Appendix 1

Cell Cycle, and proliferation analysis

Cells were irradiated with single dose of 12 or 34 Gy, trypsinized 24 h post-radiation, fixed using 70% ethanol and kept at –20 ℃ until staining. For staining, cells were washed with 1× PBS and stained with a final concentration of 50 µg/mL propidium iodide and 100 µg/mL RNAse A and kept at 4 ℃ for at least 1 h before flow cytometry analysis. Cell cycle analysis was done at the immunophenotyping platform using BD FACSCanto II.

To assess cell proliferation, vybrant 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazoliumbromide (MTT) assay was performed. Cells were irradiated at 0, 12 or 34 Gy and MTT was added at 4, 24, 48, and 72 h post-irradiation. MTT was added to cells and incubated for 4 h, DMSO was added and absorbance was measured following incubation at 560 nm using a standard microplate reader (Thermo Scientific, Multiskan Spectrum).

Protein extraction and immunoblotting

Tissues were lysed in RIPA buffer (Cedarlane, Ontarion, Canada) supplemented with phosphatase (Sigma Aldrich, Ontario, Canada) and protease inhibitors (Sigma Aldrich, Ontario, Canada) using the Speed Mill Plus Homogenizer. Homogenates were centrifuged for 15 min at 1,000 rpm. The supernatant containing the protein lysates was collected and proteins were quantified using BCA protein quantification method. Equal amounts of protein were separated by SDS-PAGE under reducing conditions and blotted onto polyvinylidene difluoride membrane. Membranes are blocked with 5% non-fat milk or 5% bovine serum albumin (BSA) and probed with primary directed against DEL-*EGFR*, L858R-*EGFR*, total-*EGFR*, phospho-*EGFR*, total-*AKT*, phospho-*AKT*, total-*ERK* 1/2, phosphor-*ERK* 1/2 (Cell Signaling Technology, MA, USA), beta-actin (Sigma, Ontario, CA, USA) served as a loading control. All primary antibodies were used at a dilution of 1:1,000 in 5% (BSA) except for beta-actin which was used at a dilution of 1:250 in 5% non-fat milk.

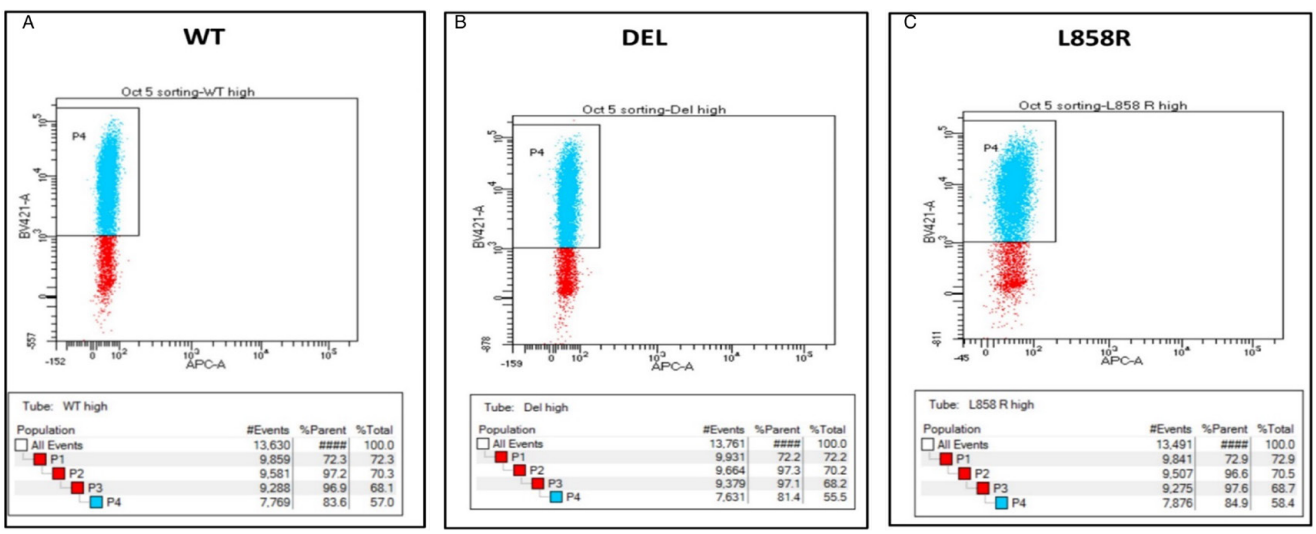

Figure S1 Cell sorting of BFP-Luciferase positive population of A549 transfected with either (A) WT-, (B) DEL-, or (C) L858R-*EGFR*.

https://dx.doi.org/10.21037/tlcr-2024-1034

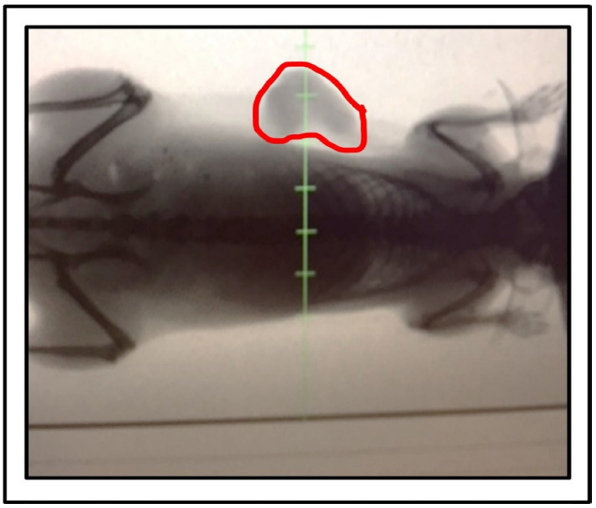

Figure S2 CT-scan image of tumor formation following subcutaneous injection of *EGFR*-mutant NSCLC into YFP-SCID mice. The red square is the subcutaneous tumour.

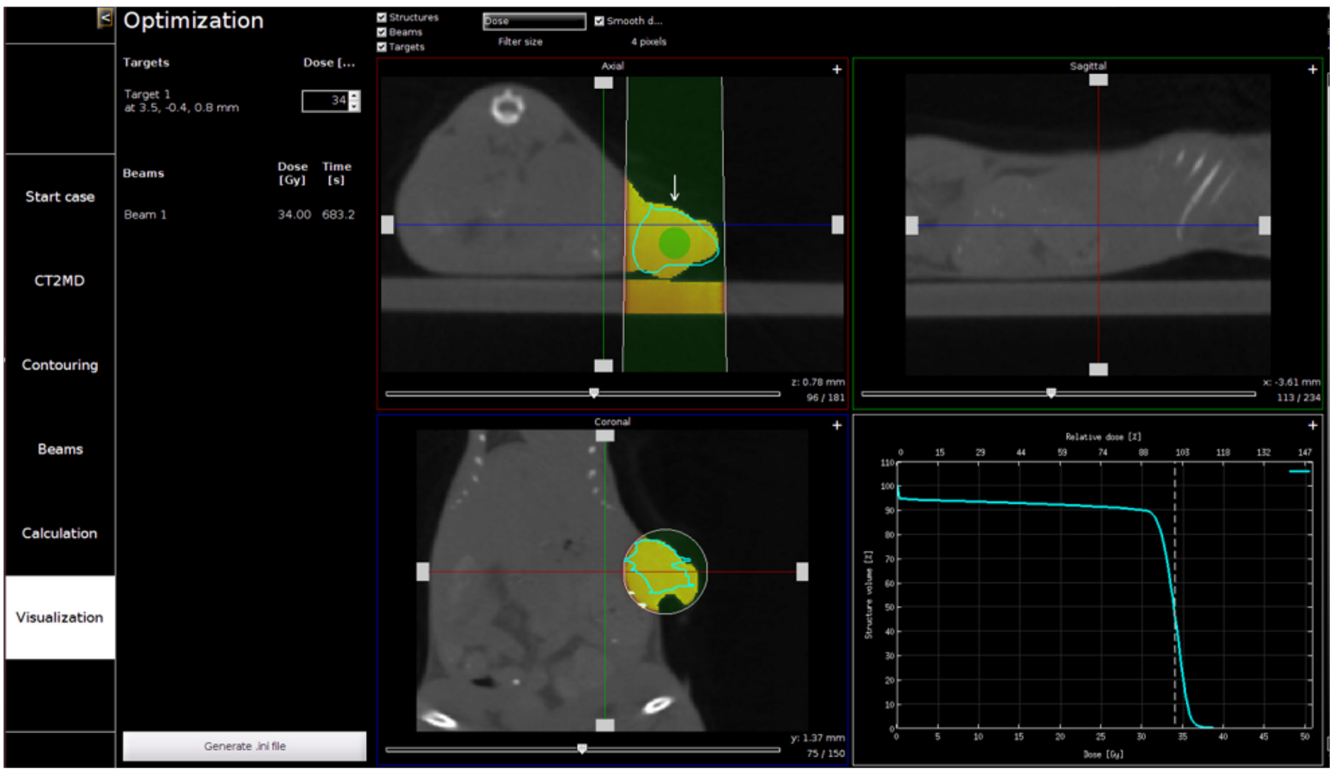

Figure S3 Treatment plan of animals treated with a single fraction of 34 Gy.

https://dx.doi.org/10.21037/tlcr-2024-1034

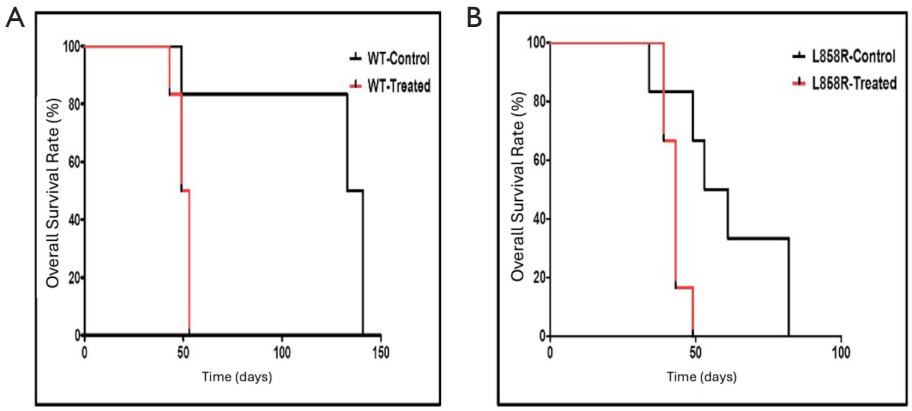

Figure S4 Overall survival data of control and SABR-treated YFP/SCID mice injected with (A) WT *EGFR* and (B) L858R NSCLC.

https://dx.doi.org/10.21037/tlcr-2024-1034