



Non-Cancer Effects: Main Achievements of DoReMi

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DoReMi TRA Questions (2010)

Questions to be addressed in DoReMi

What is the dependence on energy deposition?

What is the dependence on dose rate?

What are the tissues sensitivities?

What is the modification of risk by genetic and epigenetic factors and gender?

What is the effect of age on risk?

What is the effect of lifestyle and/or other exposures on risk?

What is the effect of physiological state?

Is there a hereditary component in risk?

What is the role of non-targeted effects in health risk?



DoReMi WP7 Programme Overview

- Exploratory workshops: 2 Tasks (7.1 & 7.2)
- Vascular effects: 3 Tasks (7.3 – 7.7 & 7.12)
- Lens opacities: 5 Tasks (7.4 – 7.4.1 – 7.8 – 7.11 & 7.13)
- Neurological/Cognitive effects: 3 Tasks (7.5 – 7.9 & 7.10)
- Inflammation: 1 Task (7.6)



DoReMi WP7: Exploratory Workshops

- To hold exploratory workshops addressing the radiation-induced vascular effects and lens opacities resulting from low dose exposure with the view of identifying the open scientific questions to be raised and provide the DoReMi consortium and the EC with recommendations about future research projects to be launched (Task 7.1 - IRSN)
- To convene a workshop of epidemiologists aiming at reviewing all existing cohorts, defining the most informative epidemiological approach for the study of vascular effects resulting from exposure to low dose of ionizing radiation, preparing a strategy for conducting molecular epidemiological studies with markers of vascular damage after the determination of the best approach, and recommending pilot studies for a future RTD call (Task 7.2 - BfS)



DoReMi WP7: Vascular Effects

- To perform experiments providing measurable and quantitative knowledge of the initial radiation response of the endothelium (Human Umbilical Vein Endothelial Cells: HUVECs) for low and high energy radiation at different radiation doses delivered through acute vs. chronic external irradiation and internal contamination, and to develop a mathematical representation of the endothelium radiation response (Task 7.3 – SU)
- To investigate the effects of low dose IR on aneurysm development using a sensitized mouse model, carrying a mutation that lowers the expression of fibulin-4, a protein that is required for aortic wall strength (Task 7.7 – ERASMUS MC)
- To evaluate the effects of low doses of X-rays on the structure and function of vascular endothelium in diabetes prone mice before and during the development of hyperglycaemia, a cause of the vascular complications in diabetes (Task 7.12 – MIHE)



DoReMi WP7: Lens Opacities

- To explore lens opacities among a small cohort of interventional radiologists and cardiologists (Task 7.4 – IRSN) and to develop a “standard” protocol for epidemiological studies on the risk of radiation-induced lens opacities among interventional cardiologists, including as well a solid protocol for the retrospective assessment of the cumulative dose to the eye lens (Subtask 7.4.1 – SCK•CEN)
- To investigate low and moderate dose radiation effects on primary Human Lens Epithelial (HLE) cells using multiple stand systems approaches to determine the low dose response of the initial cellular events and the delayed radiation effects down to 1-mGy X-irradiation (Task 7.8 – OBU)
- To validate the methodology explored in Subtask 7.4 (ELDO project) in the frame of an epidemiological pilot study on radiation-induced cataract in interventional cardiology (Task 7.11 – NIOM)
- To provide a dose-response curve for low doses of radiation (<100mGy) on the formation of cataracts, including investigations of transparency of eye lens, histology, immunochemistry, cytogenetic analysis and analyses of DNA-damage repair markers (Task 7.13 – HMGU)



DoReMi WP7: Neurological Effects

- To perform *in vitro* and *in vivo* that will provide comparative information on how neurogenesis could be affected from exposure to low dose of ionizing radiation delivered through external irradiation vs. internal contamination with drinking water contaminated with uranium (Task 7.5 – SCK•CEN)
- To study low dose induced molecular and functional alterations of the microvascular component of the brain by clarifying the role of pericytes in this system (Task 7.9 – NRIRR)
- To study the Influence of a chronic LD and LDR exposure onto the development of Parkinson symptoms in genetically predisposed Pitx3-EYL/EYL Ogg1^{-/-} mouse mutant (Task 7.10 – HMGU)



DoReMi WP7: Inflammation

- To elucidate the effects of low-dose X-radiation (LDR) over the dose range of 0.01-2 Gy on induction of anti-inflammation and better understand the mechanisms behind, with the final objective of better describing systemic radiation induced tissue and whole organism level responses and confirm or rebut system biology approaches (Task 7.6 – UKER)

DoReMi WP7 Publications vs. Questions (2015)

Questions to be addressed in DoReMi	#
What is the dependence on energy deposition?	8
What is the dependence on dose rate?	2
What are the tissues sensitivities?	1
What is the modification of risk by genetic and epigenetic factors and gender?	1
What is the effect of age on risk?	0
What is the effect of lifestyle and/or other exposures on risk?	0
What is the effect of physiological state?	0
Is there a hereditary component in risk?	0
What is the role of non-targeted effects in health risk?	1

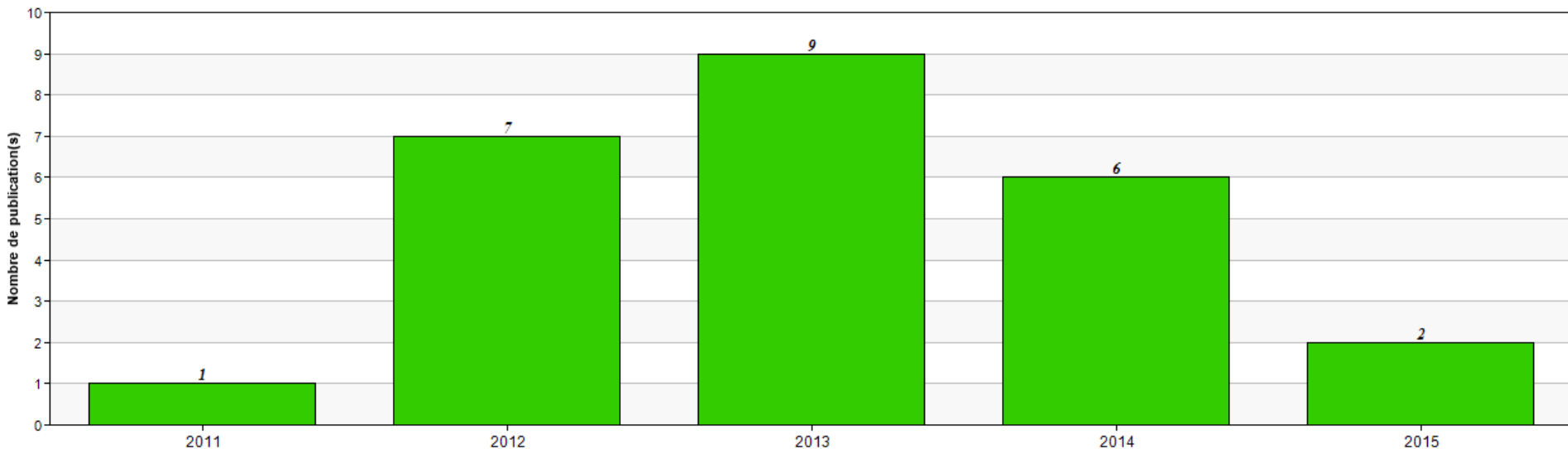


DoReMi WP7 Answers to Questions

Reference	Q1 - Dependence on energy deposition	Q2 - Dependence on dose-rate	Q3 - Tissue sensitivities	Q4 - Modification of risk by genetic and epigenetic factors and gender	Q9 - Role of non-targeted effects in health risk
Frischholz Autoimmunity 2015	U shape (no effect below 0.3 Gy and at 1 Gy, effect decreasing at 0.5 and 0.7 Gy)			Balb/c mice more radiosensitive than C57BL/6 mice	
Lödermann Int J Radiat Biol 2012	U shape (effect decreasing between 0.1 and 0.7 Gy, no effect at 1 Gy)				
Rödel Cancer Lett 2013					Multiple unresolved questions, which require further investigations
Rombouts Int J Radiat Biol 2013	Non-linear dose-response curve		Apoptosis: 0.1 Gy in EA.hy926 cells and 0.5 Gy in HUVEC	HUVEC less radiosensitive than EA.hy926	
Rombouts Int J Radiat Biol 2014	Shape of dose response curve?	Dose-rate effect on radiation-induced vascular senescence			
Ebrahimian Rad Res 2015		Dose rate effect on radiation-induced vascular function			
Samari Int J Mol Med 2013	Dose-dependence: no effect at 0.1 Gy, effects at 0.2 and 0.5 Gy				
Yentrapalli Plos One 2013		Dose-rate effects on radiation-induced vascular senescence			
Large Radiat Oncol 2014	Linearity of dose-response curve depends on post-exposure time				



DoReMi WP7 Publications (Total)



- **25 publications** reporting on DoReMi WP7 activities since the start of the project (these publications make clear reference to DoReMi)
- 1 publication on strategies for molecular epidemiological studies in Europe on cardiovascular diseases (Task 7.2) has been accepted for Mutation Research Review in March 2015



DoReMi WP7 Publications

Adhesion | Akt | [Anti inflammatory](#) | Anti inflammatory effect | [Apoptosis] | [Arm](#) | Benign painful elbow syndrome | Benign painful shoulder syndrome | Bystander | Calcaneodynia | Cardiovascular disease | Cell expose | Cell type | Cellular | [Chronic](#) | Chronic low dose rate radiation | Chronic radiation | [Cobalt] | Completion | [Comprehensive pain score](#) | Consecutive evaluable patient | [Cp](#) | Cvd | [Delay response](#) | Discontinuous dose response relationship | [Dna](#) | Dna damage | Doremi | [Dose fractionation schedule](#) | Dose range | [Dose rate](#) | Dose response | Dosimetry | Ea | Early response | Endothelial cell senescence | [[Endothelium](#)] | [Endpoint](#) | Erlangen dose optimization | Erlangen dose optimization trial | [Evaluable patient](#) | [Exceed 3](#) | Exceed 3 6 | Exert | Fr | [Fraction](#) | Fraction 3 | High dose | [Human umbilical vein endothelial cell](#) | [Huvec](#) | Hy926 | Hy926 cell | Il1beta | Immortalize | Immune system | Inflammation | Inflammatory disease | Inflammatory phenotype | Initiative | Insufficient pain remission | [Insufficient remission](#) | Integration | Ionise radiation | **[Irradiation]** | Long term | Long term response | Long term response rate | Low dose orthovoltage radiotherapy | Low dose radiotherapy | Low dose rate | Low dose rate radiation | Low dose risk | Low dose x irradiation | [[Low dose](#)] | Macrophage | [Mean cp](#) | Mean cp value | Mean vas score | [Mean vas value](#) | Mechanistic | Melodi | Mgy h | Molecular mechanism | Mononuclear | Multidisciplinary | Multidisciplinary european low dose initiative | Network | Non target | [Option](#) | Organise | Orthovoltage radiotherapy | Orthovoltage technique | P21 | [Pain](#) | [Pain reduction](#) | Painful elbow syndrome | Painful shoulder syndrome | [Pathway](#) | Premature senescence | Primary human umbilical vein endothelial cell | Pro | Prospective randomize trial | Prospective trial | Protein expression | Proteomic | Protract | Questionnaire base visual analogue scale | [Radiation protection](#) | [Radiation protection reason](#) | [Radiosensitivity] | [Radiotherapy](#) | Radiotherapy course | [Radiotherapy serie](#) | [Randomly assign](#) | Range 9 57 | Reactive oxygen specy | [Recruit](#) | [Repair] | [Response rate](#) | Road map | Rt | Rt serie | Second course | Second radiation serie | Secretion | Senescence | [Significant difference](#) | [Single dose](#) | Single dose trial arm | Single fraction | Single fraction 3 | Special emphasis | [Springer verlag](#) | Statistically significant difference | Strategic research agenda | Stress | Stuk | Summarise | [Therapy](#) | Tnf alpha | Treatment option | Trigger | Underly | University press | Urban | [Vas](#) | [Visual analogue scale](#) | Vogel | Workshop | X ray |

- The most popular concepts are:
 - “Irradiation” (24 publications)
 - “Low Dose” (17 publications)
 - “Radiation Protection” (10 publications)
 - “Radiotherapy” (9 publications)
 - “Endothelium” (9 publications)



DoReMi WP7 Key Results (Based on Publications)

Reference	Topic	Paper Topic	Experiment Type (in vivo/in vitro...)	Species	Energy	Dose	Dose rate	Main results
Frischholz Autoimmunity 2015	Inflammatory processes		ex vivo peritoneal macrophages	Mouse	X- rays	0.01, 0.05, 0.1, 0.3, 0.5, 0.7 and 1.0 Gy		Reduced secretion of IL-1 β by activated macrophages from Balb/c mice after LD-RT with 0.5 or 0.7 Gy of X-ray
Lödermann Int J Radiat Biol 2012	Inflammatory processes	Production and secretion of IL-1 β in activated macrophages are affected by low doses of X-rays	Monocytes differentiated to macrophages	Human	X-rays	0.1, 0.3, 0.5, 0.7 and 1.0 Gy		Protein levels are reduced in macrophages, with a pronounced reduction after X-ray treatment with 0.5 and 0.7 Gy
Ebrahimian Rad Res 2015	Inflammatory processes	Effect of chronic low dose-rate radiation exposure on the inflammatory response of endothelial cells and their function	HUVEC	Human	Gamma-rays (Cs-137)	0.7, 2, 4.1 Gy	1.4 and 4.1 mGy/h	Dose rate is an important parameter in the alteration of HUVEC inflammatory profile and function



DoReMi WP7 Key Results (Based on Publications)

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Ott Strahlenther Onkol 2012a	Inflammatory processes	Pain reduction of painful elbow syndrome by anti-inflammatory IR low doses		Human	Orthovoltage	6x0.5 Gy or 6x1 Gy		No statistically significant differences between the two single dose trial arms for early and delayed response
Ott Strahlenther Onkol 2012b	Inflammatory processes	Pain reduction of benign painful shoulder syndrome by RT		Human	Orthovoltage	6x0.5 Gy or 6x1 Gy		No statistically significant differences between the two single dose trial arms for early and delayed response
Ott Strahlenther Onkol 2012c	Inflammatory processes	Pain reduction for achillodynia by RT		Human	Orthovoltage	6x0.5 Gy or 6x1 Gy		No statistically significant differences between the two single dose trial arms for early and delayed response
Ott Strahlenther Onkol 2014a	Inflammatory processes	Pain reduction of benign calcaneodynia by RT		Human	Orthovoltage	6x0.5 Gy or 6x1 Gy		No statistically significant differences between the two single dose trial arms for early and delayed response
Ott Strahlenther Onkol 2014b	Inflammatory processes	Pain reduction of Radiotherapy for calcaneodynia by RT		Human	Orthovoltage	6x0.5 Gy or 6x1 Gy		No statistically significant differences between the two single dose trial arms for early and delayed response
Ott Strahlenther Onkol 2014c	Inflammatory processes	Pain reduction of benign painful elbow syndrome by RT		Human	Orthovoltage	6x0.5 Gy or 6x1 Gy		No statistically significant differences between the two single dose trial arms for early and delayed response
Ott Strahlenther Onkol 2014d	Inflammatory processes	Pain reduction of benign painful shoulder syndrome by RT		Human	Orthovoltage	6x0.5 Gy or 6x1 Gy		No statistically significant differences between the two single dose trial arms for early and delayed response

DoReMi WP7 Key Results (Based on Publications)

Reference	Topic	Paper Topic	Experiment Type (in vivo/in vitro...)	Species	Energy	Dose	Dose rate	Main results
Rombouts Int J Radiat Biol 2014	Vascular effects: premature senescence		HUVEC	Human	Gamma-rays (Cs-137)	0.7, 2, 4.1 Gy	1.4 and 4.1 mGy/h	Early stress response at one week and inflammation profile after three weeks
Yentrapalli Plos One 2013	Vascular effects: premature senescence	Involvement of the PI3K/Akt/mTOR pathway in senescence	HUVEC	Human	Gamma-rays (Cs-137)		1.4 and 2.4 mGy/h	The dose rate of 2.4 mGy/h significantly inhibited growth and induced premature senescence in HUVECs (not observed for a lower dose rate (1.4 mGy/h))
Yentrapalli Proteomics 2013	Vascular effects: proteome on senescence		HUVEC	Human	Gamma-rays (Cs-137)	0.7, 2, 4.1 Gy	4.1 mGy/h	Progressive loss of replicative capacity when the cumulative dose reaches 4 Gy (week 6), increasing appearance of senescent cells
Rombouts Int J Radiat Biol 2013	DNA damages	DSB and apoptosis	Endothelial cells (HUVEC & EA.hy926)	Human	X-rays	0.05, 0.1, 0.5 and 5 Gy	0.25 Gy/min	Acute low doses of X-rays induce DNA damage and apoptosis in endothelial cells
Large Radiat Oncol 2014	DNA damages	Dose-dependence of DNA damages, ROS levels and SOD activity	EA.hy926 cells	Human	Photons	0.3, 0.5, 0.7 and 1.0 Gy	4 Gy/min	Linear dose response characteristic of γH2AX foci at short term BUT detection at 1 h and 4 h after irradiation Non-linear regulation of ROS production and SOD activity following irradiation with doses <1 Gy

DoReMi WP7 Key Results (Based on Publications)

Reference	Topic	Paper Topic	Experiment Type (in vivo/in vitro...)	Species	Energy	Dose	Dose rate	Main results
Samari Int J Mol Med 2013	CNS: impairment of the establishment of the neuronal network	Apoptotic response mediated by N-methyl D-aspartate receptors	Primary cortical neuronal cultures	Mouse	X-rays	Cells : 0.1, 0.2 and 0.5 Gy Mouse : 0.6 Gy	5 mGy/sec	Double-strand breaks increase proportionally with the dose. Central role of calpain in radiation-induced excitotoxicity
Farah Radiat Prot Dosim 2013	Dosimetry	To estimate cumulative eye lens doses for retrospective epidemiology studies based on personal dose equivalent, Hp(10)						Practical recommendations to estimate eye lens dose are given
Rödel Cancer Lett 2013	Bystander and abscopal effects	State of art						



Observed effect depending on dose and dose-rate

Reference	Topic of the paper	Cell type	Dose	10-100 mGy	100 mGy- <1 Gy	1 Gy	Dose rate	<5 mGy/h	5 mGy/h et <1 Gy/h	>1 Gy/h
Frischholz Autoimmunity 2015	Production and secretion of IL-1 β in activated macrophages are affected by low doses of X-rays	Immune	0.01, 0.05, 0.1, 0.3, 0.5, 0.7 and 1.0 Gy	X	X	X				
Lödermann Int J Radiat Biol 2012		Immune	0.1, 0.3, 0.5, 0.7 and 1.0 Gy		X	X				
Samari Int J Mol Med 2013	Apoptotic response mediated by N-methyl D-aspartate receptors	Neuronal	Cells: 0.1, 0.2 and 0.5 Gy Mouse: 0.6 Gy		X		5 mGy/sec			X
Rombouts Int J Radiat Biol 2013	DSB and apoptosis	Endothelial	0.05, 0.1, 0.5 and 5 Gy	X	X	X	0.25 Gy/min (15 mGy/h)		X	
Rombouts Int J Radiat Biol 2014	Premature senescence	Endothelial	0.7, 2, 4.1 Gy		X	X	1.4 and 4.1 mGy/h	X		
Ebrahimian Rad Res 2015	Effect of chronic low dose-rate radiation exposure on the inflammatory response of endothelial cells and their function	Endothelial	0.7, 2, 4.1 Gy		X	X	1.4 and 4.1 mGy/h	X		
Yentrapalli Proteomics 2013	proteome on senescence	Endothelial	0.7, 2, 4.1 Gy		X	X	4.1 mGy/h	X		
Yentrapalli Plos One 2013	Involvement of the PI3K/Akt/mTOR pathway in senescence	Endothelial			X	X	1.4 and 2.4 mGy/h	X		
Large Radiat Oncol 2014	Dose-dependence of DNA damages, ROS levels and SOD activity	Endothelial	0.3, 0.5, 0.7, 1.0		X	X	4 Gy/min	X		



Observed effect depending on dose and dose rate

Reference	Topic of the paper	Cell type	Dose	10-100 mGy	100 mGy- <1 Gy	1 Gy	Dose rate	<5 mGy/h	5 mGy/h et <1 Gy/h	>1 Gy/h
Frischholz Autoimmunity 2015		Immune	0.01, 0.05, 0.1, 0.3, 0.5, 0.7 and 1.0 Gy	X	X	X				
Lödermann Int J Radiat Biol 2012	Production and secretion of IL-1 β in activated macrophages are affected by low doses of X-rays	Immune	0.1, 0.3, 0.5, 0.7 and 1.0 Gy		X	X				
Samari Int J Mol Med 2013	Apoptotic response mediated by N-methyl D-aspartate receptors	Neuronal	Cells: 0.1, 0.2 and 0.5 Gy Mouse: 0.6 Gy		X		5 mGy/sec			X
Rombouts Int J Radiat Biol 2013	DSB and apoptosis	Endothelial	0.05, 0.1, 0.5 and 5 Gy	X	X	X	0.25 Gy/min (15 mGy/h)		X	
Rombouts Int J Radiat Biol 2014	Premature senescence	Endothelial	0.7, 2, 4.1 Gy		X	X	1.4 and 4.1 mGy/h	X		
Ebrahimian Rad Res 2015	Effect of chronic low dose-rate radiation exposure on the inflammatory response of endothelial cells and their function	Endothelial	0.7, 2, 4.1 Gy		X	X	1.4 and 4.1 mGy/h	X		
Yentrapalli Proteomics 2013	proteome on senescence	Endothelial	0.7, 2, 4.1 Gy		X	X	4.1 mGy/h	X		
Yentrapalli Plos One 2013	Involvement of the PI3K/Akt/mTOR pathway in senescence	Endothelial			X	X	1.4 and 2.4 mGy/h	X		
Large Radiat Oncol 2014	Dose-dependence of DNA damages, ROS levels and SOD activity	Endothelial	0.3, 0.5, 0.7, 1.0		X	X	4 Gy/min	X		

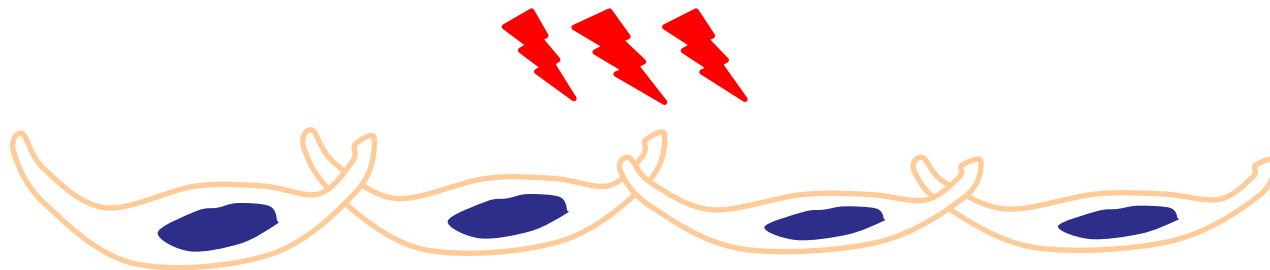
- In general, there are very few new results
- Only two publications looked at doses below 100mGy
- Majority of studies are performed on endothelial cells
- The dose rate values are not indicated in the two first publications
- Three publications showed clearly an impact of the dose rate on the observed effects (see slides on HUVEC's studies)
- No single dose, large range of dose values (up to a factor of 100)



DoReMi Task 7.3 Publications

Task 7.3 – Feasibility study towards a systems biology approach of radiation response of the endothelium

Three dose rates: 1.4, 2.4 and 4.1 mGy/h



HUVEC (Human Umbilical Vein Endothelial Cells)



		1.4 mGy/h	2.4 mGy/h	4.1 mGy/h
Cell proliferation	ERK	=	=	=
Cell cycle stopping	P21	=	=	=
Senescence	SA-β-gal	=	=	=
Replicative capacity	Population doubling	=	=	=
Inflammatory cytokines	TNFα/ IL-6/ MCP-1	=	=	=
Adhesion molecule	E-Selectin	=	=	=
Adhesion regulation	Rho GDI	=	=	↑
Cell surface marker	ICAM-1/ V-CAM-1	=	=	=
Functionality	Vascular network formation	=	=	=

Only sparse effects are observed

WEEK 1



		1.4 mGy/h	2.4 mGy/h	4.1 mGy/h
Cell proliferation	ERK			=
Cell cycle stopping	P21			=
Senescence	SA- β -gal	=	=	\uparrow
Replicative capacity	Population doubling	=	=	=
Inflammatory cytokines	TNF α / IL-6/ MCP-1	=	=	\uparrow
Adhesion molecule	E-Selectin	=	=	=
Adhesion regulation	Rho GDI			\downarrow
Cell surface marker	ICAM-1/V-CAM-1	=	=	=
Functionality	Vascular network formation	=	=	\downarrow

Effects observed for the highest dose rate only

WEEK 3



		1.4 mGy/h	2.4 mGy/h	4.1 mGy/h
Cell proliferation	ERK	=	=	=
Cell cycle stopping	P21	=	=	↑
Senescence	SA-β-gal	=	=	↑
Replicative capacity	Population doubling	=	↓	↓
Inflammatory cytokines	TNFα/ IL-6/ MCP-1	=	=	=
Adhesion molecule	E-Selectin	=	=	↑
Adhesion regulation	Rho GDI	↓	↓	↓
Cell surface marker	ICAM-1/V-CAM-1	=	=	=
Functionality	Vascular network formation	=	=	↓

Effects observed for all dose rates (more various effects for the highest dose rate)



		1.4 mGy/h	2.4 mGy/h	4.1 mGy/h
Cell proliferation	ERK	=	↓	
Cell cycle stopping	P21	↑	↑	
Senescence	SA-β-gal	=	↑	
Replicative capacity	Population doubling	=	↓	
Inflammatory cytokines	TNFα/ IL-6/ MCP-1	=	=	↑
Adhesion molecule	E-Selectin	=	=	=
Adhesion regulation	Rho GDI	↓	↓	
Cell surface marker	ICAM-1/ V-CAM-1	=	=	=
Functionality	Vascular network formation	=	=	↓

Effects observed for all dose rates

Little information for the highest dose rate (cells died?)

Cumulative dose: 2.3 Gy; 4 Gy; 6.9 Gy respectively

WEEK 10



Non-Cancer Effects: Main Achievements of DoReMi

Jean-René Jourdain

DoReMi WP7 Leader

IRSN, France

ICRR, DoReMi Session

May 27th, 2015 – Kyoto, Japan