 10.1667/RR14974.1. Epub 2018 Apr 3. PMID: 29613823.

[13] Bene, B.J., Blakely, W.F., Burmeister, D.M., Cary, L., Chhetri, S.J., Davis, C.M, Ghosh, S.P., Holmes-Hampton, G.P., Iordanskiy, S., Kalinich, J.F., Kiang, J.G., Kumar, V.P., Lowy, J.R., Miller, A., Naeem, M., Schauer, D.A., Senchak, D., Singh, V.K., Stewart, A.J., Velazquez, E.M., Xiao, M. Celebrating 60 years of accomplishments of the Armed Forces Radiobiology Research Institute. Radiation Research (2021 May 12, online ahead of print).

[14] Blakely, W.F., Bolduc, D.L., Debad, J., Sigal, G., Port, M., Abend, M., Valente, M., Drouet, M., Hérodin F. (2018). Use of proteomic and hematology biomarkers for prediction of hematopoietic acute radiation syndrome severity in baboon radiation models. Health Phys, Jul, 115(1):29-36. doi: 10.1097/HP.0000000000000819.

[15] Blakely, W.F., Port, M., Abend, M. Multiple parameter biodosimetry: Early response. J. Radiol. Prot. (accepted 19 July 2021 online ahead of press and in press).

[16] Bolduc, D.L., Blakely, W.F. (2019). Baboon radiation quality (mixed field neutron and gamma, gamma alone) dose -response model systems: Assessment of H-ARS severity using hematologic biomarkers. Radiat. Prot. Dosimetry Dec 31, 186(1): 15-23.

[17] Cavallero, S., Riccobono, D., Drouet, M. (2020). MSC-derived extracellular vesicles: New emergency treatment to limit the development of radiation-induced hematopoietic syndrome? Sabine François Health Phys Jul, 119(1):21-36. doi: 10.1097/HP.0000000000001264.

[18] Cheda, A., Nowosielska, E.M., Gebicki, J., Marcinek, A., Chlopicki, S., Janiak, M.K. (2021). A derivative of vitamin B3 applied several days after exposure reduces lethality of severely irradiated mice. Scientific Reports, 11: 7922. doi: 10.1038/s41598-021-86870-3

[19] Cruz-Garcia, L., O'Brien, G., Donovan, E., Gothard, L., Boyle, S., Laval A., Testard, I., Ponge, L., Woźniak, G., Miszczyk, L., Candéias, S.M., Ainsbury, E., Widlak, P., Somaiah, N., Badie, C. (2018). Influence of Confounding Factors on Radiation Dose Estimation Using In Vivo Validated Transcriptional Biomarkers. Health Phys, Jul, 115(1):90-101. doi: 10.1097/HP.0000000000000844.

[20] Cruz-Garcia, L., O'Brien, G., Sipos, B., Mayes, S., Love, M.I., Turner, D.J., Badie, C. (2020). Generation of a transcriptional radiation exposure signature in human blood using long-read nanopore sequencing. Radiat Res. 2020 Feb, 193(2):143-154. doi: 10.1667/RR15476.1. Epub 2019 Dec 12.5.

[21] Cruz-Garcia, L., O'Brien, G., Sipos, B., Mayes, S., Tichý, A., Sirák, I., Davídková, M., Marková, M., Turner, D.J., Badie, C. (2020). In vivo validation of alternative FDXR transcripts in human blood in response to ionizing radiation. Int J Mol Sci. 2020 Oct 23, 21(21):7851. doi: 10.3390/ijms21217851.

[22] Dainiak, N., Albanese, J., Kaushik, M., Balajee, A.S., Romanyukha, A., Sharp, T.J., Blakely, W.F. (2019). Concepts of operations for a US Dosimetry and Biodosimetry Network. Radiat. Prot. Dosimetry. Doi:10.1093/rpd/ncy294.

[23] Bolduc, D.L., Blakely, W.F., Olsen, C.H., Agay, D., Mestries, J.-C., Drouet, M., Hérodin, F. (2019). Baboon radiation quality (mixed-field neutron and gamma, gamma alone) dose-response model systems: assessment of H-ARS severity using haematologic biomarkers (2019). Radiat Prot Dosimetry, Dec 31, 186(1):15-23. doi: 10.1093/rpd/ncz048.

[24] Dörr, H., Abend, M., Blakely, W.F., Bolduc, D.L., Boozer, D., Costeira, T., Dant, T., De Amicis, A., De Sanctis, S., Dondey, M., Drouet, M., Entine, F., Francois, S., Gagna, G., Guitard, N., Hérodin, F., Hoefer, M., Lamkowski A., La Sala, G., Lista, F., Loiacono, P., Majewski, M., Martigne, P., Métivier, D., Michel, X., Pateux, J., Pejchal, J., Reeves, G., Riccobono, D., Sinkorova, Z., Soyez, L., Stricklin, D., Tichý, A., Valente, M., Woodruff, C.R. Jr, Zarybnicka, L., Port, M. (2017). Using clinical signs and symptoms for medical management of radiation casualties - 2015 NATO exercise. Radiat. Res, Mar, 187(3):273-286. doi: 10.1667/RR14619.1. Epub 2017 Feb 20.

[25] Eder, S., Hermann, C., Lamkowski, A., Kinoshita, M., Yamamoto, T., Abend, M., Shinomiya, N., Port, M., Rump, A. (2020). A comparison of thyroidal protection by stable iodine or perchlorate in the case of acute or prolonged radioiodine exposure. Arch Toxicol, Sep, 94(9):3231-3247. doi: 10.1007/s00204-020-02809-z. Epub 2020 Jul 12. PMID: 32656655; PMCID: PMC7415763.

[26] Filipova, A., Marek, J., Havelek, R., Pejchal, J., Jelicova, M., Cizkova, J., Majorosova, M., et al. (2020). Substituted piperazines as novel potential radioprotective agents. Molecules (Basel, Switzerland) 25 (3). https://doi.org/10.3390/molecules25030532.

[27] Foster, C.R.M. (2020). Emergency preparedness: Ionising radiation incidents and medical management. BMJ Military, 166:21-28. doi:10.1136/jramc-2018-000958

[28] Franchini, V., Müller, T., Haupt, J.M., Ostheim, P., Majewski, M., Lista, F., Port, M., Abend, M. (2020). Characterization of primary human dermal fibroblasts to ensure for instance emf exposure experiments under comparable cell culture condition. Health Phys, Jul, 119(1):118-127. doi: 10.1097/HP.0000000000001204. PMID: 31934933.

[29] Goh, V.S.T., Fujishima, Y., Abe, Y., Sakai A., Yoshida, M.A., Ariyoshi, K., Kasai, K., Wilkins, R.C., Blakely, W.F. (2019). Miura T. Construction of fluorescence in situ hybridization (FISH) translocation dose-response calibration curve with multiple donor data sets using, R., based on ISO 20046:2019 recommendations. Int. J. Radiat. Biol, Dec, 95(12):1668-1684.

[30] Goh, V.S.T., Nakayama, R., Blakely, W.F., Abe, Y., Chua, C.E.L., Nakata, A., Fujishima, Y., Yoshida, M.A., Kasai, K., Ariyoshi, K., Miura T. (2020). Improved harvest and fixation methodology for isolated human peripheral blood mononuclear cells in cytokinesis-block micronucleus assay. Int. J. Radiat. Biol. 97(2) 52-58.

[31] Gregoire, E., Barquinero, J.F., Gruel, G., Benadjaoud, M., Ainsbury, E., Beinke, C., Balajee A., Beukes, P., Blakely, W.F., Dominguez, I., Duy, P.N., Flegal, F.N., Gil, O.M., Güçlü, I., Guogyte, K., Hadjidekova, V., Hadjidekova, S.P., Hande, P., Jang, S., Lumniczky, K., Martinez, J.G., Meschini, R., Milic, M., Montoro, A., Moreno, M., Oestreicher, U., Pajic, J., Ricoul, M., Sabatier, L., Sebastia, N., Sommer, S., Szkarlat, Z., Testa A., Terzoudi, G., Valente, M., Venkatachalam, P., Vral, A., Wilkins, R.C., Wojcik, A., Zafiropoulos, D., Kulka, U. RENEB Inter-Laboratory Comparison 2017: limits and pitfalls of ILCs. Int J Radiat Biol (accepted 5 May 2021, posted online 10 May 2021).

[32] Haupt, J., Ostheim, P., Port, M., Abend, M. (2020). Using dicentric dose estimates and early radiation-induced blood cell count changes of real case histories for validation of the hemodose biodosimetry tool. Radiat Prot Dosimetry Jul 24, 189(4):428-435. doi: 10.1093/rpd/ncaa063. PMID: 32391564.

[33] Jones, L., Moor, D., Peacock, T., Melley, T., Foster, C., Bland, S., Gibb, I., Napier, I. (2020). Assessment of the potential impact of embedded radioactive fragments following the use of a crude radiological dispersal device (‘dirty bomb’). J. Radiol. Prot.40 1217-1235.

[34] Kaatsch, H.L., Becker, B.V., Schüle, S., Ostheim, P., Nestler, K., Jakobi, J., Schäfer, B., Hantke, T., Brockmann, M.A., Abend, M., Waldeck, S., Port, M., Scherthan, H., Ullmann R. (2021). Gene expression changes and DNA damage after ex vivo exposure of peripheral blood cells to various CT photon spectra. Sci Rep, Jun 8, 11(1):12060. doi: 10.1038/s41598-021-91023-7. PMID: 34103547; PMCID: PMC8187728.

[35] Kaatsch, H.L., Majewski, M., Schrock, G., Obermair, R., Seidel, J., Nestler, K., Abend, M., Waldeck, S., Port, M., Ullmann, R., Becker, B.V. (2020). CT Irradiation-induced changes of gene expression within peripheral blood cells. Health Phys, Jul, 119(1):44-51. doi: 10.1097/HP.0000000000001231. PMID: 32167501.

[36] Khalifa, J., François, S., Rancoule, C., Riccobono, D., Magné, N., Drouet, M., Chargari, C. (2019). Gene therapy and cell therapy for the management of radiation damages to healthy tissues: Rationale and early results. Cancer Radiother, Sep, 23(5):449-465. doi: 10.1016/j.canrad.2019.06.002.

[37] Kulka, U., Abend, M., Ainsbury, E., Badie, C., Barquinero J.F., Barrios, L., Beinke, C., Bortolin, E., Cucu, A., De Amicis, A., Domínguez, I., Fattibene, P., Frøvig, A.M., Gregoire, E., Guogyte, K., Hadjidekova, V., Jaworska, A., Kriehuber, R., Lindholm, C., Lloyd, D., Lumniczky, K., Lyng, F., Meschini, R., Mörtl, S., Della Monaca, S., Monteiro Gil, O., Montoro, A., Moquet, J., Moreno, M., Oestreicher, U., Palitti, F., Pantelias, G., Patrono, C., Piqueret-Stephan, L., Port, M., Prieto, M.J., Quintens, R., Ricoul, M., Romm, H., Roy, L., Sáfrány, G., Sabatier, L., Sebastià, N., Sommer, S., Terzoudi, G., Testa A., Thierens, H., Turai, I., Trompier, F., Valente, M., Vaz, P., Voisin, P., Vral, A., Woda, C., Zafiropoulos, D., Wojcik A. (2017). RENEB – Running the European Network of biological dosimetry and physical retrospective dosimetry. Int J Radiat Biol, Jan, 93(1):2-14. doi: 10.1080/09553002.2016.1230239. Epub 2016 Oct 6. PMID: 27707245.

[38] Kultova, G., Tichý, A., Rehulkova, H., Myslivcova-Fucikova, A (2020). The hunt for radiation biomarkers: Current situation. International Journal of Radiation Biology 96 (3): 370-82. https://doi.org/10.1080/09553002.2020.1704909.

[39] Lamkowski A., Kreitlow, M., Radunz, J., Willenbockel, M., Sabath, F., Schuhn, W., Stiemer, M., Fichte, L.O., Dudzinski, M., Böhmelt, S., Ullmann, R., Majewski, M., Franchini, V., Eder, S., Rump, A., Port, M., Abend, M. (2018). Gene expression analysis in human peripheral blood cells after 900 MHz RF-EMF short-term exposure. Radiat Res, May, 189(5):529-540. doi: 10.1667/RR14909.1. Epub 2018 Mar 6. PMID: 29509058.

[40] Lamkowski A., Kreitlow, M., Radunz, J., Willenbockel, M., Stiemer, M., Fichte, L.O., Rädel, C.F., Majewski, M., Ostheim, P., Port, M., Abend, M. (2021). Analyzing the impact of 900 MHz EMF short-term exposure to the expression of 667 miRNAs in human peripheral blood cells. Sci Rep, Feb 24, 11(1):4444. doi: 10.1038/s41598-021-82278-1. PMID: 33627699; PMCID: PMC7904780.

[41] Lierova, A., Kasparova, J., Pejchal, J., Kubelkova, K., Jelicova, M., Palarcik, J., Korecka, L., Bilkova, Z., Sinkorov, Z. (2020). Attenuation of radiation-induced lung injury by hyaluronic acid nanoparticles. Frontiers in Pharmacology. 11: 1199. doi: 10.3389/fphar.2020.01199.

[42] Lierova, A., Jelicova, M., Nemcova, M., Proksova, M., Pejchal, J., Zarybnicka, L., Sinkorova, Z. (2018). Cytokines and radiation-induced pulmonary injuries. Journal of Radiation Research 59(6): 709-53. doi: 10.1093/jrr/rry067.

[43] Majewski, M., Rozgic, M., Ostheim, P., Port, M., Abend, M. (2020). A new smartphone application to predict hematologic acute radiation syndrome based on blood cell count changes – The H-module app. Health Phys, Jul, 119(1):64-71. doi: 10.1097/HP.0000000000001247. PMID: 32484636.

[44] Manning, G., Macaeva, E., Majewski, M., Kriehuber, R., Brzóska, K., Abend, M., Doucha-Senf, S., Oskamp, D., Strunz, S., Quintens, R., Port, M., Badie, C. (2017). Comparable dose estimates of blinded whole blood samples are obtained independently of culture conditions and analytical approaches. Second RENEB gene expression study. Int J Radiat Biol, Jan, 93(1):87-98. doi: 10.1080/09553002.2016.1227105. Epub 2016 Sep 14. PMID: 27626709.

[45] Manning, G., Tichý, A., Sirák, I., Badie, C. (2017). Radiotherapy-Associated Long-term Modification of Expression of the Inflammatory Biomarker Genes ARG1, BCL2L1, and MYC. Front Immunol, Apr 10, 8:412. doi: 10.3389/fimmu.2017.00412. eCollection 2017.

[46] Marek, J., Tichý, A., Havelek, R., Seifrtova, M., Filipova, A., Andrejsova, L., Kucera, T. et al. (2020). A novel class of small molecule inhibitors with radioprotective properties. European Journal of Medicinal Chemistry 187: 111606. doi: 10.1016/j.ejmech.2019.111606.

[47] Moquet, J., Higueras, M., Donovan, E., Boyle, S., Barnard, S., Bricknell, C., Sun, M., Gothard, L., O'Brien, G., Cruz-Garcia, L., Badie, C., Ainsbury, E., Somaiah, N. (2018). Dicentric dose estimates for patients undergoing radiotherapy in the RTGene study to assess blood dosimetric models and the new Bayesian method for gradient exposure. Radiat Res, Dec, 190(6):596-604. doi: 10.1667/RR15116.1. Epub 2018 Sep 20.

[48] Nikovics, K., Favier, A.L., Barbier, L., Drouet, M., Riccobono, D. (2021). Characterization of macrophages, giant cells and granulomas during muscle regeneration after irradiation. Cytokine, Jan, 137:155318. doi: 10.1016/j.cyto.2020.155318. Epub 2020 Oct 9. PMID: 33045525.

[49] Nikovics, K., Morin, H., Riccobono, D., Bendahmane A., Favier, A.L. (2020). hybridization-chain-reaction is a relevant method for in situ detection of M2D-like macrophages in a mini-pig model. FASEB J. Dec, 34(12):15675-15686. doi: 10.1096/fj.202001496R. Epub 2020 Oct 20. PMID: 33078886.

[50] Nowosielska, E.M., Cheda, A., Zdanowski, R., Lewicki, S., Scott, B.R., Janiak, M.K. (2018). Effect of internal contamination with tritiated water on the neoplastic colonies in the lungs, innate anti-tumour reactions, cytokine profile, and haematopoietic system in radioresistant and radiosensitive mice. Radiation and Environmental Biophysics, 57:251-264. doi: 10.1007/s00411-018-0739-4.

[51] Nowosielska, E.M. Cheda, A., Pociegiel, M., Cheda, L. Szymański, P., Wiedlocha, A. (2021). Effects of a unique combination of the whole-body low dose radiotherapy with inactivation of two immune checkpoints and/or a heat shock protein on the transplantable lung cancer in mice. International Journal of Molecular Sciences, 22:12, 6309. doi: 10.3390/ijms22126309 (IF 4.556).

[52] O'Brien, G., Cruz-Garcia, L., Majewski, M., Grepl, J., Abend, M., Port, M., Tichý, A., Sirak, I., Malkova, A., Donovan, E., Gothard, L., Boyle, S., Somaiah, N., Ainsbury, E., Ponge, L., Slosarek, K., Miszczyk, L., Widlak, P., Green, E., Patel, N., Kudari, M., Gleeson, F., Vinnikov, V., Starenkiy, V., Artiukh, S., Vasyliev, L., Zaman A., Badie, C. (2018). FDXR is a biomarker of radiation exposure in vivo. Sci Rep, Jan 12, 8(1):684. doi: 10.1038/s41598-017-19043-w. PMID: 29330481; PMCID: PMC5766591.

[53] Ostheim, P., Coker, O., Schüle, S., Hermann, C., Combs, S.E., Trott, K.R., Atkinson, M., Port, M., Abend, M. (2021). Identifying a diagnostic window for the use of gene expression profiling to predict acute radiation syndrome. Radiat Res, Jan 1, 195(1):38-46. doi: 10.1667/RADE-20-00126.1. PMID: 33181834.

[54] Ostheim, P., Mallawaratchy A.D., Müller, T., Schüle, S., Hermann, C., Popp, T., Eder, S., Combs S.E., Port, M., Abend, M. (2021). Acute radiation syndrome-related gene expression in irradiated peripheral blood cell populations. Int J Radiat Biol, 97(4):474-484. doi: 10.1080/09553002.2021.1876953. Epub 2021 Mar 3. PMID: 33476246.

[55] Ostheim, P., Haupt, J., Herodin, F., Valente, M., Drouet, M., Majewski, M., Port, M., Abend, M. (2019). miRNA expression patterns differ by total- or partial-body radiation exposure in baboons. Radiat Res, Dec, 192(6):579-588. doi: 10.1667/RR15450.1. Epub 2019 Sep 26. PMID: 31556848.

[56] Ostheim, P., Haupt, J., Schüle, S., Herodin, F., Valente, M., Drouet, M., Majewski, M., Port, M., Abend, M. (2020). Differentiating total- or partial-body irradiation in baboons using mRNA Expression Patterns: A Proof of Concept. Radiat Res, Nov 10, 194(5):476-484. doi: 10.1667/RADE-20-00121.1. PMID: 32991726.

[57] Ostheim, P., Majewski, M., Gluzman-Poltorak, Z., Vainstein, V., Basile, L.A., Lamkowski A., Schüle, S., Kaatsch, H.L., Haimerl, M., Stroszczynski, C., Port, M., Abend, M. (2021). Predicting the radiation sensitivity of male and female rhesus macaques using gene expression. radiat res, Jan 1, 195(1):25-37. doi: 10.1667/RADE-20-00161.1. PMID: 33181854.

[58] Ostheim, P., Tichý, A., Sirak, I., Davidkova, M., Stastna, M.M., Kultova, G., Paunesku, T., Woloschak, G., Majewski, M., Port, M., Abend, M. (2020). Overcoming challenges in human saliva gene expression measurements. Sci Rep, Jul 7, 10(1):11147. doi: 10.1038/s41598-020-67825-6. PMID: 32636420; PMCID: PMC7341869.

[59] Peacock, T., Jones, L., Foster, C. (2017). Consequences to patient and surgeon from embedded radioactive shrapnel. DSTL/CR100581 V1.031 March 2017.

[60] Polozov, S., Cruz-Garcia, L., Badie, C. (2019). Rapid gene expression based dose estimation for radiological emergencies. Radiat Prot Dosimetry, Dec 31, 186(1):24-30. doi: 10.1093/rpd/ncz053.

[61] Port, M., Abend, M. (2018). Clinical triage of radiation casualties-the hematological module of the bundeswehr institute of radiobiology. Radiat Prot Dosimetry, Dec 1, 182(1):90-92. doi: 10.1093/rpd/ncy141. PMID: 30165461.

[62] Port, M., Haupt, J., Ostheim, P., Majewski, M., Combs, S.E., Atkinson, M., Abend, M. (2021). Software Tools for the Evaluation of Clinical Signs and Symptoms in the Medical Management of Acute Radiation Syndrome-A Five-year Experience. Health Phys, Apr 1, 120(4):400-409. doi: 10.1097/HP.0000000000001353. PMID: 33315652.

[63] Port, M., Hérodin, F., Drouet, M., Valente, M., Majewski, M., Ostheim, P., Lamkowski A., Schüle, S., Forcheron, F., Tichý, A., Sirak, I., Malkova, A., Becker, B.V., Veit, D.A., Waldeck, S., Badie, C., O'Brien, G., Christiansen, H., Wichmann, J., Beutel, G., Davidkova, M., Doucha-Senf, S., Abend, M. (2021). Gene expression changes in irradiated baboons: A summary and interpretation of a decade of findings. Radiat Res, Jun 1, 195(6):501-521. doi: 10.1667/RADE-20-00217.1. PMID: 33788952.

[64] Port, M., Hérodin, F., Valente, M., Drouet, M., Lamkowski A., Majewski, M., Abend, M. (2017). Gene expression signature for early prediction of late occurring pancytopenia in irradiated baboons. Ann Hematol, May, 96(5):859-870. doi: 10.1007/s00277-017-2952-7. Epub 2017 Feb 24. PMID: 28236054; PMCID: PMC5371629.

[65] Port, M., Hérodin, F., Valente, M., Drouet, M., Ostheim, P., Majewski, M., Abend, M. (2018). Persistent mRNA and miRNA expression changes in irradiated baboons. Sci Rep, Oct 18, 8(1):15353. doi: 10.1038/s41598-018-33544-2. PMID: 30337559; PMCID: PMC6194144.

[66] Port, M., Hérodin, F., Valente, M., Drouet, M., Ullmann, R., Majewski, M., Abend, M. (2017). Pre-exposure gene expression in baboons with and without pancytopenia after radiation exposure. Int J Mol Sci, Mar 2, 18(3):541. doi: 10.3390/ijms18030541. PMID: 28257102; PMCID: PMC5372557.

[67] Port, M., Majewski, M., Abend, M. (2019). Radiation dose is of limited clinical usefulness in persons with acute radiation syndrome. Radiat Prot Dosimetry, Dec 31, 186(1):126-129. doi: 10.1093/rpd/ncz058. PMID: 31330030.

[68] Port, M., Ostheim, P., Majewski, M., Voss, T., Haupt, J., Lamkowski A., Abend, M. (2019). Rapid high-throughput diagnostic triage after a mass radiation exposure event using early gene expression changes. Radiat Res, Aug, 192(2):208-218. doi: 10.1667/RR15360.1. Epub 2019 Jun 18. PMID: 31211643.

[69] Port, M., Pieper, B., Dörr, H.D., Hübsch, A., Majewski, M., Abend, M. (2018). Correlation of radiation dose estimates by DIC with the METREPOL hematological classes of disease severity. Radiat Res, May, 189(5):449-455. doi: 10.1667/RR14936.1. Epub 2018 Mar 1. PMID: 29494324.

[70] Port, M., Pieper, B., Knie, T., Dörr, H., Ganser, A., Graessle, D., Meineke, V., Abend, M. (2017). Rapid prediction of hematologic acute radiation syndrome in radiation injury patients using peripheral blood cell counts. Radiat Res, Aug, 188(2):156-168. doi: 10.1667/RR14612.1. Epub 2017 Jun 7. PMID: 28590841.

[71] Rump, A., Becker, B., Eder, S., Lamkowski, A., Abend, M., Port M. (2018). Medical management of victims contaminated with radionuclides after a "dirty bomb" attack. Mil Med Res, Aug 6, 5(1):27. doi: 10.1186/s40779-018-0174-5. PMID: 30086798; PMCID: PMC6080556.

[72] Rump, A., Eder, S., Hermann, C., Lamkowski, A., Kinoshita, M., Yamamoto, T., Abend, M., Shinomiya, N., Port M. (2021). A comparison of thyroidal protection by iodine and perchlorate against radioiodine exposure in Caucasians and Japanese. Arch Toxicol, May 18. doi: 10.1007/s00204-021-03065-5. Epub ahead of print. PMID: 34003340.

[73] Rump, A., Eder, S., Lamkowski A., Hermann, C., Abend, M., Port M. (2019). A quantitative comparison of the chemo- and radiotoxicity of uranium at different enrichment grades. Toxicol Lett, Oct 1, 313:159-168. doi: 10.1016/j.toxlet.2019.07.004. Epub 2019 Jul 2. PMID: 31276769.

[74] Rump, A., Eder, S., Lamkowski A., Kinoshita, M., Yamamoto, T., Abend, M., Shinomiya, N., Port M. (2019). Development of New Biokinetic-Dosimetric Models for the Simulation of Iodine Blockade in the Case of Radioiodine Exposure in Man. Drug Res (Stuttg), Oct, 69(11):583-597. doi: 10.1055/a-0960-5590. Epub 2019 Aug 7. PMID: 31390663.

[75] Rump, A., Ostheim, P., Eder, S., Hermann, C., Abend, M., Port M. (2021). Preparing for a "dirty bomb" attack: The optimum mix of medical countermeasure resources. Mil Med Res, Jan 17, 8(1):3. doi: 10.1186/s40779-020-00291-3. PMID: 33455578; PMCID: PMC7812656.

[76] Rump, A., Stricklin, D., Lamkowski A., Eder, S., Abend, M., Port M. (2017). Analysis of the antidote requirements and outcomes of different radionuclide decorporation strategies for a scenario of a "dirty bomb" attack. Am J Disaster Med, Fall, 12(4):227-241. doi: 10.5055/ajdm.2017.0276. PMID: 29468625.

[77] Rump, A., Stricklin, D., Lamkowski A., Eder, S., Abend, M., Port M. (2017). The incorporation of radionuclides after wounding by a "dirty bomb": The impact of time for decorporation efficacy and a model for cases of disseminated fragmentation wounds. Adv Wound Care (New Rochelle), Jan 1, 6(1):1-9. doi: 10.1089/wound.2016.0693. PMID: 28116223; PMCID: PMC5220565.

[78] Sicherre, E., Favier, A.L., Riccobono, D., Nikovics, K. (2021). Non-specific binding, a limitation of the immunofluorescence method to study macrophages in situ. Genes (Basel). Apr 27, 12(5):649. doi: 10.3390/genes12050649. PMID: 33925331

[79] Šinkorová, Z., Filipová, A., Vávrová, J., Pejchal, J., Andrejsová, L., Jeličová, M., Marek, J. et al. (2019). Investigation of the radioprotective effect of orthovanadate in mice after total body irradiation. Radiation Protection Dosimetry, 186 (2-3): 149-54. https://doi.org/10.1093/rpd/ncz192.

[80] Subramanian, U., O'Brien, B., McNamara, M., Romanyukha-L, Bolduc, D.L., Olsen, C., and Blakely, W.F. (2020). Automated dicentric aberration scoring for triage dose assessment: 60Co gamma rays dose-response at different dose rates. Health Physics, July: 119(1): 52-58.

[81] Tichý, A., Kabacik, S., O'Brien, G., Pejchal, J., Sinkorova, Z., Kmochova, A., Sirak, I., Malkova, A., Beltran, C.G., Gonzalez, J.R., Grepl, J., Majewski, M., Ainsbury, E., Zarybnicka, L., Vachelova, J., Zavrelova, A., Davidkova, M., Markova Stastna, M., Abend, M., Pernot, E., Cardis, E., Badie, C. (2018). The first in vivo multiparametric comparison of different radiation exposure biomarkers in human blood. PLoS One, Feb 23, 13(2):e0193412. doi: 10.1371/journal.pone.0193412. eCollection 2018.

[82] Tichý, A., Marek, J., Havelek, R., Pejchal, J., Seifrtova, M., Zarybnicka, L., Filipova, A., Rezacova, M., Sinkorova, Z. (2018). New light on an old friend: Targeting PUMA in radioprotection and therapy of cardiovascular and neurodegenerative diseases. Current Drug Targets 19 (16): 1943-57. https://doi.org/10.2174/1389450119666180406110743.

[83] Tichý, A., Kabacik, S., O'Brien, G., Pejchal, J., Sinkorova, Z., Kmochova, A., Sirak, I., et al. (2018). The first in vivo multiparametric comparison of different radiation exposure biomarkers in human blood. PloS One 13 (2): e0193412. doi: 10.1371/journal.pone.0193412.

[84] Williams, K., Jeggo, P.A., Hammond, E.M., West, C., Badie, C., Anderson, R.M. (2020). Meeting report of the 16th international congress of radiation research and the 12th international symposium on chromosomal aberrations. J Radiol Prot, Mar, 40(1):361-365. doi: 10.1088/1361-6498/ab52de. Epub 2020 Feb 21.

[85] Ziegler, A.K, Abend, M., Port, M., Dammann, E., Homeyer, R.S., Eder, M., Ganser, A., Schrem, H., Koenecke, C. (2019). Cumulative dosages of chemotherapy and radiotherapy exposure, and risk of secondary malignancies after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant, Apr, 54(4):635-640. doi: 10.1038/s41409-018-0359-2. Epub 2018 Oct 18. PMID: 30337701.