Combination treatment with radiotherapy and oncolytic reovirus generates CD8+ T cell infiltration in primary and abscopal tumours in an organoid model of basal-like breast cancer

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Introduction
Basal-like breast cancer (BLBC) is defined by its gene expression profile and usually does not express oestrogen, progesterone or HER2 receptors2, so has limited treatment options. Immune checkpoint inhibitors with atezolizumab has recently been approved for the treatment of advanced triple-negative breast cancer, however only a proportion of patients respond to treatment and novel treatments are needed to enhance immunotherapy response rates. Oncolytic reovirus has been shown to mediate innate and adaptive immune responses, immunologically prime for checkpoint blockade3, and enhance the cytotoxicity of radiotherapy4.

Methods
• In vitro: Tumour organoids were generated from a spontaneous mammary tumour arising in a BLC-Cre;Bra Intactp535–/– genetically engineered mouse. Organoids were incubated with medium containing reovirus (Dearing type 3, Oncolytics Biotech) and cell survival assessed at 4 days. Irradiation was performed using an AGD 250 kV X-ray machine and cell survival assessed at 10 days. Cell survival was determined by CellTiter-Fluor 3D Cell Viability Assay.

• In vivo: Organoids were orthotopically injected into the right 5th (105 cells; primary site) and left 4th (5x104 cells; abscopal site) mammary fat pads of C57BL/6 mice. Radiotherapy was delivered to the primary site using an Xstrahl Small Animal Radiotherapy Research Platform, under isocurative anaesthesia. Reovirus and vehicle (PBS) were administered intratumoural (IT) injection to the primary site, and α-PD-1 (clone RMP1-14, BioXcell), by intraperitoneal injection. For survival cohorts (n=8 per group), mice were cycled when either tumour reached 14mm in diameter. For IHC analysis (n=4-6 per group), 2 days after the final radiotherapy treatment, tumours were fixed for 24h in 10% neutral-buffered formalin Immunohistochemistry (IHC) was performed via standard techniques.

BLBC organoids are sensitive to reovirus and radiotherapy in vitro
Organoids generated from a tumour arising in a BLC-Cre;Bra Intactp535–/– genetically engineered mouse, implanted into C57BL/6 mice, develop tumours (A), which display a basal-like phenotype by IHC analysis (negative for oestrogen receptor (B), Her2 (C), and positive for CK14 (D)). In vitro, the BLBC organoids are sensitive to monotherapies with either reovirus (E) or radiotherapy (F), in a dose-dependent manner.

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• BLBC organoids were orthotopically injected bilaterally in the mammary fat pad of C57BL/6 mice. When the primary tumour reached 100-200 mm3, mice were randomised to treatment with either sham radiotherapy, IT reovirus (3 x 105 pfu), radiotherapy (3 x 8 Gy), or radiotherapy + reovirus (A).

• Single-agent treatment with either radiotherapy or reovirus significantly reduced the growth rate of primary (treated) tumours compared with controls, though there was no additive effect of combination treatment (B). There were no significant differences between treatment groups in abscopal tumour growth rates (C). P values represent comparison between treatment groups by two-way ANOVA calculated using TumGrowth4. Piecewise growth curve regression was applied with a breakpoint at the time of the first measurement after the start of treatment. All groups compared to all other groups and Holm-Bonferroni method applied for multiple comparisons (adjusted p values shown).

Radiotherapy and reovirus + reovirus treatment led to significantly longer survival than controls. Combination treatment generated longer survival than radiotherapy alone, though this difference was not statistically significant (D). Survival comparisons were by log-rank test with all groups compared to all other groups, and Bonferroni correction for multiple comparisons (adjusted alpha level: 0.0006).

In primary tumours, neither single treatment significantly changed the CD8+ T cell infiltration, however combination treatment generated a 15-fold increase in CD8+ cell infiltration compared with controls (47±3 vs 31 cells per mm2 of tumour; p = 0.02 [E, F]). In abscopal tumours, CD8+ T cell infiltration was increased by either reovirus alone, or combination treatment, though the effect was smaller than in primary tumours (G, H). Comparisons by one-way ANOVA with Tukey’s test for multiple comparisons (adjusted p values shown).

Conclusions
• In an organoid model of murine BLBC, combination treatment with reovirus and radiotherapy generated CD8+ T cells infiltrates in primary and abscopal tumours, suggesting a local and systemic immune response to treatment.
• Combination treatment with radiotherapy, reovirus and α-PD-1 significantly prolonged survival though did not generate cures or long-term tumour control.
• Investigations are ongoing to explore the mechanisms for these effects and identify immune pathways which may be targeted to further improve treatment responses.

References