**FLASH radiation effects on ocular tissues***Beatrice Di Marco, Giulia Salamone, Damiano Del Sarto, Elisa De Santis, Raffaele Mazziotti, Gabriele Sansevero, Beatrice D’Orsi, Fabio Di Martino, Simone Capaccioli, Fabiola Paiar, Mario Costa, Enrica Strettoi.*

Uveal melanoma is the most common primary intraocular malignancy in adults. Eye enucleation has long been the gold standard for treatment but adverse effects have shifted recommendations toward eye preservation and radiotherapy. This can also lead to complications like radiation retinopathy, retinal detachment and optic neuropathy. To overcome the limitations of conventional radiotherapy (CONVRT), flash radiotherapy (FLASHRT) might represent a promising alternative. This novel technology involves the ultrafast delivery of radiation at dose rates much higher than those used in CONVRT (40 Gy/s versus 0.5–5 Gy/min, respectively), causing less damage to healthy tissues but achieving similar disease control (the so-called flash effect).
In this study, we employed a dedicated Linear Accelerator (Linac) with a triode gun, enabling in vitro and in vivo studies and able to switch rapidly between ultra-high and conventional modalities under controlled conditions. We compared the effects of FLASH and conventional radiations in healthy ARPE-19 cells, modeling the human retinal pigment epithelium (RPE), and in the retina and RPE of living, healthy mice, using various radiation protocols. Our long-term aim is to develop a protocol for ocular melanoma treatment. We demonstrated the successful occurrence of a flash effect on ARPE-19 cells under specific doses and discovered novel effects of the radiations with the protocols used. Stemming from these data, we initiated an in vivo toxicity study on whole-brain irradiated mice using 20 Gy and 15 Gy with either conventional or flash methods, performing chronic and acute observations. Notably, we observed no significant differences in the morphology of the RPE from FLASH versus CONV mice in both acute and chronic groups. This may be due to a dose exceeding the range necessary to generate a flash effect; the outcomes of lower doses are presently under investigation. A morphological analysis of the retinal tissues labelled with antibodies against Iba1, a protein specifically expressed by microglia and macrophages, demonstrated a lower inflammatory response in the retina of animals treated with Flash compared to mice irradiated using conventional methods. Molecular analyses to confirm these data are currently in progress.