Effective combination immunotherapy using OnCARlytics and ARTEMIS® CD19 T cells against hepatocellular carcinoma (HCC)

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Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the sixth most common cancer worldwide. Currently, there are six systemic therapies available for patients with advanced disease including atezolizumab in combination with bevacizumab, lenvatinib, regorafenib, cabozantinib, and ramucirumab, in addition to the traditional treatments including ablation, surgical resection, and liver transplantation. CD19 targeting chimeric antigen receptor (CAR) T cell therapy has demonstrated impressive clinical outcomes but translating this therapy to solid-tumor cancers has been met with various challenges, including the immunosuppressive microenvironment, on-target off-tumor toxicity, and antigen heterogeneity. To date, CAR T cell therapies against HCC targeting antigens such as alpha-fetoprotein and glypican-3 have shown nominal efficacy in clinical trials. Therefore, development of novel and innovative therapeutic approaches against HCC is desperately needed to overcome the challenges and improve clinical outcomes.

Oncolytic viruses (OV) are a novel and attractive form of immunotherapy due to the ability to target tumor cells selectively, even in the absence of tumor specific antigens, and they can deliver genes of interest for therapeutic intervention. We have harnessed this capability of OV and developed a chimeric vaccinia-based OV called CF33-CD19t (onCARlytics) that delivers a non-signaling, truncated CD19 (CD19t) antigen to solid tumors allowing CD19-specific T cells to target them. In order to target CD19t expressed on the surface of solid tumor cells, we combined onCARlytics with CD19 ARTEMIS® T cell, a CD19-targeting adoptively engineered T cell powered by the ARTEMIS® antibody-T cell receptor (AbTCR) platform. The ARTEMIS® AbTCR distinguishes itself from CAR by its recruitment of endogenous CD3 complex and utilizing the same activation and regulatory signaling pathways used by natural TCRs. Tumors infected with onCARlytics induced robust CD19t surface-antigen expression prior to virus-induced tumor lysis. Co-cultured CD19 ARTEMIS® T cells secreted cytokines and elicited potent cytolytic activity against infected HCC tumor cells. In addition, this combination approach demonstrated impressive *in vivo* anti-tumor responses in a human xenograft HepG2 tumor model. By using this combination OV and adoptively engineered T cell strategy, we have now broadened the utility of CD19 ARTEMIS® T cells to otherwise target-less tumors such as HCC, which we anticipate can be applied to a wide array of solid tumors as an effective immunotherapy approach.

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

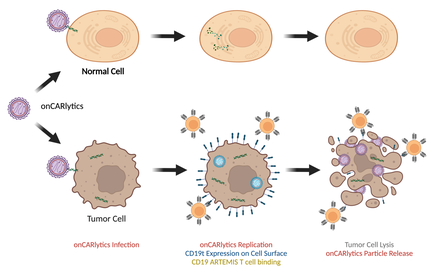
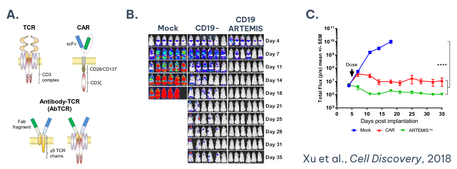


Figure 2

CD19 ARTEMIS® T cells (Eureka Therapeutics, Inc)

(**A**) Schematic of ARTEMIS® platform compared to a TCR and a second-generation CAR platform. CD19 ARTEMIS® T cell therapy shows efficacy in a Raji lymphoma model. (**B**) Bioluminescent images and (**C**) total flux over time of Raji-luc implanted mice intravenously administered with 5 x 10^6 Mock (untransduced) T cells, CD19 CAR-T cells, or CD19 ARTEMIS® T cells (n = 6).



Xu et al., *Cell Discovery*, 2018

Figure 3

CD19 ARTEMIS® T cells effectively target 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® T (Eureka), 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 Mock (D45757), COH CD19-CAR (D45757), CD19 ARTEMIS® T (D45757), or CD19 ARTEMIS® T (D45758) 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.

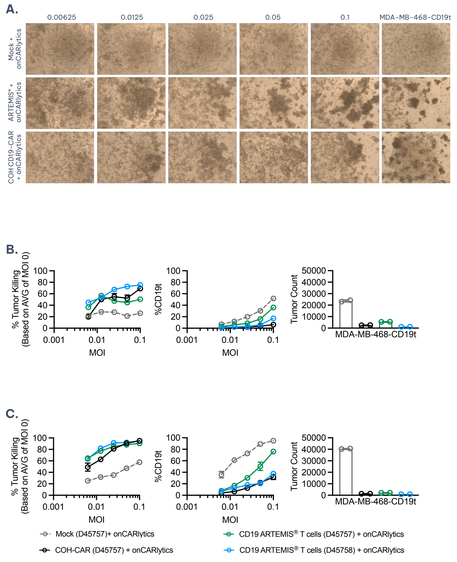


Figure 4

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), COH CD19-CAR (D45757), CD19 ARTEMIS® (D45757), or CD19 ARTEMIS® (D45758) 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), COH CD19-CAR (D45757), CD19 ARTEMIS® (D45757), or CD19 ARTEMIS® (D45758) T cells measured at 24h (left) and 48h (right) by ELISA. (**D**) IFNy and (**E**) IL-2 production following *in vitro* co-culture of MDA-MB-468-CD19t with Mock (D45757), COH CD19-CAR (D45757), CD19 ARTEMIS® (D45757), or CD19 ARTEMIS® (D45758) T cells at 24h (left) and 48h (right) by ELISA.

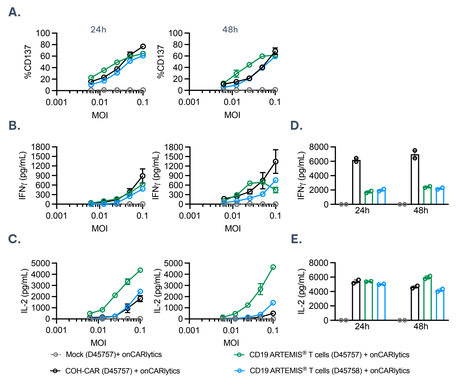


Figure 5

CD19 ARTEMIS® T cells effectively target 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, COH CD19-CAR, or CD19 ARTEMIS® 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.

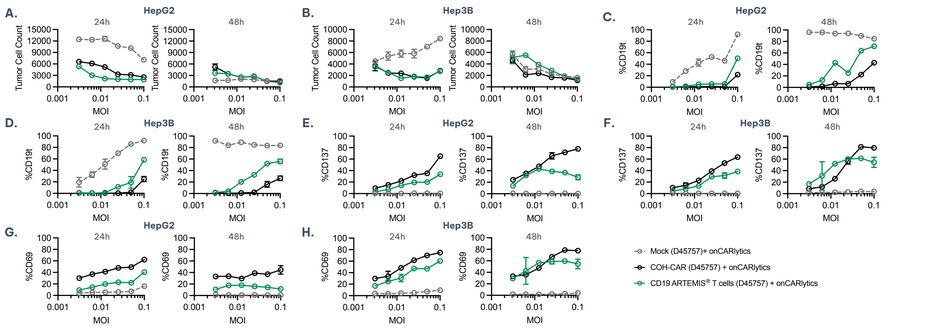


Figure 6

***In vivo* activity and CD19t expression in HepG2 tumor model 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 infection to determine CD19t expression via flow cytometry. (**C**) Tumor volumes were measured to determine *in vivo* efficacy of onCARlytics against subcutaneous HepG2 tumors.

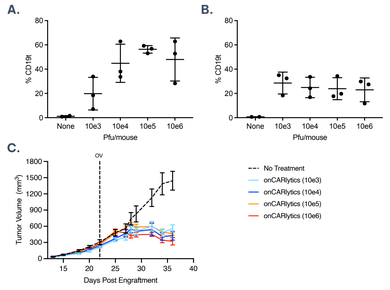
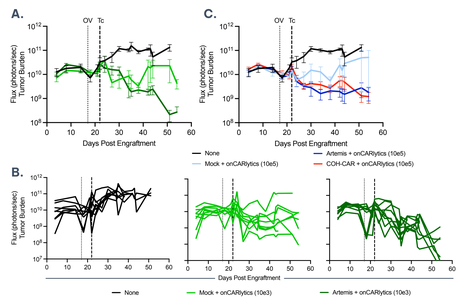


Figure 7

***In vivo* anti-tumor efficacy of CD19 ARTEMIS® T cells and onCARlytics against HepG2 tumor cells**

NSG mice were subcutaneously engrafted with HepG2 tumors. Tumors were treated onCARlytics intratumorally when tumor flux reached an average of approximately 10^9 photons/sec followed by T cells 5 days later. Tumor measurement was determined using non-invasive bioluminescent imaging. Average tumor flux treated with either (**A**) 10^3 or (**C**) 10^5 pfu per mouse is shown, and (**B**) flux of individual mouse for 10^3 pfu treatment.



Summary

- OnCARlytics can target triple-negative breast cancer cell line MDA-MB-468 to express CD19t as an antigen for engineered T cells in an MOI-dependent manner.
- OnCARlytics can target hepatocellular carcinoma cell lines HepG2 and Hep3B to express CD19t as an antigen for engineered T cells in an MOI-dependent manner.
- Eureka's CD19 ARTEMIS® T cells in combination with onCARlytics demonstrated greater *in vitro* killing efficacy against MDA-MB-468, HepG2, and Hep3B tumor cell lines compared to onCARlytics alone.
- There is a trend of increasing CD19 ARTEMIS® T cell activation in an onCARlytics MOI-dependent manner.
- CD19 ARTEMIS® T cells demonstrated a 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*.
- Combining CD19 ARTEMIS® T cells and onCARlytics demonstrated enhanced anti-tumor efficacy *in vivo* against HepG2 hepatocellular carcinoma tumors.

References

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