In Vivo Engineering of Hematopoietic Stem Cells with Virus-Like Particles to Generate Multi-Lineage CAR Immune Cell Therapy for Cancer

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Abstract

Background Chimeric antigen receptor-engineered T (CAR-T) cell therapy shown limited efficacy in solid tumors, owing to an immunosuppressive tumor microenvironment and inefficient trafficking of CAR-Ts to tumor. **Methods** We developed a virus-like particle (VLPs) platform using helper-dependent adenovirus to enable *in vivo* engineering of hematopoietic stem cells (HSCs). These VLPs have a large cargo capacity of up to 35 kilobases, enabling construction of single- or multi-cellular CAR sequences under distinct lineage-specific promoters for precise immune cell engineering. **Results** To achieve selective therapeutic payload expression, we identified and validated lineage-restricted promoters with myeloid- or T/NK-cell-specific activity in primary human and murine immune cells. CAR constructs driven by monocyte- or T/NK-restricted promoters successfully generated functional CAR myeloid (M), T and NK cells, respectively. To assess activity *in vivo*, human CD46+ (hCD46+) mouse hematopoietic stem and progenitor cells (HSPCs) were transduced with VLPs encoding CAR driven by a ubiquitous promoter (CAG) or lineage restricted regulatory elements and transplanted into irradiated recipient mice to assess HSPC-derived CAR+ immune cell generation. While the ubiquitous CAG promoter drove CAR expression across all immune cell lineages, myeloid- and T/NK-restricted promoters confined CAR expression to their respective lineages. These lineage-specific CAR immune cells exhibited on-target tumor cytotoxicity comparable to CAG-driven CAR while minimizing off-target expression. Tumor-infiltrating CAR+ effector cells displayed a proinflammatory phenotype compared to their CAR- counterparts. Furthermore, concatenation of myeloid- and T/NK-restricted promoters enabled generation of multi-lineage CAR immune cells from a single VLP.

Engenious™ Platform for *in vivo* gene delivery

Virus-like particle (VLP)

Evolved capsid
Adenoviral vector built on evolved, high efficiency gene delivery vectors

Hematopoietic Tropism
Highly preferential transduction of HSCs & derived lineages

"Gutless" vector
Contains no viral genes, resulting in low immunogenicity & high payload capacity

35 kB Payload Capacity
Enables multiplexed gene insertion controlled by distinct regulatory elements

Engenious™ Platform for Immuno-Oncology

- **Multiple immune cell types** engineered *in vivo*
- **Regulatory elements** enable cell type-specific multiplexing of anti-tumor modalities
- **Self-renewing** source of HSC-derived engineered effector cells

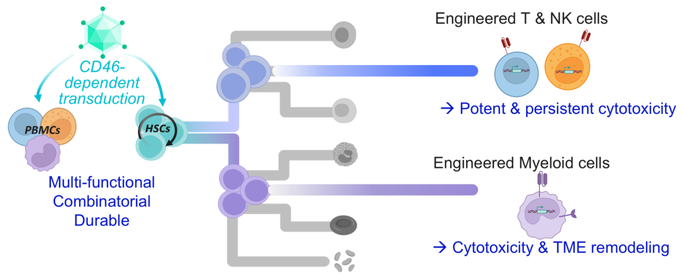

Figure 1. Mobilization of HSCs into peripheral blood enables *in vivo* VLP targeting of both primitive progenitors and mature immune cells. Direct transduction of circulating myeloid, T, and NK cells generates a population of armed effector cells within days of VLP administration. Transduced HSCs home to the bone marrow where integrated HSCs give rise to engineered immune cell lineages. Long-term HSCs comprise a self-renewing pool of effector cells, conferring durable anti-tumor activity from a single VLP dose.

Lineage-specific promoters are active in mature immune cells *in vivo*

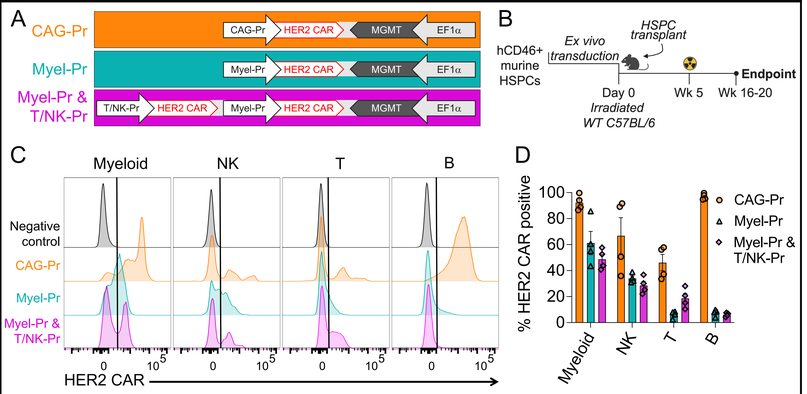

Figure 2. (A) VLP vector encoding anti-HER2 CAR(s) driven by CAG, T/NK- and/or myeloid- (Myel-) promoter (Pr), along with an EF1α driven MGMT^{P140K} cassette to enable chemotherapy-based enrichment of integrated cells. **(B)** Schematic of *ex vivo* VLP treatment of hCD46+ murine HSPCs and transplant into irradiated C57BL/6 mice. Fluorescence histogram **(C)** and frequency **(D)** of HER2 CAR+ cells in blood at 16 weeks post-HSPC transplant.

In vivo engineered HSCs give rise to lineage-specific HER2 CAR-M, -NK & -T cells

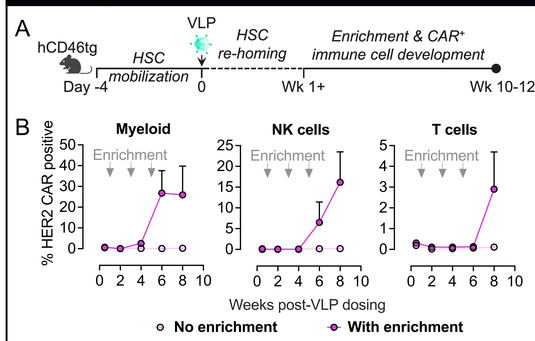

Figure 3. (A) Experimental timeline to assess *in vivo* VLP HSC transduction, enrichment, and CAR expression in mature immune cell lineages. HSC mobilized hCD46 transgenic (hCD46tg) mice dosed with VLP vector encoding anti-HER2 CARs driven by T/NK-Pr and Myel-Pr, and an EF1α driven MGMT^{P140K} cassette to enable chemotherapy-based enrichment of integrated cells. **(B)** Frequency of HER2 CAR in myeloid, NK & T cells in peripheral blood of mice dosed with VLP. Chemotherapy dosing for enrichment of integrated cells indicated by vertical arrows.

Additional regulatory elements reduce Myel-Pr leakiness in HSPCs

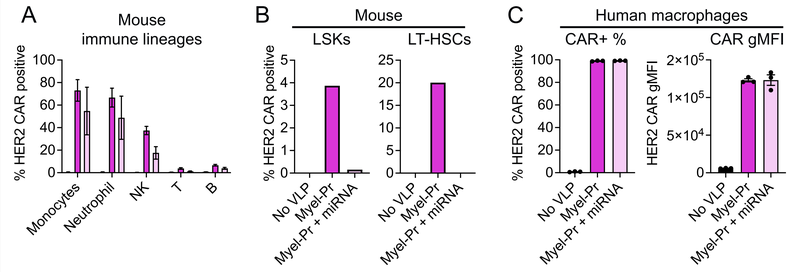

Figure 4. Frequency of HER2 CAR expression in peripheral blood immune cell types **(A)** and bone marrow cells **(B)** at 10- and 6.5-weeks post-HSPC transplant, respectively. Bone marrow cells include Lineage-, Sca1+, c-Kit+ (LSK) and long-term hematopoietic stem cell (LT-HSCs). Experiment outlined in Figure 2B. **(C)** HER2 CAR frequency (left) geometric mean fluorescence intensity (gMFI; right) in human CD14+ monocytes treated with VLP and differentiated to macrophages. VLP vector encoding anti-HER2 CAR driven by Myel-Pr with or without binding sites for candidate miRNA, anti-HER2 CAR driven by T/NK-Pr, and an EF1α driven MGMT^{P140K} cassette.

In vivo generated CAR M and CAR T exhibit potent antigen-dependent tumor cell cytotoxicity

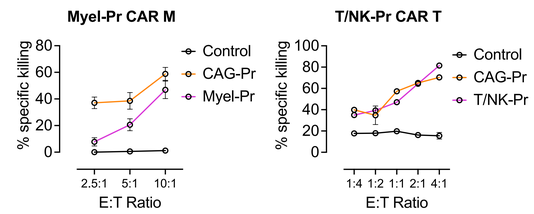

Figure 5. T cells or macrophages isolated from spleen or bone marrow, respectively, of gene marked mice. Study design in Figure 2B. VLPs encoding anti-HER2 CAR(s) driven by Myel-Pr and/or T/NK-Pr, and an EF1α driven MGMT^{P140K} cassette. Co-cultures were setup with HER2+ tumor cells and CAR effector cells (CAR M or CAR T) at normalized ratios.

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

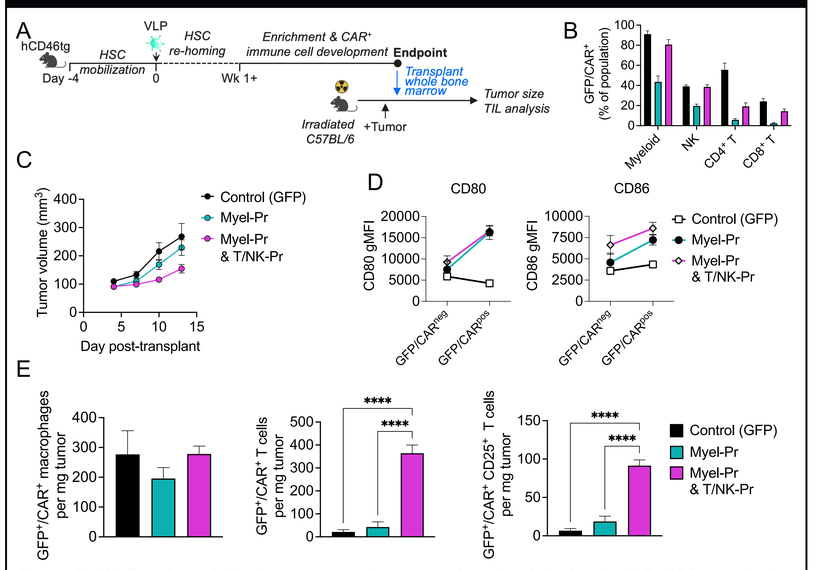

Figure 6. (A) Experimental timeline to assess tumor control mediated by *in vivo* VLP HSC transduction, enrichment, and CAR expression in mature immune cell lineages. Bone marrow transplanted into HER2+ tumor bearing and irradiated C57BL/6 mice to assess tumor control. **(B)** Composite data of tumor growth over time. Tumors were harvested on Day 25 post-transplant and processed to single cells for flow cytometric analysis. **(C)** Geometric mean fluorescence intensity (gMFI) of CD80 (left) and CD86 (right) on non-gene marked and gene-marked macrophages in tumor. **(D)** Absolute count of GFP+ or CAR+ T cells in tumors. **(E)** Absolute count of CD25+ GFP+ or CAR+ T cells. **(F)** Absolute count of CD8+ GFP+ or CAR+ T cells.

Conclusions

- **A single *in vivo* dose** of VLPs generates a multi-cellular HER2 CAR+ population of immune effectors, including **CAR-M, CAR-NK & CAR-T cells**
- Lineage-specific promoters direct CAR expression to discreet mature 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**

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