Amphiphile-Peptide Boosting with FMC63-binding Surrogate Peptide Mimotopes Induces Activation and Potent Effector Function in CAR-T Cells Targeting CD19

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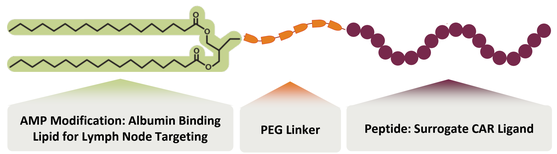
Overview

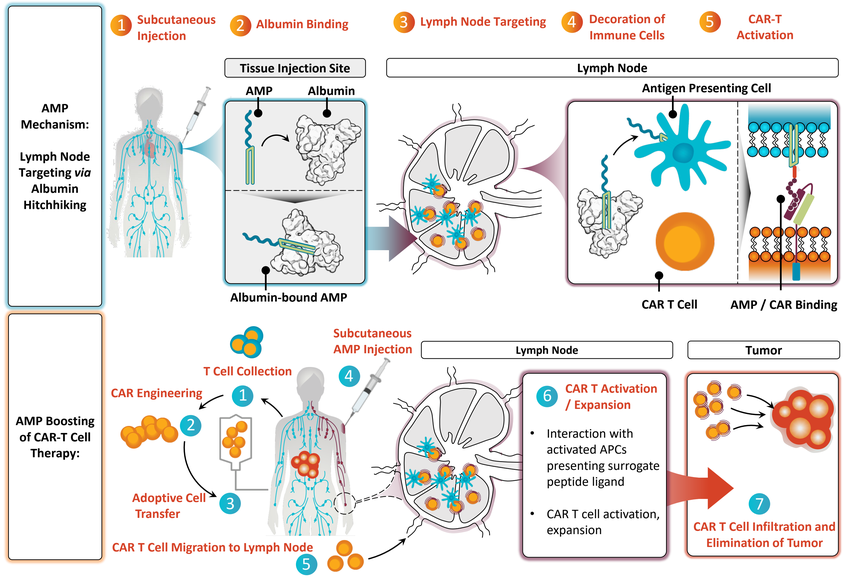
Genetic engineering of T cells to express anti-CD19 Chimeric Antigen Receptors (CAR-T cells) has been FDA approved for treatment of refractory/relapsing acute lymphocytic leukemia and diffuse large B cell lymphoma. With more patients receiving treatment with CAR-T cells it has been observed that approximately 10–20% of patients fail to enter remission after therapy [1], and 30–50% of patients who achieve remission with anti-CD19 CAR T cells have disease relapse [2]. In prior studies, CAR-binding Amphiphile (AMP)-peptides were shown to effectively localize in lymph nodes (LN), where they decorate endogenous antigen presenting cells (APC) and stimulate CAR signaling to promote potent CAR-T responses against solid tumors [3]. In this study we describe how CD19 mimotope peptides specific for FMC63-based CARs can be modified with AMP technology to enhance peptide accumulation in LNs, enable presentation on APCs to CAR-Ts, and promote activation and effector functionality of CAR-T cells.

The AMP Platform – Designing a Lymph Node Targeted AMPlifier for CAR-T Therapy

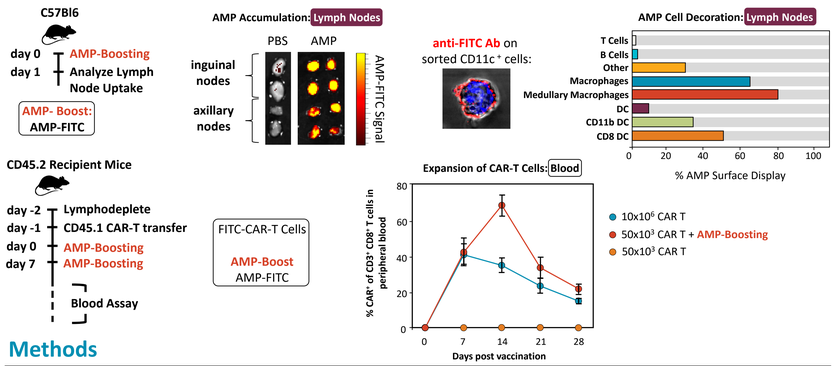
AMP-Peptide : Peptide Surrogate Ligand

- Surrogate peptide ligand (mimotope) for CAR antigen-specific binding domain
- AMP modification gives >10-fold improved lymph node targeting





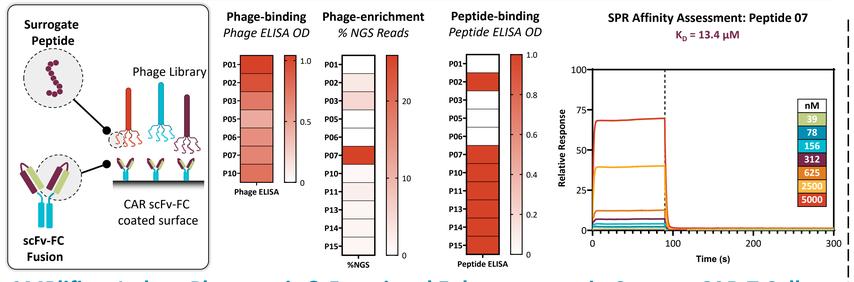
AMP Lymph Node Targeting, APC Surface Decoration, and CAR-T Expansion[3]



Methods

We performed phage-screening and enrichment for CD19 surrogate peptides recognized by FMC-63-scFv. Surface Plasmon Resonance (SPR) was utilized to evaluate affinity of the peptides to immobilized FMC-63. AMP versions of candidate peptides were generated. *In vitro*, human dendritic cells (DCs) were preconditioned with AMP-CD19 or soluble peptides and cocultured with autologous T cells engineered to express CD19 CARs (FMC63-28z and FMC63-41BBz). Markers for activation, proliferation, cytotoxicity, and effector functions were evaluated.

Phage Display Screening Yields FMC63-specific CD19 Surrogate Peptide Ligands



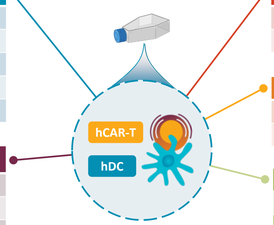
AMPlifiers Induce Phenotypic & Functional Enhancements in Cognate CAR-T Cells

T Cell Activation

Readout	Function
41BB	Activation marker
OX40	Activation marker
CD25	Activation marker
CD69	Activation marker
CD40L	Activation marker

T Cell Effector Function

Readout	Function
TNF-α	Effector cytokine
IFN-γ	Effector cytokine
IL-2	Effector cytokine



T Cell Cytotoxic Function

Readout	Function
CD107a	Degranulation
Granzyme	Cytotoxic effector

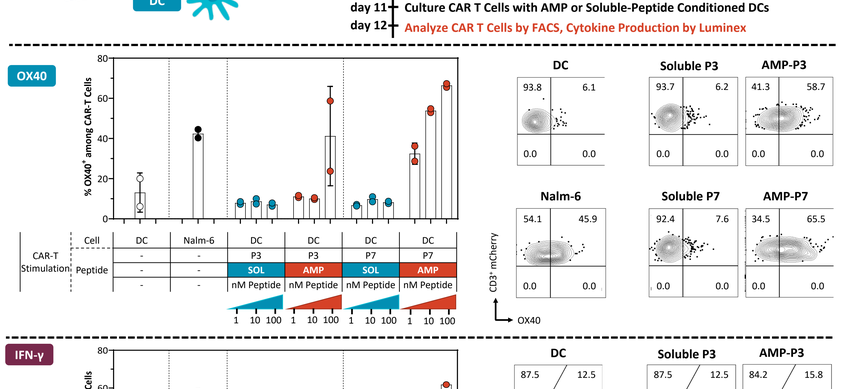
T Cell Phenotype and Memory

Readout	Function
CCR7	Memory phenotype
CD45RO	Memory phenotype

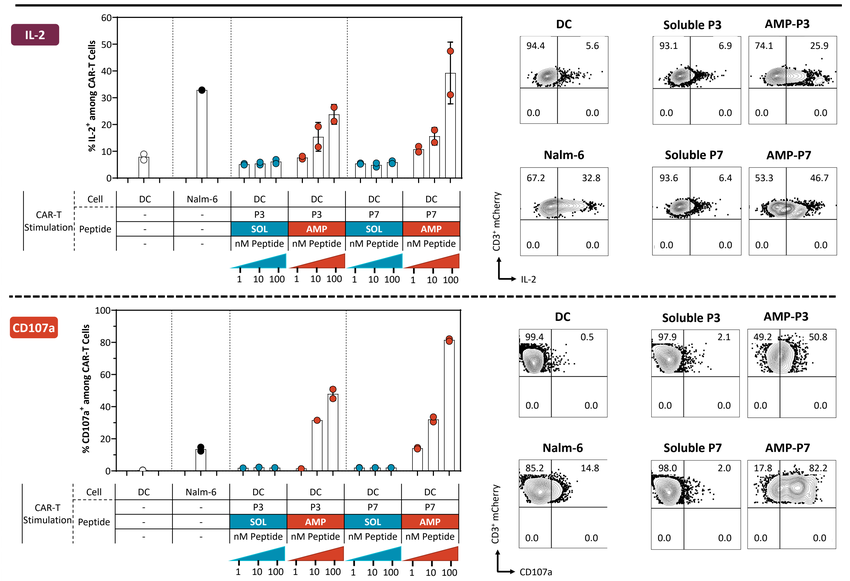
T Cell Proliferation

Readout	Function
Ki67	Proliferation
CFSE	Proliferation

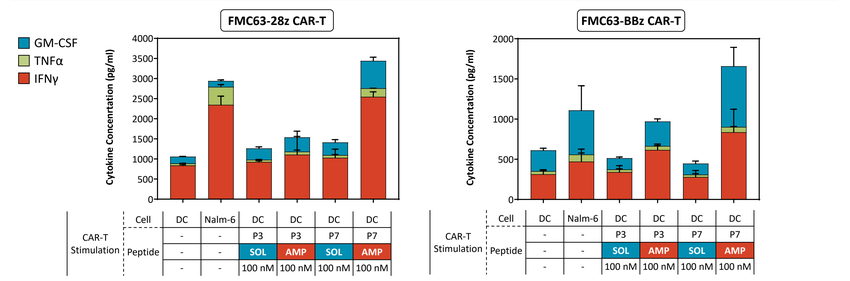
CD19 AMPlifiers Stimulate Human FMC63-28z CAR-T cells



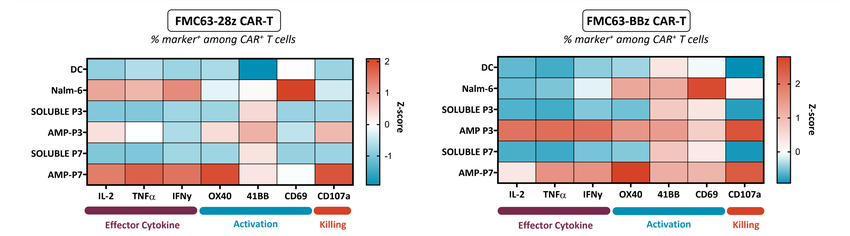
CD19 AMPlifiers Stimulate Human FMC63-28z CAR-T cells



CD19 AMPlifiers Stimulate Cytokine Secretion by Human FMC63-28z CAR-T cells



CD19 AMPlifiers Induce Coordinated CAR-T Activation and Effector Function



Summary

- AMP-peptides (AMPlifiers) effectively accumulate in lymph nodes, decorate lymph node resident APCs and boost CAR-T activation, and expansion *in vivo*.
- Phage display screening produces surrogate peptide mimotopes with specific binding affinity for CAR scFv domains such as FMC63.
- *In vitro,* CD19 AMPlifiers induce phenotypic activation, cytotoxic and effector function in cognate CD19 CAR-T cells with 28z or BBz signaling domains.
- The AMP platform can potentially be utilized as a mechanism to expand and functionally enhance CAR-T cells *in vivo* targeting blood and solid tumors.

1. Maude SL *et al.* Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N. Engl. J. Med* 378, 439–448 (2018).
2. Park JH *et al.* Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N. Engl. J. Med* 378, 449–459 (2018).
3. Ma L *et al.* Enhanced CAR–T cell activity against solid tumors by vaccine boosting through the chimeric receptor. *Science* 365(6449):162-168 (2019).