This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 662287.

EJP-CONCERT

European Joint Programme for the Integration of Radiation Protection Research

H2020 – 662287

D9.53 - Progress summary and actions - year 2 (Report)

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Reviewer(s): CONCERT coordination team

Work package / Task	WP 9	T 9.5
Deliverable nature:	Report	
Dissemination level: (Confidentiality)	Public	
Contractual delivery date:	M24 (CONCERT M43)	
M24 (CONCERT M43)	M24 (CONCERT M43)	
Version:	1	
Total number of pages:	6	
Keywords:	LDLensRad, Ionising Radiation; Lens; Cataract; Mouse Models	
Approved by the coordinator:	CONCERT M43	
Submitted to EC by the coordinator:	CONCERT M43	

Abstract

Studies of human populations (for example, Atomic Bomb survivors, clinical staff, pilots and victims of the Chernobyl accident) have led to the conclusion that the lens of the eye is more sensitive to ionising radiation exposure than previously thought. Revised and substantially reduced dose limits were put into effect in Europe in early 2018. However, it is still very unclear how lower dose ionising radiation might cause or be involved in the development of cataracts. This is an important current public health issue, particularly for medical radiation workers, many of whom will need to amend their working practices despite a clear lack of understanding of the effects of chronic, lower dose, ionising radiation exposure on the lens.

The EU CONCERT funded 'LDLensRad' project aims to bring together experts from across Europe to answer a number of key research questions on the topic of radiation exposure of the lens, including the mechanisms of how low-medium dose radiation causes cataracts and how genetic background and age influences cataract development after radiation exposure. Outcomes are anticipated to include information regarding the shape of the dose response curve and thus the risk of radiation cataract at low doses (relevant for EU radiation workers), thereby strengthening the evidence base for informed radiation protection.

Following a detailed programme of experimental set up and optimisation during year 1 of the project, data gathering continues in earnest. All experiments are proceeding as planned, and, indeed, some promising initial data are starting to emerge – with several presentations at international scientific conferences taking place during this period. The project teams are now beginning to consider the first publications. Importantly, the work planned to take place during year 3 will consolidate findings across the range of different experimental endpoints and, we predict, will enable concrete conclusions to be drawn.

This deliverable summarises LDLensRad progress during the last 12 months, year 2 (of 3) of the project. Overall, the LDLensRad project is progressing very well to date, and integration of the partners within the collaboration has been highly successful. It is anticipated that the proposed work plan will be completed as per the original schedule of milestones and deliverables, with no significant problems or delays.

Progress summary

LDLensRad: Towards a full mechanistic understanding of low dose radiation induced cataracts, is a multidisciplinary research project focused on a number of key research questions regarding the mechanisms of low dose radiation cataract induction.

Following a detailed programme of experimental optimisation and set up during 2017, in 2018 the partners have made excellent progress in data generation and collection. In terms of long-term mouse models, at HMGU and ENEA, irradiations and imaging are progressing according to plan, with additional groups of mice added after irradiation at 2 days after birth in order to investigate early susceptibility. At PHE, irradiations and imaging is all on schedule but problems with acquisition of 129Sv mice have meant that the 0.5 Gy and 1 Gy dose endpoints will be delayed, with efforts now being concentrated on the 0 and 2 Gy endpoints for both dose rates, for all experiments.

With minimal data collected to date, background levels of lens density assessed by Scheimpflug imaging are remarkably consistent across the different strains and labs, within the expected limits, due to genetic variation. No significant radiation induced differences in lens density have been observed for the HMGU Ercc2+/- or wildtype (WT) groups, the PHE C57BL/6J groups, or the ENEA adult Ptch1+/- on CD1 and C57BL/6J background. However, the CD1 background mice show significantly higher basal mean lens opacity compared with C57BL/6J mice. After neonatal irradiation Ptch1 mutants on CD1 background and their WT counterpart did show increased lens densities compared to unirradiated groups, with a potential dose rate effect also suggested. Overall, the albeit very early results suggest a relatively large effect of genetic background, but many questions remain regarding damage repair pathways and genetic control of these.

Initial data on the histological analyses and additional retinal investigations have been performed at HMGU. No cataracts have been detected in the 4 and 12-month mice, but some interesting features have been observed, including large cells at the posterior suture, fibre cells with nuclei at both the anterior and posterior part of the lens, and epithelial-like cells at the posterior. Retinal measurements by optical coherence tomography also indicate retinal thickening in some mice, but further work is needed.

Initial data on DNA damage responses assessed by 53BP1 has also been collated, including some very interesting initial observations on the difference between the lower (0.063 Gy/min) and higher (0.3 Gy/min) dose rate exposures – with the dose rate effect seemingly dominating the dose effect in some circumstances. Effects observed in the C57BL/6J mice from PHE have also thus far been mirrored in the Ptch1+/- samples received thus far from ENEA, but they are not seen in C57BL/6J lymphocytes; however, a lot of further work (including in vitro experiments) is required to identify whether this effect is real and then what the mechanism might be. In terms of the effects of radiation on lens density in vivo, initial data following whole mounting and 3D optical imaging, suggest increased densities around the germinative zone, at very short time periods after 0.25 Gy X-rays. The mechanism is as yet unclear and further experiments are also underway.

Initial next generation sequencing on ENEA samples has also been carried out. Large strain differences have been observed here too, together with some initial indications of radiation differences, but with several genes/miRNAs involved and no obvious pathways identified as yet. Validation of the early gene expression results with quantitative polymerase chain reaction and pathway analysis is ongoing, with more targeted analyses intended going forward.

In terms of in vitro experiments to support the mouse model programme, proteo- and lipidomic analysis is progressing incredibly well, with identification of differential oxysterol responses to radiation doses as low as 100 mGy in vivo. Work is ongoing to investigate the hypothesis that ionizing radiation causes oxidative stress in the lens, leading to cholesterol oxidation into various oxysterols which might gradually lead to cataractogenesis. Oxysterols identified in the lens are those implicated in the literature in a number of other health effects, suggesting that the results may have wider impact. The potential use of mitigators (tocopherol and ascorbate) is also being actively explored. Work on this assay will shortly begin in lens samples taken from Mayak PA workers, in partnership with SUBI. The Ki-67 protocol for detection of cell proliferation has also now been successfully optimised.

In vitro validation and additional experimentation on a number of extra endpoints has now started at ENEA (intracellular communication endpoints) but the bulk of the remaining work will now be completed by OBU in partnership with PHE.

Considering whether lens changes might be used as biomarkers of global radiation sensitivity, LDLensRad will build heavily on the INSTRA project behavioural testing based on the hypothesis that radiation affects adult neurogenesis and causes neuro-inflammation, demonstrating significant changes in mouse behaviour at 12 months following 0.5 Gy irradiation, a dose effect over time, and late genotype-dose interaction. For LDLensRad, data have only been collected up to 4 months, but already there is evidence of dose and dose rate effects with 2 Gy having a significant impact, and further work needed to clarify the observations to date. Brain analyses at ENEA have thus far focused on the susceptibility of the Ptch1+/- mice to development of medulloblastoma (amongst other effects) following radiation exposure. To date no dose or dose rate effect has been observed, but the genetic effect is large. Collection of brains for histology, immunohistochemistry and molecular analysis is ongoing.

In terms of statistical analysis, provision of accurate uncertainties associated with individual experiments is the responsibility of the labs/individuals carrying out those experiments. Within the next 3 months, work will start on the 'systems' style models promised in the original application. To a large extent the shape of the model(s) depends very much on what the data look like, and it is too early to tell in most cases, but initial model development will be with 'MANOVA' style, multivariate statistical approaches followed by pathway-based and network-based analysis methods.

Communication within the project has been excellent: project partners have met a number of times, including at the project wide progress meeting (Munich, June 2018). In addition, regular email contact is maintained between the partners carrying out the day-to-day laboratory work and between all project partners and Advisory Board members through regular updates from the PI.

In terms of data sharing within and external to the project partners, the project has an active ResearchGate site (https://www.researchgate.net/project/LDLensRad-the-European-CONCERT-project-starting-in-2017-Towards-a-full-mechanistic-understanding-of-low-dose-radiation-induced-cataracts) which has 58 followers and has been viewed over 1000 times. LDLensRad partners have also been actively engaging with representatives of STORE to develop a plan of action to use this facility to share data in an open manner in the community. An initial meeting took place in January 2018, followed by wider discussion at the AM 2018, and the formal data management policy is now being finalised.

In terms of wider stakeholder engagement, during this period, the LDLensRad 'Medical Professional, Public and Patient' involvement event took place at PHE on the 24th May 2018. Invitees from each of these important stakeholder groups listened to presentations on the LDLensRad project and associated

work programmes at PHE, then were given the opportunity to comment. All attendees supported the project as important research to support public, patient and medical professional health protection and a good use of tax-payers money, and were interested to find out more, so a future event will be organised (e.g. in partnership with the Royal Society of Medicine), with contributions from additional partners.

Education and training during the project has focused so far on ‚on the job training' for early career and 'in house' training for PhD students (a total of 5 PhD students), in addition to provision of as many opportunities as possible for early career scientists to contribute to scientific and public dissemination, as detailed below. In the last 12 months, partners have presented at over 15 national or international project meetings, workshops or conferences during the last period, including at the ERPW 2018 (Rovinj, October 2017), the CNSC Webinar on Lens Effects (April 2018) and the EPRBiodose 2018 meeting (June 2018). Increased representation at international scientific meetings including a Special Interest Symposium at the European Association for Vision and Eye Research (EVER) 2019 meeting; https://www.ever.be/) and other wider events is anticipated as the project progresses.

Administratively, OBU and DU have now joined the project as formal LTP, and work to ensure the full integration of these partners is progressing well.

The project Advisory Board members have been hugely important to the successful progression of the project to date, with more detailed consideration of their contribution given in D9.56.

Overall, the LDLensRad project is progressing very well to date, and integration of the partners within the collaboration has been highly successful. It is anticipated that the proposed work plan will be completed as per the original schedule of milestones and deliverables, with no problems or delays.