AbstractID: 3955 Title: In vitro model to study the biological effect of dose gradients on a cell culture system

Purpose: Coupled with improved accuracy of radiation therapy delivery, margins around tumour volumes tend to be much tighter. Radiobiological assessment of the changes in cell death at boundaries between the high and low dose regions would improve our knowledge on what is an adequate treatment margin. We have developed a new method of cellular analysis to assess the spatial response of cells across a gradient radiation dose.
Method and Materials: A549 non-small cell lung cancer cell line was used in a 6 well plate format. The cells were bedded down and maintained at a constant position in the well over the time frame of analysis. Wells 1 and 4 were the unirradiated control wells, wells 2 and 5 were half irradiated and wells 3 and 6 were fully irradiated. Different radiation doses were delivered to the cells in various phases of growth and cell density. The cells were left for various times after irradiation before analysis. The cells were washed with the protein specific Crystal Violet stain and then scanned at 570nM to obtain the protein levels in the well.
Results: Crystal Violet cell viability correlated with the MTT cell viability assay. An exponential decrease in cell viability was observed when increasing the dose up to 15Gy. The intensity of Crystal Violet stain across the half-irradiated wells showed a sigmoid shape at the interface with close correlation between the slope of the stain and the radiation dose.
Conclusion: This method provides valuable biological information on the effects of the radiation dose at the beam edge. The results indicate that there may be inter-cellular communication across the gradient dose interface. This may need to be considered when defining the tumour region.