In vivo HSC engineering generates lineage-restricted, multiplexed CAR-M, NK, and T cells that mediate synergistic anti-tumor activity in pre-clinical models

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Abstract

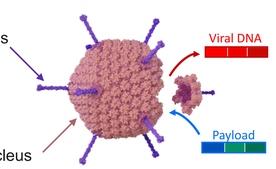
Background The clinical success of cancer immunotherapy, including chimeric antigen receptor (CAR) cell therapy, has revolutionized treatment paradigms and patient outcomes. While hematological tumors have benefited most from these approaches, such transformative success has yet to be achieved for refractory solid tumors. Limited access, complex manufacturing, and high cost of therapy exacerbate the burdens faced by these patients. **Methods** To address the unmet need of advanced solid tumor patients, we developed a helper-dependent adenovirus (HDAd) platform of virus-like particles (VLP) harboring a 35-kb cargo capacity. The VLP preferentially targets CD46 which is highly expressed on primitive hematopoietic stem cells (HSCs). Mobilization of HSCs into the bloodstream enables in situ transduction followed by stable transgene integration, ultimately aiming to generate a continuous supply of HSC-derived engineered immune effectors from a single VLP dose. **Results** With the goal of programming a lineage-specific and potent multi-cellular anti-tumor immune response, we identified and validated genetic regulatory elements that drive functional CAR expression in human. We next built a vector comprising concatenated myeloid- and lymphoid-restricted HER2 CARs to generate a multiplexed CAR-M, NK, and T cell therapy. The lineage-restricted, multiplexed HER2 CAR VLPs were administered to HSC-mobilized, immune competent mice expressing human CD46. Peripheral blood monitoring at Weeks 6 to 12 post-VLP dose revealed HER2 CAR-expressing myeloid, NK, and T cells; total immune cell blood counts were equivalent between VLP-HER2 CAR-treated animals and VLP-GFP or mock controls, demonstrating successful generation of HSC-derived engineered immune cells concomitant with normal hematopoiesis. Importantly, CAR expression in B cells and HSCs was below the limit of detection in vivo, demonstrating lineage-selective promoter specificity. At the study endpoint, bone marrow from CAR- or GFP-expressing mice was transplanted to EO771/huHER2 orthotopic tumor-bearing mice. The multiplexed CAR-M, NK, and T cell therapy mediated superior tumor control compared to single-lineage CAR alone or GFP control. In the tumor microenvironment, HER2 CAR-M displayed a pro-inflammatory phenotype coupled to a significant increase in CAR-T and NK cell abundance and activation. **Conclusions** These data establish robust proof of concept for the generation and anti-tumor potency of HSC-derived, lineage-restricted CAR-M, NK, and T cell therapy generated via a single dose of VLPs in vivo. HSCs comprise a promising self-renewing reservoir of CAR-M, NK, and T cells mediating synergistic anti-tumor activity. This platform has the potential to overcome myriad therapeutic challenges in advanced solid tumors and provide an innovative off-the-shelf therapy for expanded patient access.

Engenious™ Platform for *in vivo* gene delivery

Virus-like particle (VLP)

LT-HSC targeting
Ad35++ fiber targets CD46 on HSCs

Delivery to nucleus
Ad5 capsid shuttles DNA to cell nucleus

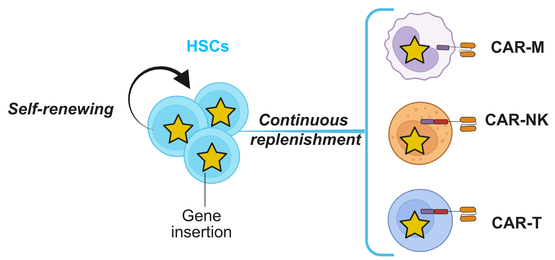


"Gutless" vector
Contains no viral genes

35 kB payload capacity
Enables multiplexed gene insertion

Multi-lineage CAR strategy for solid tumors

HSC engineering generates a ***self-renewing, multi-cellular*** therapy



Validation of lineage-specific promoters driving robust on-target HER2 CAR expression *in vitro*

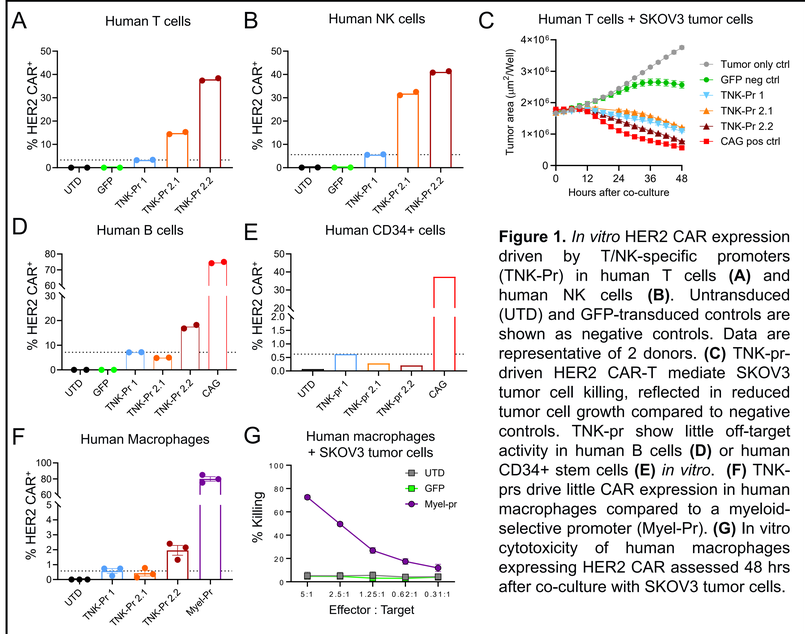


Figure 1. *In vitro* HER2 CAR expression driven by T/NK-specific promoters (TNK-Pr) in human T cells **(A)** and human NK cells **(B)**. Untransduced (UTD) and GFP-transduced controls are shown as negative controls. Data are representative of 2 donors. **(C)** TNK-pr-driven HER2 CAR-T mediate SKOV3 tumor cell killing, reflected in reduced tumor cell growth compared to negative controls. TNK-pr show little off-target activity in human B cells **(D)** or human CD34+ stem cells **(E)** *in vitro*. **(F)** TNK-prs drive little CAR expression in human macrophages compared to a myeloid-selective promoter (Myel-Pr). **(G)** In vitro cytotoxicity of human macrophages expressing HER2 CAR assessed 48 hrs after co-culture with SKOV3 tumor cells.

Lineage-specific promoters drive restricted HER2 CAR expression *in vivo*

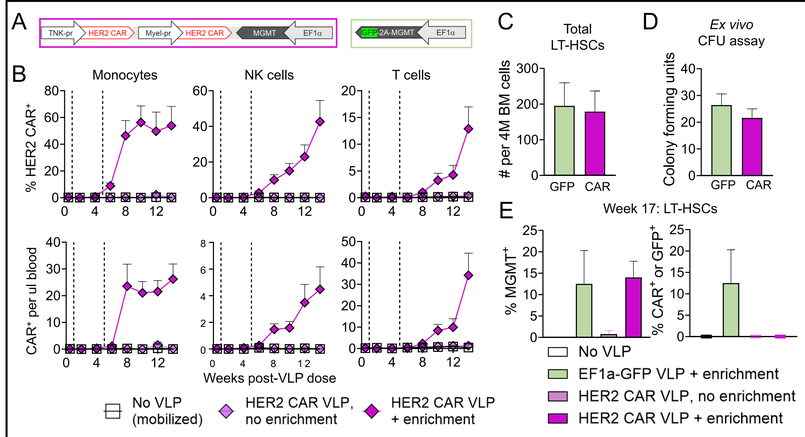


Figure 2. (A) VLP encoding anti-HER2 CAR(s) driven by T/NK- and myeloid- (Myel-) promoters (Pr), along with an EF1α driven MGMT^{P140K} cassette to enable chemotherapy-based enrichment of integrated cells. A vector encoding GFP serves as a control. **(B)** HSC-mobilized hCD46tg mice were dosed with VLPs and administered enrichment chemotherapy at Weeks 1 and 5 post-VLP (vertical dashed lines) Peripheral blood was collected biweekly to quantify the frequency (top panel) and absolute number (bottom panel) of HER2 CAR-expressing monocytes, NK cells, and T cells. HER2 CAR expression is not detected in mock mice or VLP-dosed mice without enrichment (dotted lines). **(C)** Absolute number of LT-HSCs quantified in the bone marrow at Week 17 were equivalent in GFP & HER2 CAR-expressing mice. **(D)** Equivalent colony forming units (CFUs) were generated *ex vivo* by bone marrow expressing control the GFP transgene or the lineage-restricted HER2 CAR payload. **(E)** Bone marrow LT-HSCs express the EF1α-driven MGMT payload but no cell surface HER2 CAR, demonstrating successful lineage restriction *in vivo*.

Multiplexed CAR-M, NK, & T cells mediate anti-tumor activity and remodel the tumor microenvironment *in vivo*

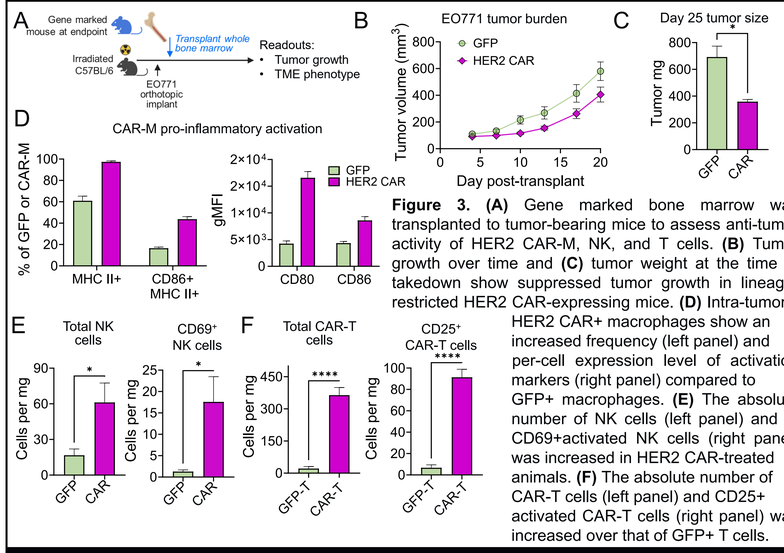


Figure 3. (A) Gene marked bone marrow was transplanted to tumor-bearing mice to assess anti-tumor activity of HER2 CAR-M, NK, and T cells. **(B)** Tumor growth over time and **(C)** tumor weight at the time of takedown show suppressed tumor growth in lineage-restricted HER2 CAR-expressing mice. **(D)** Intra-tumoral HER2 CAR+ macrophages show an increased frequency (left panel) and per-cell expression level of activation markers (right panel) compared to GFP+ macrophages. **(E)** The absolute number of NK cells (left panel) and of CD69+activated NK cells (right panel) was increased in HER2 CAR-treated animals. **(F)** The absolute number of CAR-T cells (left panel) and CD25+ activated CAR-T cells (right panel) was increased over that of GFP+ T cells.

In vivo generated CAR-M & CAR-T cells mediate potent ex vivo tumor cell killing

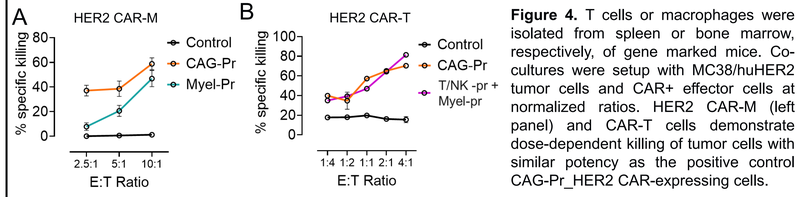


Figure 4. T cells or macrophages were isolated from spleen or bone marrow, respectively, of gene marked mice. Co-cultures were setup with MC38/huHER2 tumor cells and CAR+ effector cells at normalized ratios. HER2 CAR-M (left panel) and CAR-T cells demonstrate dose-dependent killing of tumor cells with similar potency as the positive control CAG-Pr_HER2 CAR-expressing cells.

Conclusions

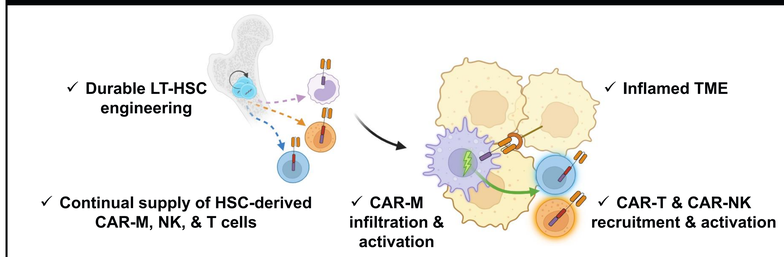


Figure 5. *In vivo* engineered HSCs are a self-renewing source of CAR-M, NK, and T cells, mediating synergistic anti-tumor activity in mice

- **A single *in vivo* dose** of VLPs generates a multi-cellular HER2 CAR+ population comprising **CAR-M, CAR-NK & CAR-T cells**
- Lineage-specific promoters direct CAR expression to discreet effector immune cells, enabling **regulated expression of multiplexed therapeutic payloads**
- *In vivo* generated HER2 CAR-M/NK/T cells **infiltrate and remodel the TME, resulting in anti-tumor efficacy in mice**