COMBINATION IMMUNOTHERAPY USING A NOVEL CHIMERIC ONCOLYTIC VIRUS (ONCARLYTICS) TO REDIRECT CD19 BISPECIFIC T-CELL ENGAGERS TO TARGET SOLID TUMORS

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Introduction

Bispecific T-Cell engager (BiTE) monoclonal antibodies have emerged as a promising immunotherapy strategy for the treatment of hematological malignancies. Blinatumomab, an FDA approved BiTE carrying CD19 and CD3 scFv's has shown durable clinical responses for the treatment of B-Cell acute lymphoblastic leukemia (B-ALL) and non-Hodgkins lymphomas. Despite a wide array of research in hematological malignancies, BiTE therapies for the treatment of solid tumors have remained a significant challenge in demonstrating comparable efficacy. Solid tumors often lack amenable and targetable tumor antigens, and in many tumor types the tumor microenvironment (TME) is largely known to be immunologically "cold" and a barrier to immunotherapy responses.

Oncolytic viruses have recently gained traction in the field for the treatment of solid tumors because of their ability to target tumor-intrinsic properties and reshape the immunosuppressive TME. We have previously described the use of a chimeric oncolytic vaccinia virus (OV), CF33, for the treatment of a variety of tumor cell types, including triple-negative breast cancer, lung cancer, and liver cancer. Building on this, we generated an OV that expresses a non-signaling, truncated CD19 (CD19t) antigen called onCARlytics (CF33-CD19t), onto the surface of infected tumor cells prior to virus mediated tumor lysis, which redirected CD19-targeting chimeric antigen receptor (CAR) T Cell activity against solid tumors (Park et al. STM 2020). Using this OV, we have created a universal system that is agnostic to solid tumor type and can be provided with a targetable and well-characterized antigen. We now demonstrate that onCARlytics can redirect cytolytic functions of blinatumomab. We have demonstrated that tumors infected with onCARlytics in combination with blinatumomab show improved tumor cell killing, comparable to CD19-CAR T Cell. Using this approach, we show that a clinically-approved CD19-directed BiTE can be combined with onCARlytics to activate endogenous immune responses against solid tumors.

Figure 1

Delivering truncated CD19t (CD19t) to tumor cells using oncolytic virus (OV) as a target for bispecific T-Cell engagers (BiTEs)

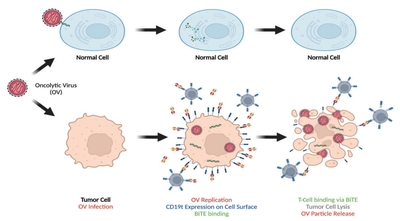


Figure 2

CD19t expression following onCARlytics infection leads to naïve T-Cell activation in combination with blinatumomab

A Quantification of T-Cells activation following in vitro co-culture (48h) of infected MDA-MB-468 triple negative breast cancer cells at varying MOIs of CF33-CD19t in the presence or absence of blinatumomab in combination with untransduced T-Cells. B C IFNγ and IL-2 production following in vitro infection of MDA-MB-468 tumor cells with CF33-CD19t in the presence or absence of blinatumomab in combination with untransduced T-Cells, measured at indicated time points by ELISA.

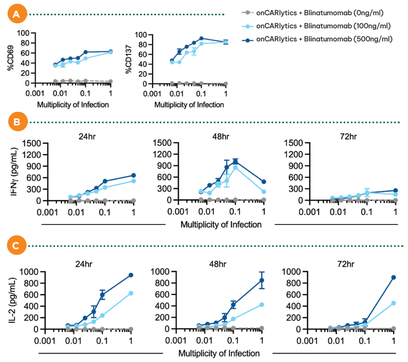


Figure 3

Blinatumomab-mediated T-Cell killing of triple negative breast cancer cell line following onCARlytics infection

Bright-field microscopy (10x magnification) of MDA-MB-468 tumor cells at 48h following CF33-CD19t infection (MOI 0, 0.0125, 0.5, and 1) or MDA-MB-468-CD19t (positive control lentivirally transduced to stably express CD19t) in the presence or absence of blinatumomab in combination with untransduced T-Cells or CD19-CAR T Cell.

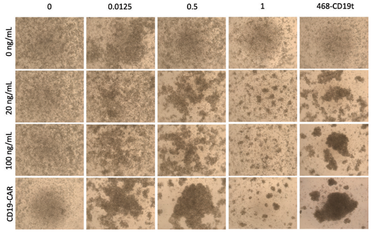


Figure 4

T-Cells specifically target and kill CD19t expressing tumor cells following onCARlytics infection in combination with blinatumomab

Killing assay combining varying MOIs of CF33-CD19t in the presence or absence of blinatumomab with naïve T-Cells against MDA-MB-468 tumor cells. A Tumor killing percentage relative to uninfected tumor cell count and B CD19t expression post CF33-CD19t infection.

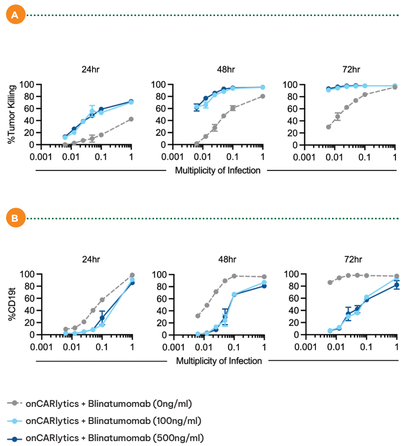


Figure 5

In vivo studies testing onCARlytics and blinatumomab combination therapies

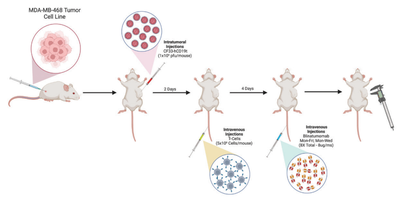


Figure 6

Anti-tumor activity of onCARlytics in combination with blinatumomab and PBMCs in human xenograft TNBC tumor model

Mice were engrafted with subcutaneous MDA-MB-468 ($5x10^6$ cells) and were intratumorally treated with 0 or 10^6 pfu of CF33-CD19t per mouse. Mice were intravenously treated with PBMCs ($5x10^6$ cells) followed by blinatumomab (8 ug/mouse) treatment. A Lines represent tumor volumes of individual mice per group (n=5-11) and B average of each group.

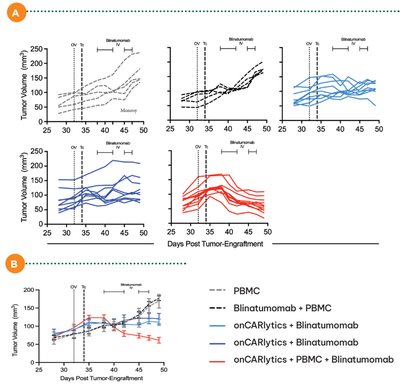
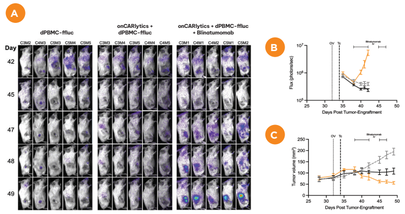


Figure 7

Blinatumomab dependent T-Cell infiltration following onCARlytics infection

Mice were engrafted with subcutaneous MDA-MB-468 ($5x10^6$ cells) on day 0 and were intratumorally treated with 0 or 10^6 pfu of CF33-CD19t per mouse on day 39. Mice were intravenously treated with depleted PBMCs (dPBMC) expressing firefly luciferase (ffluc) ($5x10^6$ cells) on day 41 followed by blinatumomab (8 ug/mouse) treatment from day 45. A Flux imaging tracking T-Cells after treatment with dPBMC-ffluc alone, dPBMC-ffluc with CF33-CD19t, and dPBMC with CF33-CD19t and blinatumomab. B Quantification of T-Cell flux from the regions of interest shown in A. C Average tumor volumes.



Summary

- T-Cell activation markers along with IFNγ and IL-2 secretion increase in response to blinatumomab in a onCARlytics dose-dependent manner in co-culture.
- Blinatumomab initiates T-Cell- mediated tumor killing in onCARlytics infected tumor cells.
- Blinatumomab treatment following onCARlytics infection and T-Cell treatment shows a significantly higher tumor regression compared to onCARlytics, blinatumomab, or T-Cells alone in xenograft models of TNBC.
- This combination immunotherapy approach shows that blinatumomab treatment leads to significantly higher T-Cell infiltration following OV-mediated delivery of CD19t antigen with onCARlytics when compared to OV alone.

References

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