CF33-CD19T ONCOLYTIC VIRUS (onCARlytics) TARGETS HEPATOCELLULAR CARCINOMA (HCC) AND IN COMBINATION WITH CD19 ARTEMIS® T-CELLS RESULTS IN SIGNIFICANT TUMOR KILLING

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Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths in the world with a 5-year survival rate at less than 12%. Currently, curative treatments include ablation, surgical resection, and liver transplantation. For majority of patients with advanced-stage disease, treatment with agents such as sorafenib, lenvatinib, and atezolizumab/bevacizumab and other investigational agents yield modest success rates and justify the need for further development of new therapies. T-Cell therapy against HCC targeting antigens such as alpha-fetoprotein (AFP) and glypican-3 (GPC-3) have shown some efficacy in clinical trials with conventional challenges against solid tumors including antigen heterogeneity, the immunosuppressive tumor microenvironment, and off-tumor on-target activity. Therefore, novel therapies are desperately needed to improve clinical outcomes for patients with HCC.

We have developed a novel chimeric vaccinia-based oncolytic virus, called onCARlytics (CF33-CD19t, Imugene Limited in collaboration with City of Hope®), that delivers a non-signaling, truncated CD19t (CD19t) antigen to tumors that allows for targeting of solid tumors by CD19 T-Cells. Once the CD19t is expressed on solid tumor cells, to enable cell killing, we have combined onCARlytics with CD19 ARTEMIS® T-Cell, a CD19-targeting adoptive engineered T-Cell powered by the ARTEMIS® antibody-T-Cell receptor (AbTCR) platform (Eureka Therapeutics®, Inc). ARTEMIS® AbTCR is distinct from CAR by recruiting the endogenous CD3 complex and utilizing the same activation and regulatory signaling pathways employed by natural TCRs, which enables both potent killing activity against CD19+ tumor cells and a superior safety profile. When administrated after onCARlytics, CD19 ARTEMIS® T-Cells were able to induce potent cytolytic activity against triple negative breast cancer and HCC tumor cells. OnCARlytics demonstrated expression of CD19t and robust in vivo anti-tumor efficacy against human HCC tumor xenografts. In summary, CD19 ARTEMIS® T-Cells combined with onCARlytics is a potentially effective immunotherapy strategy for the treatment of patients with HCC and can be applied to other solid tumors.

Figure 1

Delivering truncated CD19t (CD19t) to tumor cells using oncolytic virus (OV) as a target for CD19 ARTEMIS® T-Cells.

onCARlytics selectively infect solid tumor cells and deliver truncated CD19 (CD19t) as a target for CD19 ARTEMIS® T-Cells.

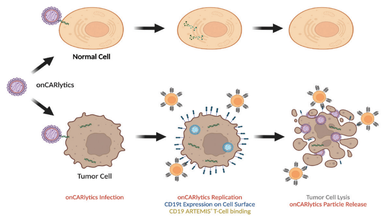


Figure 2

CD19 ARTEMIS® T-Cells (Eureka Therapeutics®, Inc)

Schematic of A ARTEMIS® platform compared to B TCR and C second-generation CAR platform.

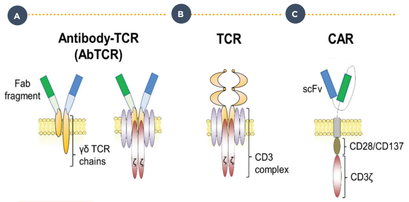


Figure 3

CD19 ARTEMIS® T-Cells effectively targets triple negative breast cancer cell line MDA-MB-468 following onCARlytics infection

A Bright-field microscopy (10X magnification) of MDA-MB-468 tumor cells at 24h following onCARlytics infection or MDA-MB-468-CD19t (positive control lentivirally transduced to stably express CD19t) in the presence of Mock (untransduced), CD19 ARTEMIS®, or City of Hope® (COH) CD19-CAR T Cells using donor D45757. B In vitro killing assay at 24h and C 48h of MDA-MB-468 or MDA-MB-468-CD19t tumor cells infected with onCARlytics and treated with Mock (D45757), CD19 ARTEMIS® (D45757), or COH CD19-CAR (D45757) T-Cells. Graphs on the left represents tumor killing, and in the middle represents CD19t expression on tumor cells. Graphs on the right represents tumor count against MDA-MB-468-CD19t treated with Mock (D45757), CD19 ARTEMIS® (D45757), or COH CD19-CAR (D45757) T-Cells.

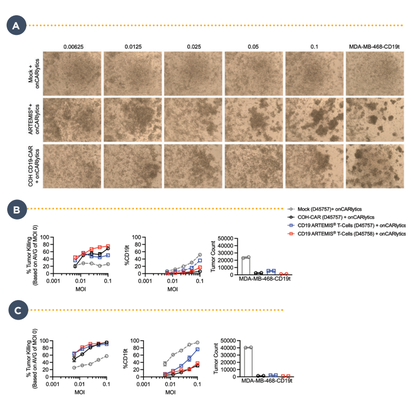


Figure 4

Activation of CD19 ARTEMIS® T-Cells by targeting of triple negative breast cancer cell line MDA-MB-468 expressing CD19t following onCARlytics infection

A Expression of activation marker (CD137) on Mock (D45757), CD19 ARTEMIS® (D45757), CD19 ARTEMIS® (D45758), or COH CD19-CAR (D45757) T-Cells following 24h (left) and 48h (right) in vitro co-culture with MDA-MB-468 tumor cells infected with onCARlytics. B IFNγ and C IL-2 production following in vitro infection of MDA-MB-468 tumor cells with onCARlytics in the presence of Mock (D45757), CD19 ARTEMIS® (D45757), CD19 ARTEMIS® (D45758), or COH CD19-CAR (D45757) T-Cells measured at 24h (left) and 48h (right) by ELISA. D IFNγ and E IL-2 production following in vitro co-culture of MDA-MB-468-CD19t with Mock (D45757), CD19 ARTEMIS® (D45757), CD19 ARTEMIS® (D45758), or COH CD19-CAR (D45757) T-Cells measured at 24h (left) and 48h (right) by ELISA.

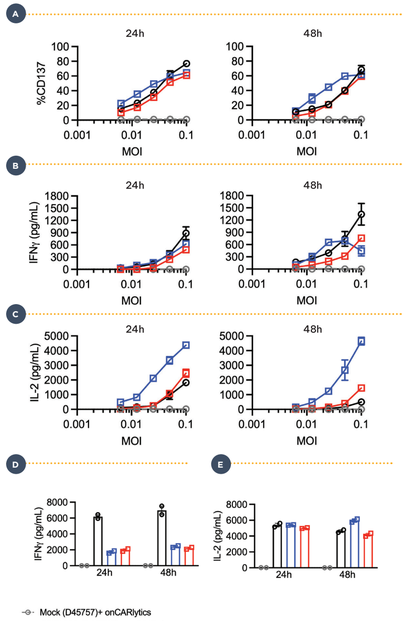


Figure 5

CD19 ARTEMIS® T-Cells effectively targets hepatocellular carcinoma tumor cell lines HepG2 and HEP3B following onCARlytics infection

In vitro killing assay combining onCARlytics and CD19 ARTEMIS® T-Cells at 24h and 48h against A HepG2 and B Hep3B. CD19t expression on C HepG2 and D Hep3B tumor cells following onCARlytics infection at varying MOIs (0.003125, 0.00625, 0.0125, 0.025, 0.05, and 0.1) co-cultured with untransduced (mock) T-Cells, CD19 ARTEMIS®, or COH CD19-CAR T Cells. Activation marker CD137 E and CD69 G expression on T-Cells following co-culture with HepG2 tumor cells infected with onCARlytics. F H Co-culture against Hep3B tumor cells.

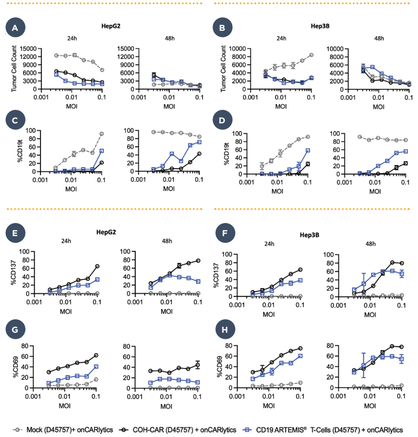
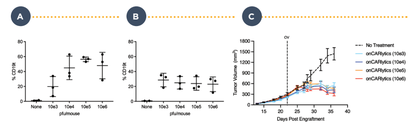


Figure 6

In vivo efficacy and CD19t expression of HepG2 tumor cells following onCARlytics infection

NSG mice were subcutaneously engrafted with HepG2 tumors. Tumors were treated with 10^3, 10^4, 10^5, and 10^6 plaque-forming units (pfu) per mouse of onCARlytics intratumorally when tumor volumes reached approximately 250 mm^3. Tumors were harvested A 3 or B 7 days following onCARlytics treatment to determine CD19t expression via flow cytometry. C Tumor volumes were measured to determine in vivo efficacy of onCARlytics against subcutaneous HepG2 tumors.



Summary

- onCARlytics can target triple negative breast cancer cell line MDA-MB-468 to express CD19t as a target for engineered T-Cells in an MOI-dependent manner.
- onCARlytics can target hepatocellular carcinoma cell lines HepG2 and Hep3B to express CD19t as a target for engineered T-Cells in an MOI-dependent manner.
- Eureka's CD19 ARTEMIS® T-Cells in combination with onCARlytics demonstrated greater in vitro efficacy against MDA-MB-468, HepG2, and Hep3B tumor cell lines compared to onCARlytics alone.
- There is an increasing trend in CD19 ARTEMIS® T-Cell activation in an onCARlytics MOI-dependent manner.
- CD19 ARTEMIS® T-Cells demonstrated higher trend of IL-2 production and lower IFNγ production compared to COH CD19-CAR T Cells when co-cultured with onCARlytics.
- CD19t expression was detected in tumors following onCARlytics infection in vivo.
- CD19 ARTEMIS® T-Cells and onCARlytics combination therapy efficacy will be tested in multiple in vivo models.

References

1. Park AK, et al. Effective combination immunotherapy using oncolytic viruses to deliver CAR targets to solid tumors. Sci Transl Med. 2020. **2.** McCart JA, Ward JM, Lee J, Hu Y, Alexander HR, Libutti SK, Moss B, Bartlett DL. Systemic cancer therapy with a tumor-selective vaccinia virus mutant lacking thymidine kinase and vaccinia growth factor genes. Cancer Res. 2001. **3.** Chaurasiya S, et al. A chimeric poxvirus with J2R (thymidine kinase) deletion shows safety and anti-tumor activity in lung cancer models. Cancer Gene Ther. 2020. **4.** O'Leary MP, Warner SG, Kim SI, Chaurasiya S, Lu J, Choi AH, Park AK, Woo Y, Fong Y, Chen NG. A Novel Oncolytic Chimeric Orthopoxvirus Encoding Luciferase Enables Real-Time View of Colorectal Cancer Cell Infection. Mol Ther Oncolytics. 2018. **5.** Yiyang Xu, et al. A novel antibody-TCR (AbTCR) platform combines Fab-based antigen recognition with gamma/delta-TCR signaling to facilitate T-Cell cytotoxicity with low cytokine release. Cell Discovery. 2018.