Are DNA repair defects an indication for proton or heavy ion therapy?

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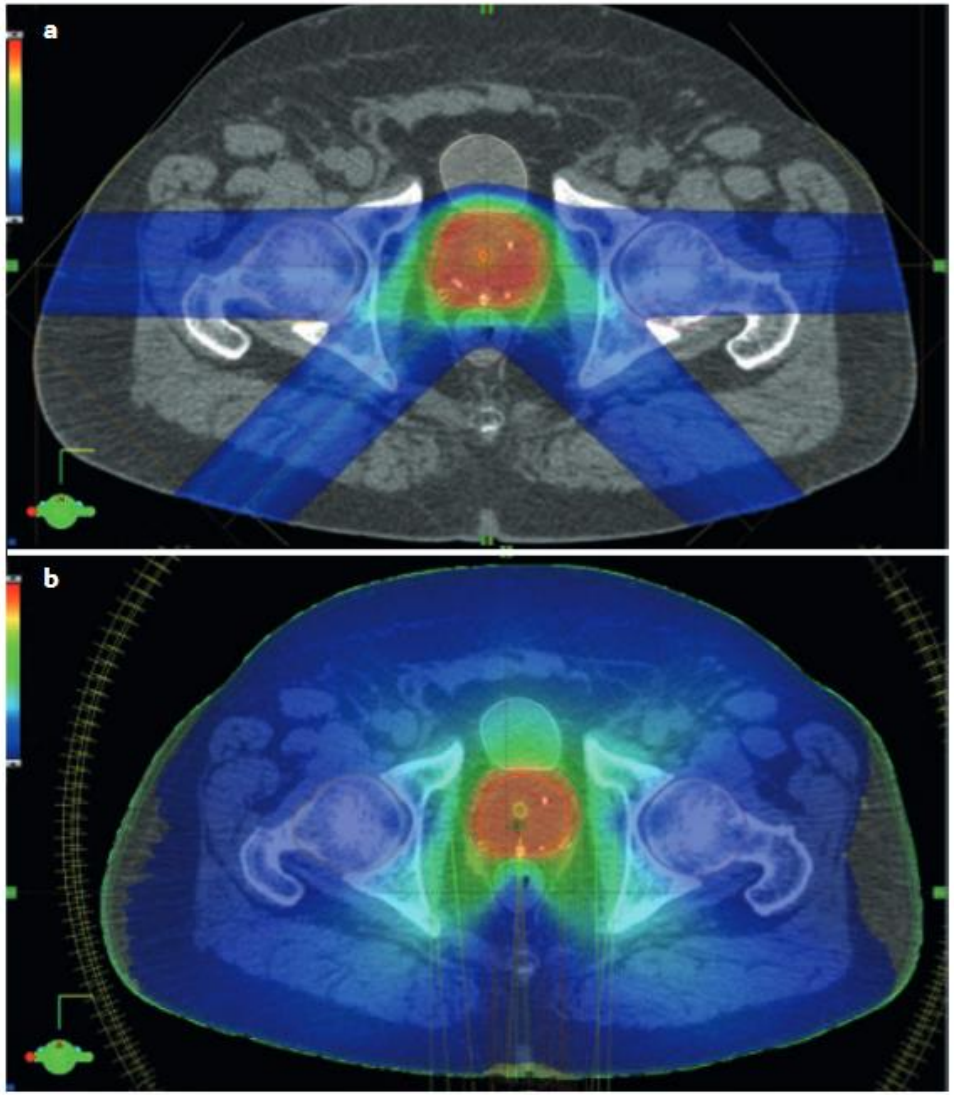
Background

Particle therapy has significant dosimetric benefits compared to X-ray therapy.

They also offer radiobiological advantages, due to their elevated Relative Biological Effectiveness (RBE).

Much work has focused on the physical dependence of RBE, but less so on underlying biology.



Comparison of 4-field proton (top) and VMAT photon (bottom) plans showing significant reduction in dose to normal tissues. From *Durante et al, Nat Rev Clin Oncol, 2017*

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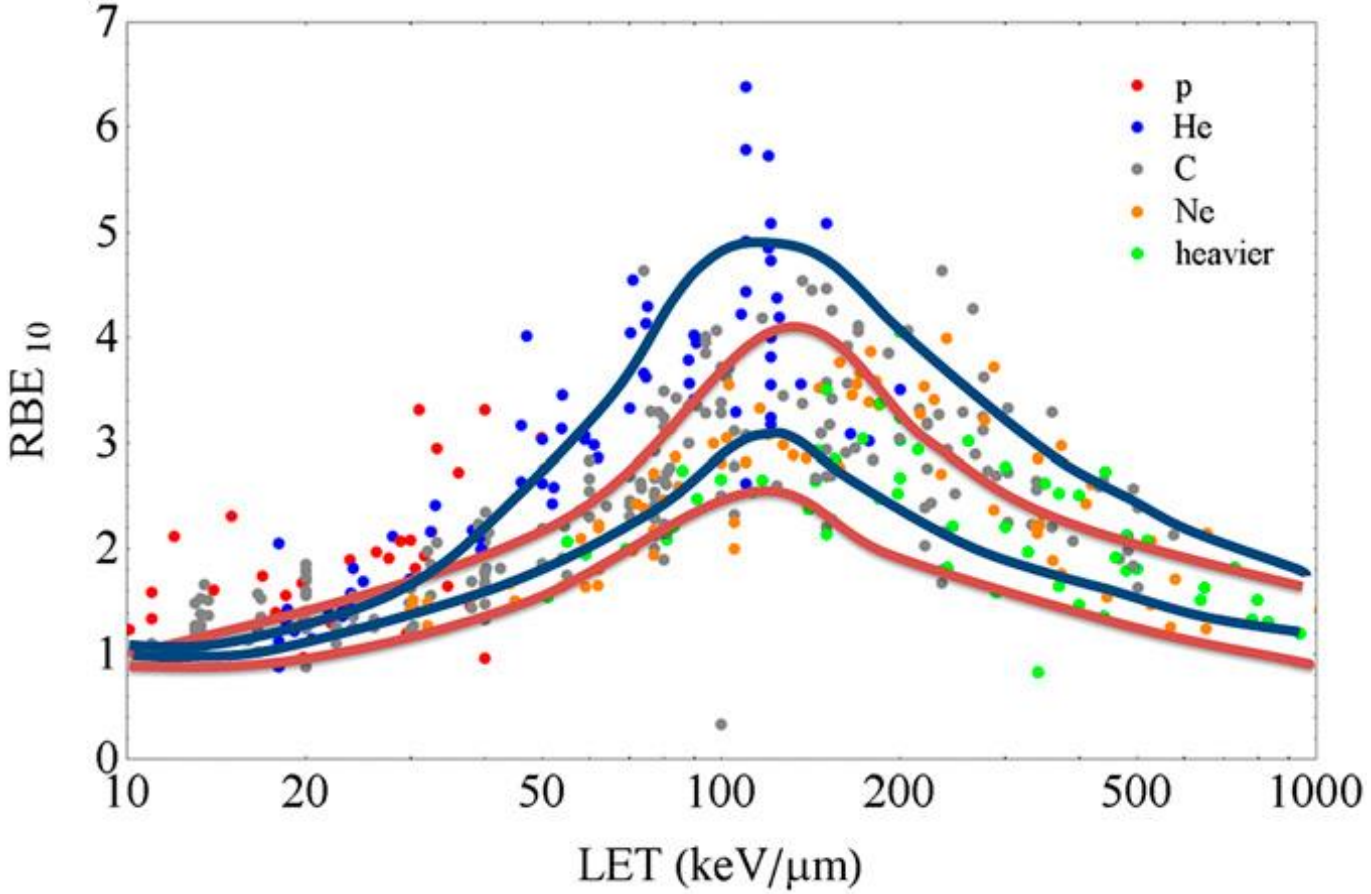

Exploration of RBE-LET relationship for different ions from the PIDE database. Common trend is clearly visible, but with significant heterogeneity. From *Durante, Br J Radiol, 87, 2014*

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Elevated LET and damage complexity

One suggestion for the increasing effect of high LET radiations is that their denser energy deposition leads to more complex damage – moving from simpler DSBs to complex DSBs and DSB clusters.

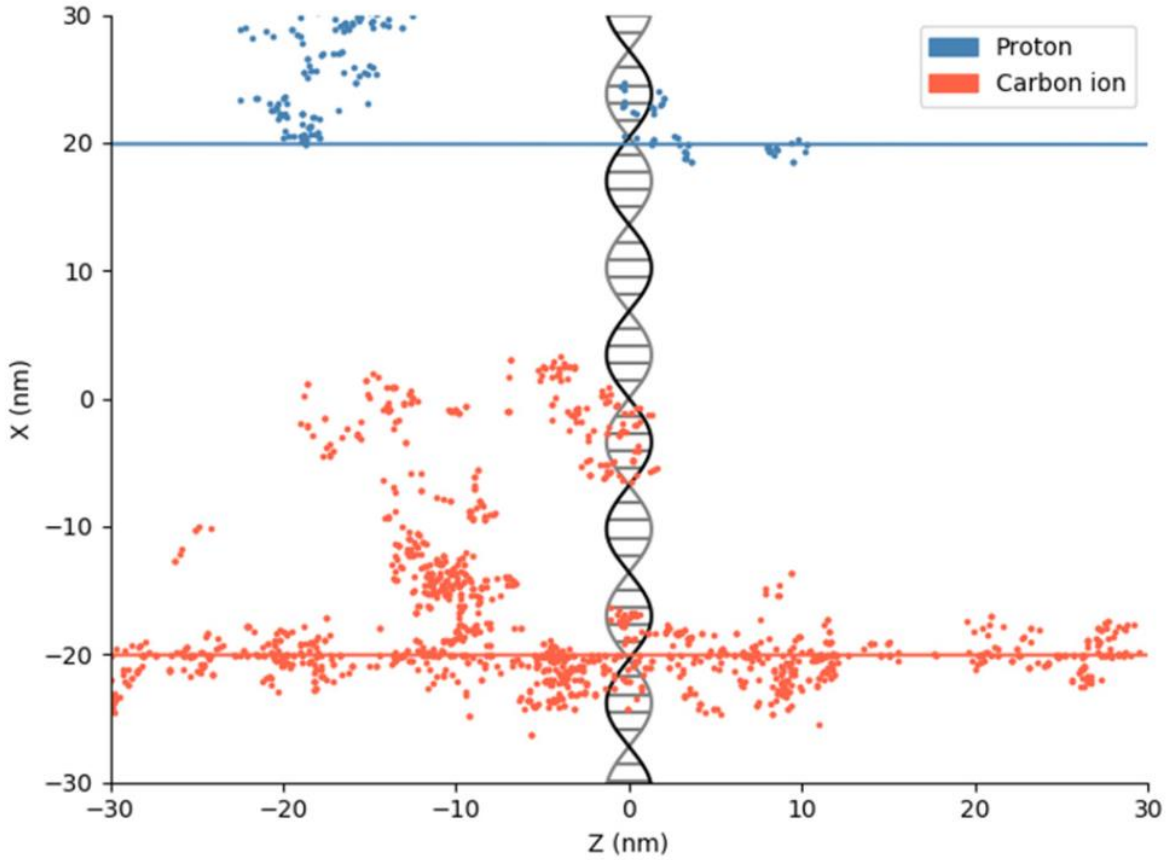

Illustration of ionisation patterns around a 10 MeV proton (top) and 200 MeV carbon ion (Bottom) track. Each point is an energy deposition event, with a DNA strand presented for scale. Events are much denser within the DNA. From: *McMahon & Prise, Cancers, 2019*

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Relationship with DNA repair

DNA Double Strand Breaks (DSBs) induced by ionising radiation are repaired through three main pathways.

Homologous Recombination (HR) is more accurate, but slower and only available in some cell cycle phases, while Nonhomologous End Joining (NHEJ) is faster and available through the cell cycle, but prone to small errors.

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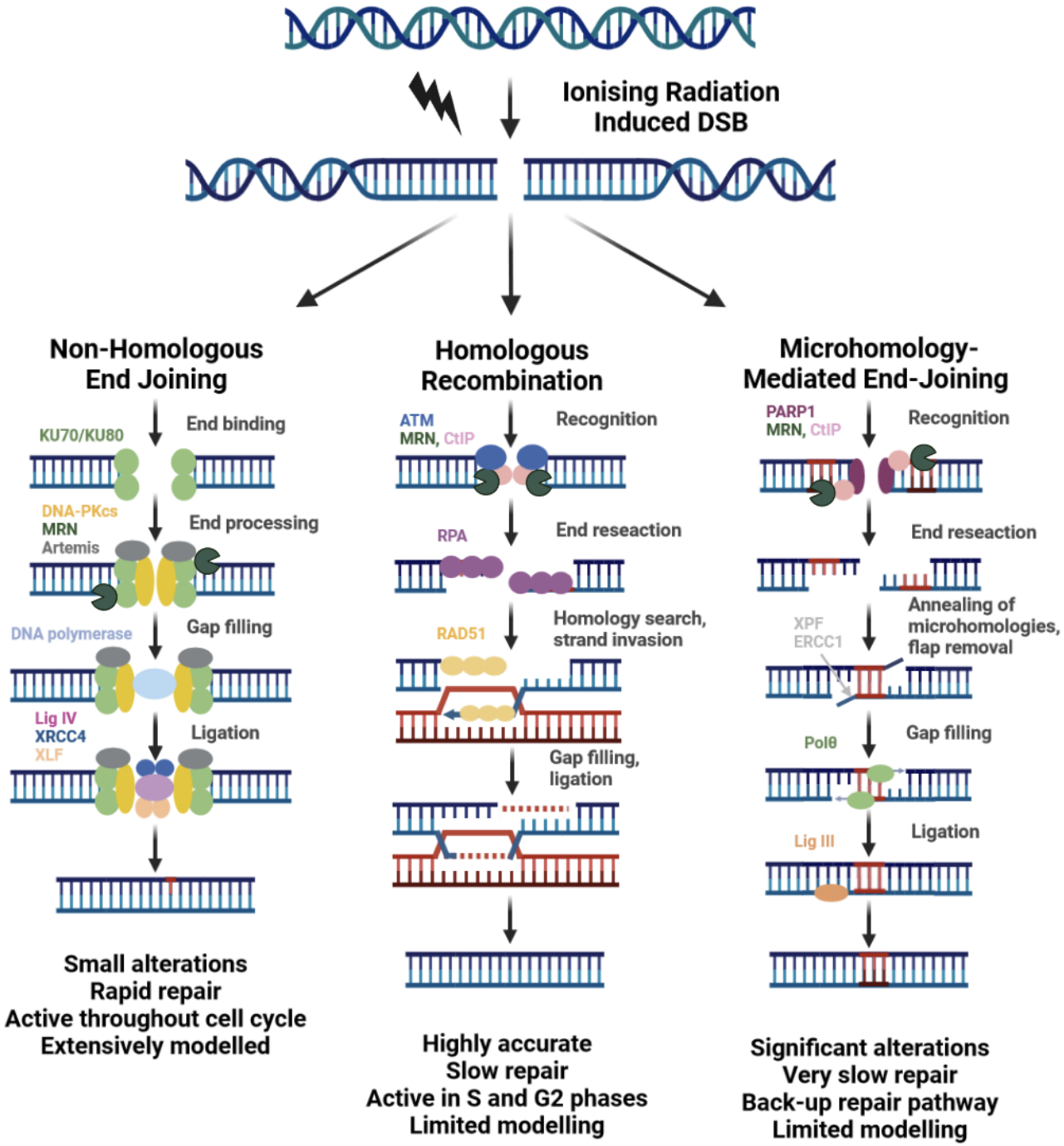

Relationship with DNA repair

Several publications have suggested that HR becomes more important as LET increases.

However, a number have also reported contradictory results, indicating little effect or even an apparent preference for NHEJ.

Highlights a need for more insight in this area.

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The question

How do DNA repair defects impact on the relative sensitivity to X-rays and particle therapy?

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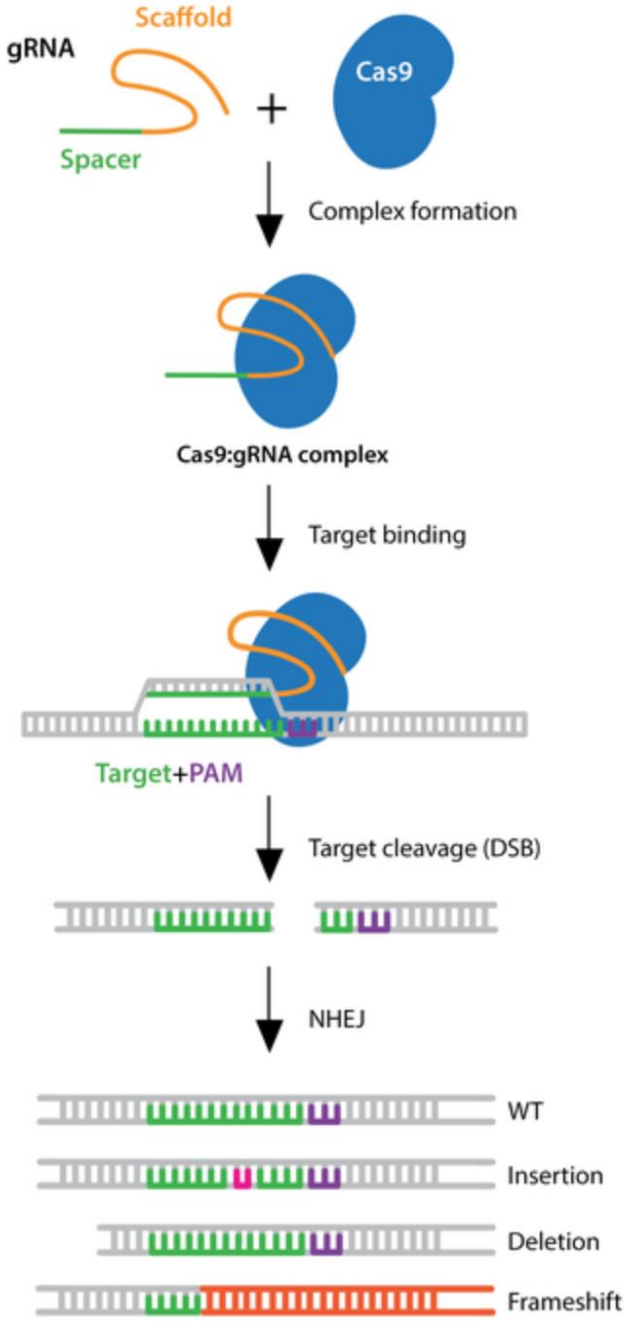
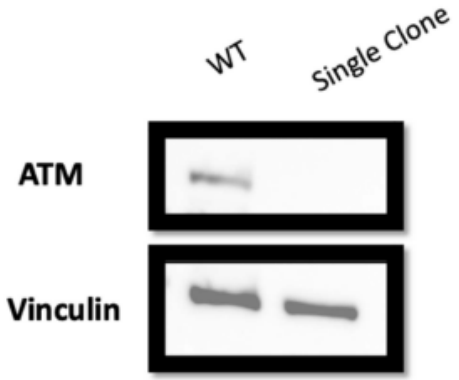
Cell model generation

We used CRISPR-Cas9 to perform targeted gene knockouts in genes associated with DNA repair in RPE-1 (Retinal Epithelium) cells.

Genes targeted were:

- TP53 (Checkpoint control);
- ATM (DNA Damage Sensor);
- PRKDC (NHEJ Pathway);
- LIG4 (NHEJ Pathway);
- DCLRE1C (NHEJ Pathway);
- BRCA1 (HR Pathway);





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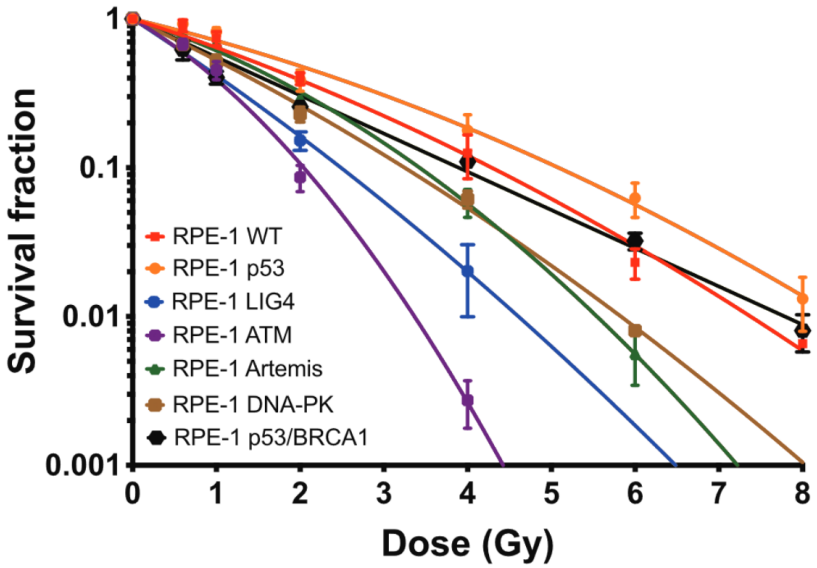
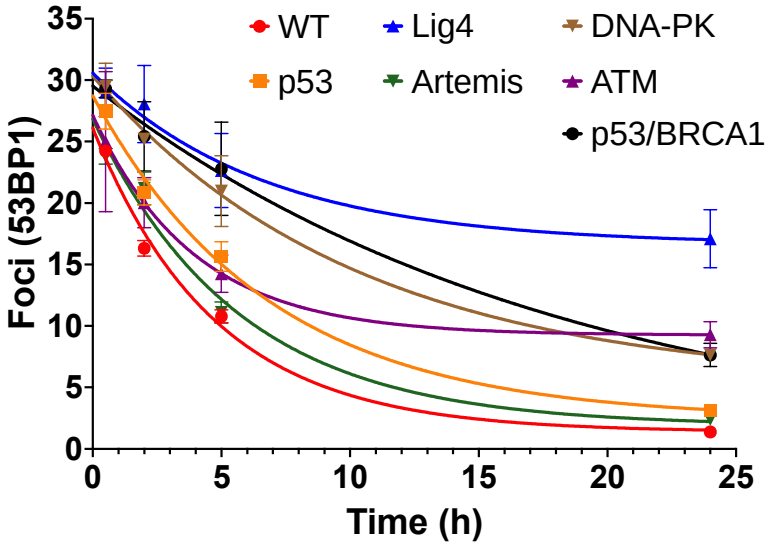
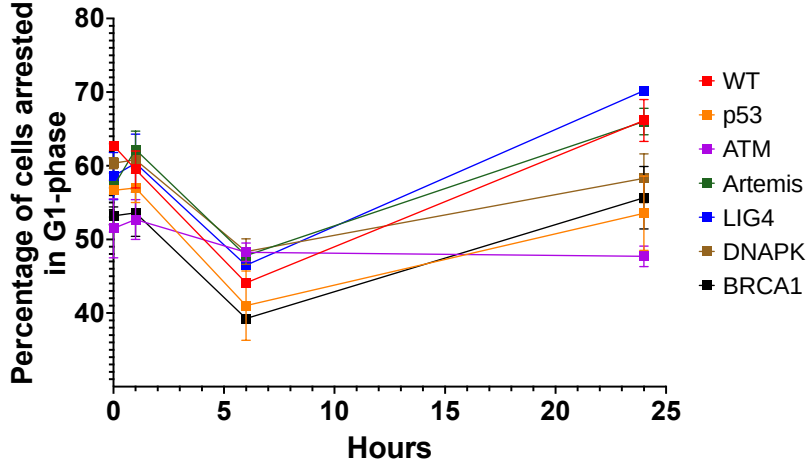
From: Liberal & McMahon, IJMS, 2023

Initial X-ray Characterisation







DNA repair defects significantly impact on X-ray radiation responses. **Left**: Clonogenic survival shows small protection from p53 knockout, and significant sensitisation from most DNA repair defects; **Middle**: DNA repair kinetics show similar impacts according to pathway knocked out; **Right**: Some differences are seen in cell cycle distribution, but substantial effects only observed for ATM-defective lines.

From: Liberal & McMahon, IJMS, 2023

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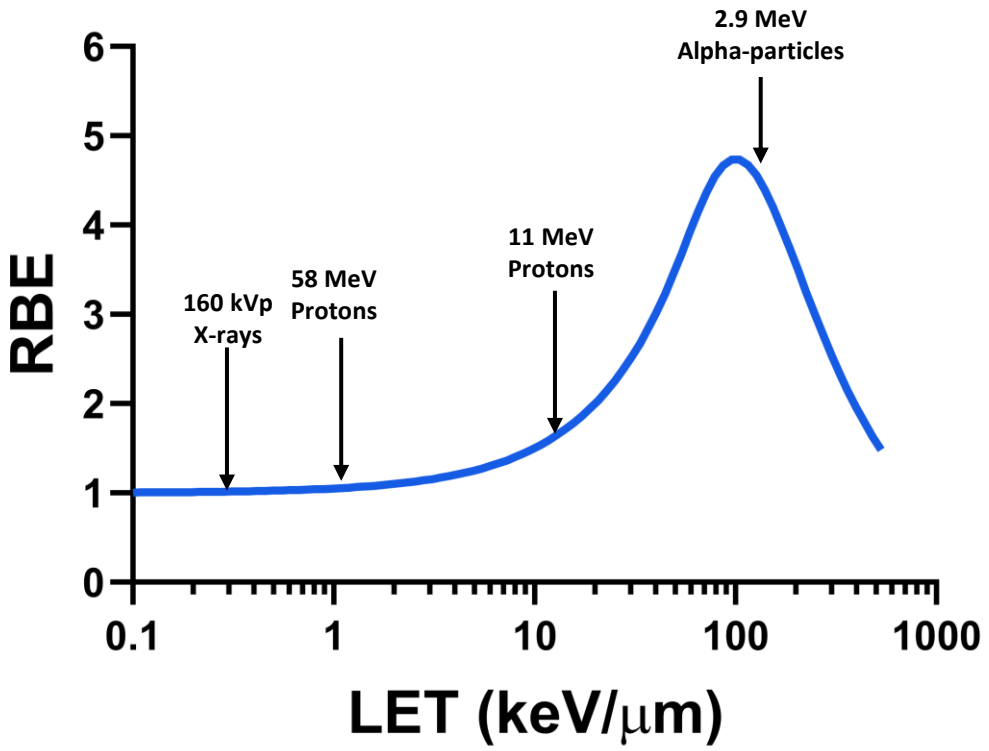
High-LET irradiations

We then mapped these effects as a function of LET, comparing:

- 160 kVp X-rays (~0.3 keV/µm effective);
- 58 MeV protons (~1 keV/µm);
- 11 MeV protons (~11 keV/µm);
- 2.9 MeV alpha particles (~129 keV/µm)



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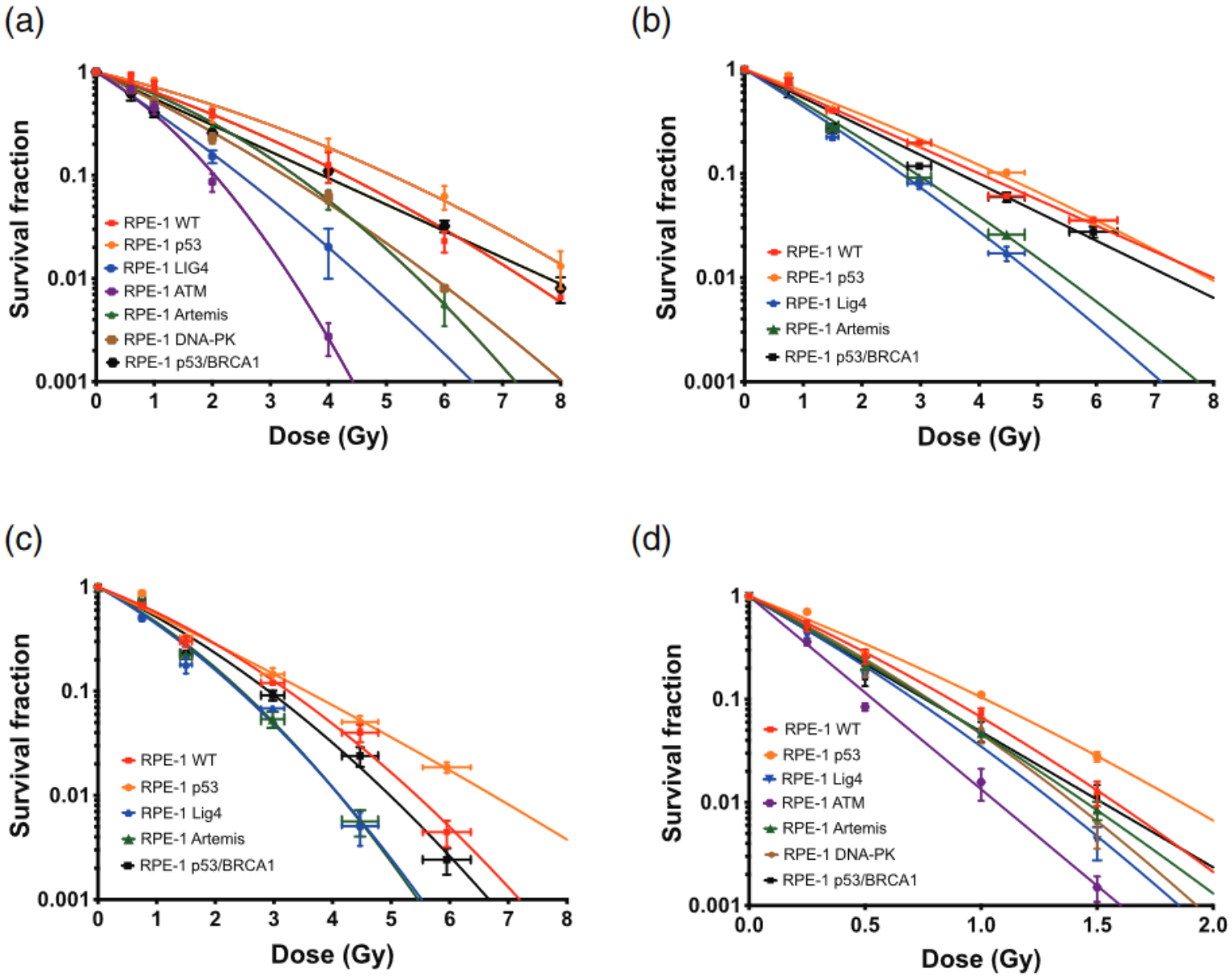
High-LET survival

We obtained clonogenic survival for X-rays (a), low and high LET protons (b, c) and alpha particles (d – note reduced X-axis scale).

As expected, sensitivity increased with increasing LET.

However, it can be seen qualitatively here that the ordering of cell lines did not change significantly between different radiation qualities.



Liberal et al, Medical Physics, 2023

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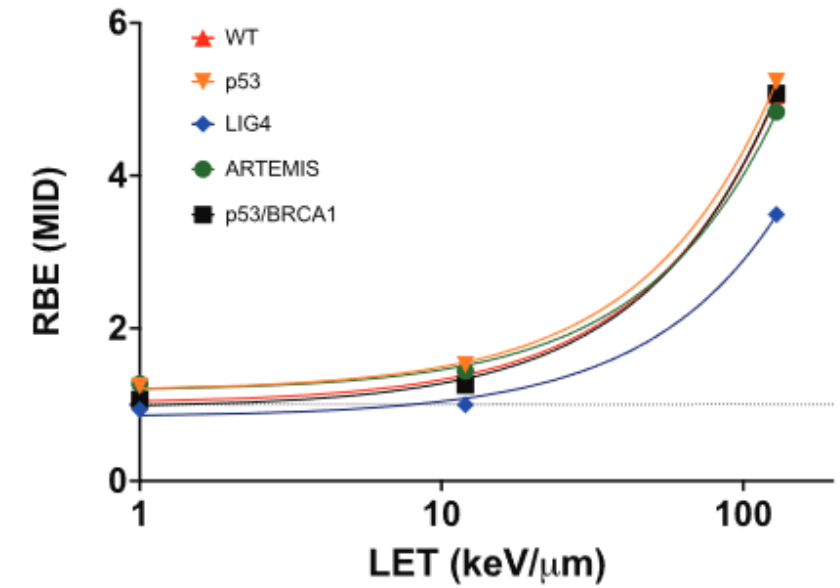
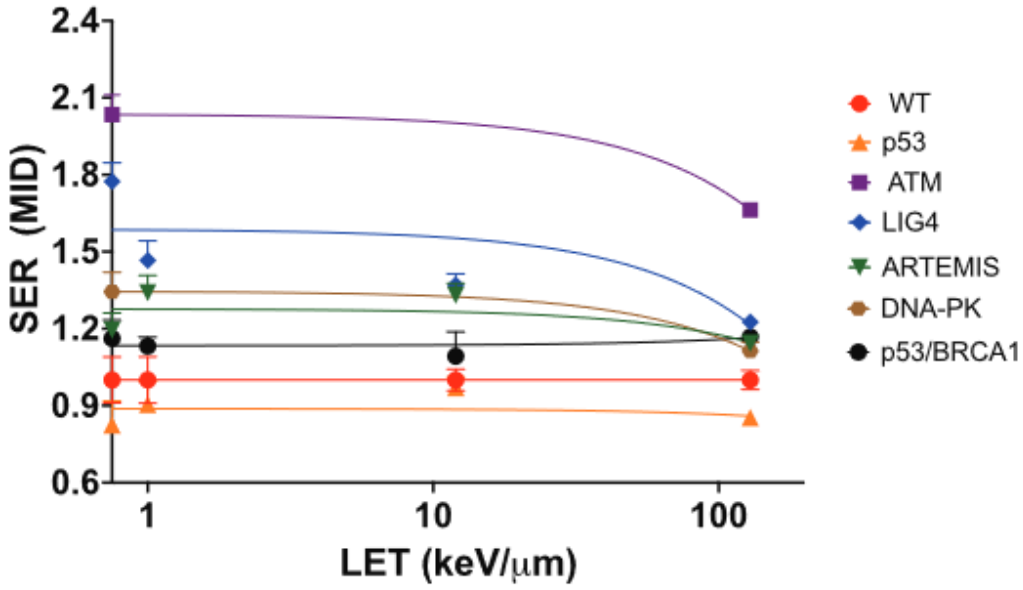
RBE and SER

We then calculated RBE and Sensitiser Enhancement Ratios (SER) for these lines.

RBE showed the expected increase with LET (top), with a reduced magnitude in LIG4 defective lines.

SER showed that all genes had a constant or decreasing impact on sensitivity with increasing LET, with no pathway showing elevated importance.





Liberal et al, Medical Physics, 2023

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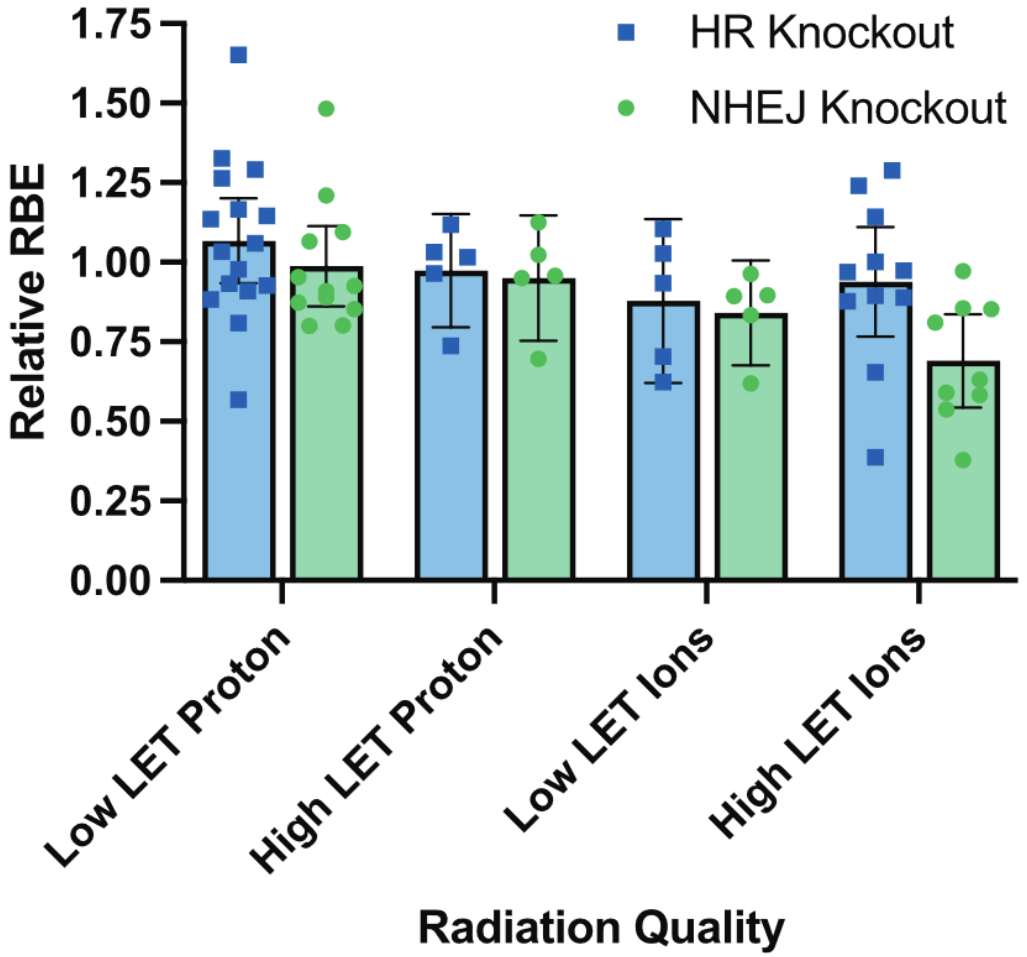
Comparison with literature

Although a relationship between DNA repair pathway and LET is often assumed, a survey suggests evidence is more equivocal.

Analysing all papers which has wild-type and DNA repair defective lines where Relative RBEs can be calculated for different radiation qualities, there is no evidence of a significant preference for HR at any LET. NHEJ defects reduce RBE at the highest LETs due to ‘overkill’ effects.



Liberal et al, Medical Physics, 2023

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Conclusions

- DNA repair capability significantly moderates overall cellular sensitivity
- The relative sensitivity of cell lines is approximately independent of LET, regardless of DNA repair pathway is defective
- NHEJ defects have a greater impact at all LETs, with no elevated importance of HR
- Suggests that, contrary to some reports, targeting HR-defective cancers may not be an effective way to allocate particle therapy, and that more resistant repair-competent cancers would see greater benefit.

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Liberal & McMahon, *Characterization of Intrinsic Radiation Sensitivity in a Diverse Panel of Normal, Cancerous and CRISPR-Modified Cell Lines*, Int J Mol Sci. 2023 May; 24(9): 7861.

Liberal, Parsons & McMahon, *Most DNA repair defects do not modify the relationship between relative biological effectiveness and linear energy transfer in CRISPR-edited cells, Med Phys. 2023 Sep 27.*