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D 9.52 - Progress summary and actions - year 1 (Report)

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Abstract

The lens of the human eye is known to be more radiosensitive than previously thought but, despite a substantial reduction in occupational dose limits based on recent epidemiological information and reanalyses, the mechanisms of low dose radiation cataract induction are still unclear. This is an important current public health issue, for instance for medical radiation workers, many of whom will need to amend their working practices despite a clear understanding of the effects of chronic, low dose, ionising radiation exposure.

The LDLensRad project aims to bring together experts from across Europe to answer a number of key research questions on this topic, including: how does low dose radiation cause cataracts; is there a dose rate effect, and how does genetic background influence cataract development after radiation exposure. CONCERT Deliverable 9.51, 5.1.1 of the project, describes the detailed work plan and timing for irradiations of mice for long term and short term models of cataract initiation and development.

The experiments are currently being carried out in 6 different mouse strains, as described in the Gantt charts in Deliverable 9.51, to test the impact of genetic background and further inform the mechanistic understanding. Mice will be exposed to doses of 0 to 2 Gy at an acute, high dose rate (0.3 Gy/min) or at a more protracted, low dose rate (0.063 Gy/min), to assess the effect of dose protraction on the dose response for radiation cataractogenesis. Mice are being irradiated at 10 weeks (when they have fully developed lenses) at PHE and HMGU. At ENEA, mice are being irradiated at neonatal age (postnatal day 2), the age of peak susceptibility to radiation lens injury, and at 10 weeks, specifically in order to investigate the ageing effect in this particularly age-sensitive strain. The mice are then being followed for up to 18 mth post exposure, with Scheimpflug imaging taking place at 1 mth intervals to track the appearance and development of cataracts. Behavioural testing will be carried out concurrently and, at the end of the long term study, the lenses of surviving mice will be analysed for histological and morphological changes and the results compared with wider existing and newly collected data on wider systemic effects, to test the hypothesis that lens effects can be used as an indicator of global radiation effects.

In addition, for each strain, dose and dose rate, lenses extracted from groups of mice will be assessed for: initial DNA damage at 4 and 24 hrs following exposure; intracellular communication, cell cycle effects, biochemical analyses and genetic pathway analyses at 4 and 24 hrs, 4 and 12 mth; proliferative and morphological effects at 24 hrs, 4 and 12 mth; miRNA content using Next Generation Sequencing (NGS) at 4 hrs and qRT-PCR at 24 hrs, 4 and 12 mth, with appropriate sham irradiated controls for all endpoints. The results and associated analysis of these studies will be made available as further Deliverables as the project progresses.

This Deliverable summarises LDLensRad progress during the last 12 months, year 1 (of 3) of the project. During the first year of the LDLensRad project, work has progressed well, with the original project milestones of the kick-off meeting (M1), mouse acquisition/breeding and genotyping (M2) and progress meeting (M3) all completed on schedule. In accordance with original plans, the first year has been focused on optimization and irradiations, so no data is presented in this report, rather a summary of the progress towards facilitating data collection in year 2 is presented.

Progress summary

The LDLensRad project, 'Towards a full mechanistic understanding of low dose radiation induced cataracts' is now approaching the end of the first full year of the project, out of a total of three years. During the first year, work has advanced incredibly well, and progress is in line with the original plans, with all deliverables and milestones expected to be completed on time. Year one has focused chiefly on optimization of experimental protocols and irradiation of mice in order to be ready to begin large scale data collection in year two.

Following initial work to complete the administrative requirements in January 2017, an initial 'Implementation meeting' was held in February 2017 in London. This meeting was attended by representatives from each partner centre (PHE, HMGU, ENEA, DU and OBU) and the original plans were discussed in detail, to ensure that each partner was clear as to their responsibilities regarding irradiation, sampling, distribution of samples, analysis, reporting of results, and contribution to milestones and deliverables. The project Gantt charts were reviewed and minor amendments agreed (see Deliverable 9.51). To aid day to day communication, it was agreed that a SharePoint site would be set up, and the partners would have responsibility for storage and analysis of their own data but that discussions with STORE representatives would take place to attempt to make best use of this data storage and sharing resource to support the project. The role of the Advisory Board (AB), initial plans for meetings throughout the project period, plans for stakeholder engagement and dissemination were also discussed.

At the formal Kick-off meeting, which took place in Oxford in May 2017, project partners presented detailed background relevant to, project plans and progress against each of the tasks under each WP detailed in the original proposal. Following discussion with the partners and AB members, minor changes were made to a number of tasks, with the largest change being that due to OBU's uncertain status as a project partner, HMGU would take responsibility for the OBU tasks associated with the in vivo samples. In addition, it was decided to carry out validation testing with the NGS at ENEA, to decide whether to apply this at 4 hours (as planned) or at 24 hours, as the later time point might yield more relevant information. Testing following the meeting confirmed that 24 hours was more appropriate, so the experimental plans were amended accordingly. Calibration and dosimetric issues were also discussed in detail. In addition, each of the above points were revisited and formal plans for day to day project management and data sharing were elaborated. Following the meeting, protocols for irradiation, sampling and dissemination of samples to the project partners were standardized and shared between the partners on the SharePoint site. UNIPV was also welcomed as an informal project partner, to assist with analysis of NGS data undertaken at ENEA.

In June 2017, the project lead from PHE, Dr Liz Ainsbury, presented an outline of the LDLensRad project to the UK Association for Radiation Research Annual Meeting. The presentation was well received.

In September, Dr Ainsbury attended the first CONCERT Stakeholder meeting, to present plans for the LDLensRad project and receive comments and suggestions from the chiefly academic and industrial stakeholders present at the meeting. Shortly after this, in October, a progress meeting took place at the ICRP-European Radiological Protection Research Week (ERPW) 2017 meeting in Paris. At this meeting, each partner presented progress to date. The planned irradiations (at PHE, HMGU and ENEA) and sampling from the irradiated mice are progressing according to the original schedules. HMGU and ENEA have started disseminating samples to the other partners for analysis, and it was agreed that samples from all centres would be distributed in bulk every 2-3 months. No problems or delays are expected for Work Package 1 – mouse irradiation, sampling and analysis. Optimization of analysis

protocols has also taken place, including for DNA damage responses, immunohistochemistry, proteomics, lipidomics and genomic analyses, with data from in vitro samples (Work Package 2) beginning to be collated. Lipidomic results and pathway analysis in particular have highlighted some very promising lines of further inquiry. Discussion took place regarding how to classify cataracts in the most appropriate manner according to the Scheimpflug (quantitative lens opacity data). Further discussion of each of the above points is expected at the next Annual Meeting, planned to take place in Munich from the 5th – 7th June 2018.AB member Dr Tamara Azizova from SUBI was also present at the ERPW meeting, and so it was possible to discuss further the potential for project partners to make use of human lens samples taken from the former Mayak Production Association Workers as part of a separate SUBI project. Only small amounts of material will be available to start with, so it was agreed that these will go to DU to complement potentially very exciting initial observations regarding the proteomic and lipidomic responses of the lens to ionizing radiation.

In addition, a formal response to the written AB comments following the Kick-off meeting was composed and accepted, as was an initial publication plan. Finally, plans for an initially UK based Medical, Patient and Public stakeholder group, to complement the CONCERT stakeholder group, were also elaborated on at ERPW. PHE colleagues have made initial contact with a number of potential contributors including those in the ophthalmological community, and a workshop is expected to take place during the first half of 2018. ERPW also afforded the project partners the opportunity to present the project to the wider radiation protection community, in a session dedicated to lens effects. The wider context of the project – that radiation protection of the lens should be underpinned by the best possible science – remains key, and project partners have also since discussed the need to ensure that the wider community is engaged going forward. This will be aided by the appointment of a 'data wrangler' at each partner centre, to take responsibility for ensuring that all data are collated and deposited into the STORE facility – to facilitate both within project analysis and also data sharing to the wider community. STORE training for the data wranglers is planned in early 2018.

In conclusion, the LDLensRad project has progressed very well during the first year – with optimization of protocols for irradiation, sampling, distribution of samples, analysis and sharing of data complete. Indeed, the irradiations of mice are now well underway, and some initial results are also starting to be collected for the in vitro experiments investigating the potential mechanistic chain of events from irradiation through to appearance of a cataract. It looks likely that at least some of the results will be able to be validated in human lens samples. Communication between the project partners has been excellent, and the AB has been incredibly well engaged. Plans for stakeholder engagement are maturing well, and plans for publication and other means for dissemination of data are also in development. The partners are looking forward to sharing progress with the AB at the next annual meeting, planned to take place in Munich on the 5th – 7th June 2018 and to sharing initial data with the radiation emergency dosimetry community at the EPRBioDose meeting in June 2018 and the wider community at later meetings in 2018 and 2019.