In vivo VLP-based engineering generates multi-lineage CAR-immune cells that mediate potent and durable anti-tumor activity

Corinne Decker, Cristina Santoriello, Chirayu Chokshi, Richard Davidson, Jessica Kohler, Charles Fox, Adam Fisher, Chapman Wright, Emilio Flano, and Robert Peters

Ensoma, Inc. Boston, MA USA

Abstract

Background The clinical success of cancer immunotherapy, including 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 compound 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-kilobase cargo capacity. These VLPs target CD46-positive cells including mature immune cells and hematopoietic stem cells (HSCs). Mobilization of HSCs into the blood facilitates efficient VLP transduction *in vivo*, followed by HSC-derived engineered immune cell development and renewal. The VLP's large capacity permits the inclusion of a tumor-targeting chimeric antigen receptor (CAR) under the control of genetic regulatory elements, enabling a precise and potent multi-cellular anti-tumor immune response. **Results** VLPs encoding an anti-HER2 CAR were administered to HSC-mobilized, immune competent mice expressing human CD46. Mature immune cells in the blood were efficiently transduced, showing 10-20% CAR positivity on Day 3 post-VLP dosing. HSC-derived CAR-positive myeloid, NK, and T cells were detected in the blood within 8 to 10 weeks of treatment and maintained through 5 months post-dose, illustrating stable transgene integration. Normal long-term function of engineered HSCs was further evidenced by equivalent multi-lineage immune cell development in mock and VLP-dosed mice as well as secondary transplant recipients. Control and HER2 CAR-expressing mice were challenged with human HER2+ EO771 orthotopic mammary tumors or MC38 colon carcinoma. HER2 CAR immune cells mediated robust tumor control, accompanied by inflammatory cytokine release and CAR-positive immune cell activation in the solid tumor microenvironment. **Conclusions** Our data demonstrate compelling proof-of-concept for efficient *in vivo* engineering of functional immune cells mediating anti-tumor efficacy. Complementing direct immune cell transduction, HSCs comprise a self-renewing reservoir of engineered cells to mediate long-term tumor surveillance and control. 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)

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

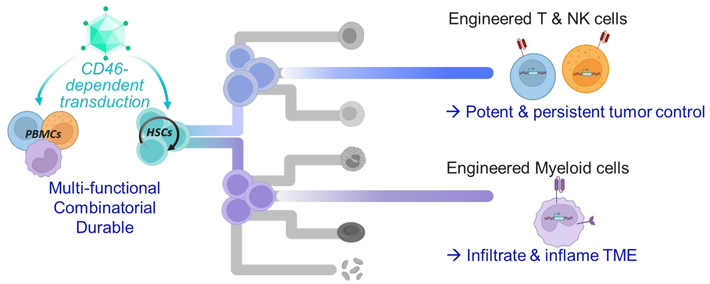

Figure 1. Mobilization of HSCs into peripheral blood enables *in vivo* VLP targeting of both primitive progenitors and mature immune cells. Direct transduction of circulating myeloid, T, and NK cells generates a population of armed effector cells within days of VLP administration. Transduced HSCs home 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.

VLPs efficiently deliver HER2 CAR to immune cells *in vivo*

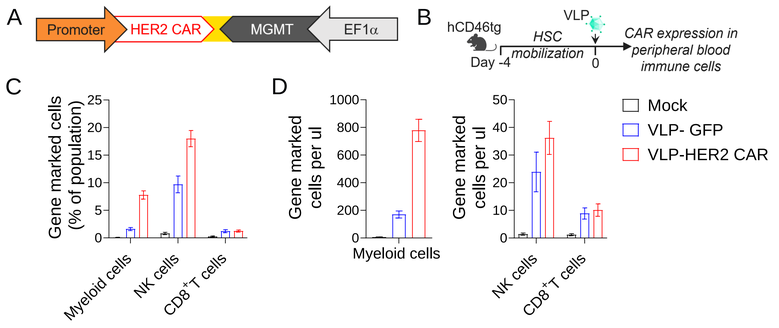

Figure 2. (A) VLP vector encoding an anti-HER2 CAR to mediate tumor targeting and an MGMT^{P140K} cassette to enable chemotherapy-based enrichment of integrated cells. **(B)** Experimental timeline of *in vivo* VLP dosing in HSC-mobilized human CD46 transgenic (hCD46tg) immune competent mice. Frequency **(C)** and absolute number **(D)** of HER2 CAR-expressing myeloid, NK and CD8$^+$ T cells in the blood on Day 3 post-VLP dosing.

In vivo CAR engineered HSCs give rise to multi-lineage CAR-expressing immune cells

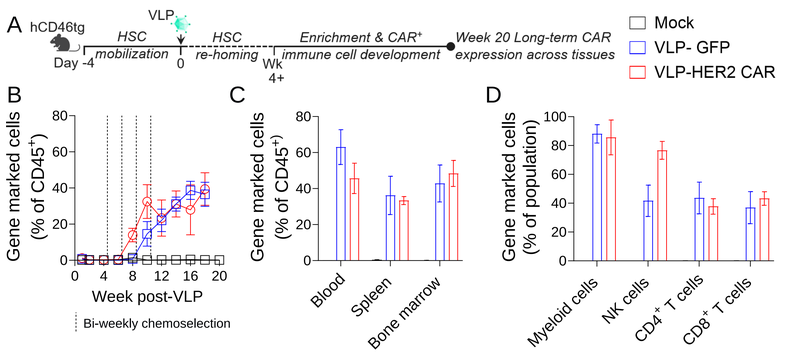

Figure 3. (A) Experimental timeline to assess *in vivo* VLP HSC transduction, enrichment, and CAR expression in mature immune cell lineages. **(B)** HER2 CAR expression in peripheral blood CD45$^+$ immune cells over time indicates long-term, stable integration in HSCs and derived lineages. **(C)** CD45$^+$ cell gene marking in blood and lymphoid tissues is similar at Week 20 post VLP dose. **(D)** HER2 CAR is detected in HSC-derived immune cells.

In vivo engineered HSCs provide a durable, self-renewing source of CAR$^+$ immune effectors

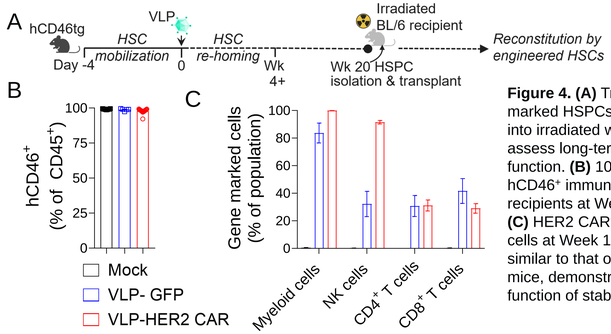

Figure 4. (A) Transplantation of gene marked HSPCs generated in Figure 3 into irradiated wild-type BL/6 mice to assess long-term engineered HSC function. **(B)** 100% reconstitution of hCD46$^+$ immune cells in BL/6 recipients at Week 18 post-transplant. **(C)** HER2 CAR$^+$ expression in immune cells at Week 18 post-transplant is similar to that of primary VLP-dosed mice, demonstrating long-term function of stably-integrated HSCs.

Multi-cellular *in vivo* HER2 CAR mediates tumor control

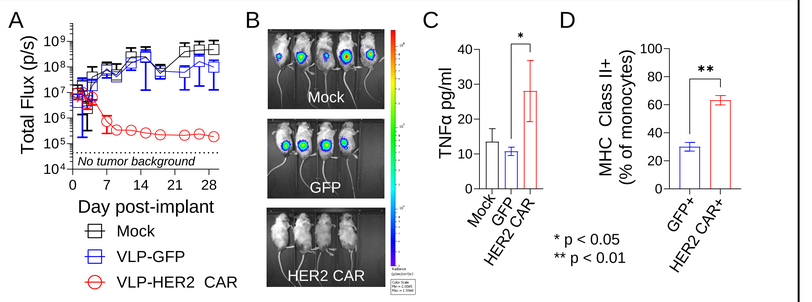

Figure 5. (A) *In vivo* generated HER2 CAR induces durable MC38/huHER2/FLuc tumor regression. **(B)** BLI captured on Day 12 post-tumor implant illustrates tumor control in 100% of HER2 CAR mice. **(C-D)** Gene marked mice were implanted with EO771/huHER2 tumors in the mammary fat pad to assess immune cell activation in the orthotopic tumor microenvironment. Increased serum TNF-α on Day 3 post-implant **(C)** and MHC Class II expression on intra-tumoral monocytes **(D)** demonstrates infiltration & antigen-dependent activation of HER2 CAR$^+$ immune cells.

Lineage-specific promoter drives restricted CAR expression sufficient to suppress tumor growth *in vivo*

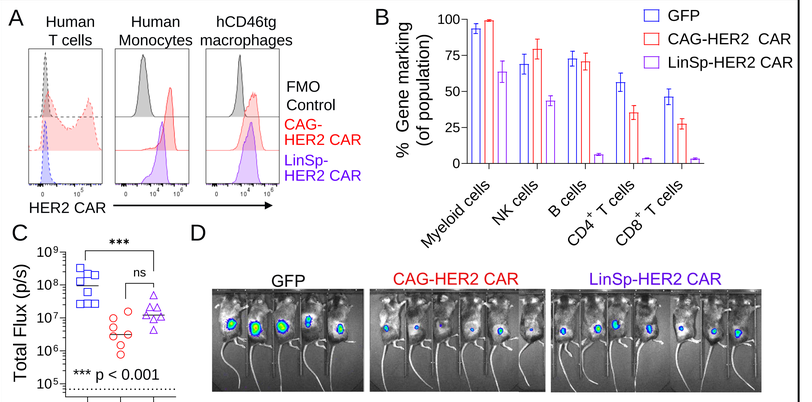

Figure 6. (A) HER2 CAR driven by a native lineage-specific (LinSp) promoter is active in human CD14+ monocytes & hCD46tg mouse macrophages but not T cells *in vitro*. **(B)** hCD46tg HSPCs were transduced with VLPs *ex vivo* and transplanted to irradiated BL/6 mice. Gene marking of HSPC-derived immune cell lineages at Week 12 post-transplant demonstrates limited activity of the lineage-specific promoter in myeloid & NK cells compared to pan-lineage expression of CAG-HER2**. (C)** MC38/huHER2-Fluc tumor growth is suppressed in mice expressing HER2 CAR driven by the lineage-specific promoter. **(D)** BLI shows reduced tumor burden in HER2 CAR$^+$ mice.

Summary

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**

In vivo generated HER2 CAR immune cells infiltrate a forming solid tumor and mediate **durable tumor control**

Lineage-specific promoters direct CAR expression to discreet mature immune cells, enabling **regulated expression of multiplexed therapeutic payloads**

Created with BioRender.com

ensoma

CONFIDENTIAL