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AMPHIPHILE-PEPTIDE BOOSTING WITH FMC63-BINDING SURROGATE PEPTIDE MIMOTOPES INDUCES ACTIVATION AND POTENT EFFECTOR FUNCTION IN CAR-T CELLS TARGETING CD19Peter DeMuth, Amy Tavares, Ana Castano*. *Elicio Therapeutics, Cambridge, MA, USA*

Background Genetic engineering of T cells to express anti-CD19 Chimeric Antigen Receptors (CAR-T cells) has been FDA approved for the 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.

Methods We performed phage-screening and enrichment for CD19 surrogate peptides recognized by FMC-63-scFv. Surface Plasmon Resonance (SPR) was utilized to evaluate the affinity of the peptides to immobilized FMC-63. AMP versions of 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. In vivo experiments were performed to evaluate the biodistribution of peptides. Luciferase-expressing murine CAR-T cells were engineered to evaluate the expansion and biodistribution of CAR-T cells in combination with AMP or soluble regimens.

Results We found surrogate CD19 peptide mimotopes that bind to FMC-63 with different affinities evaluated by ELISA and SPR. Assessment in human autologous DC/CAR-T cell cocultures demonstrated that AMP-CD19 peptides can decorate DCs effectively and promote potent activation (OX40, 41BB, CD69), proliferation, cytokine production (IFN γ , TNF α , and IL2), cytotoxicity (CD107a), and phenotypic enhancement of CD19-specific CAR-T cells. Assessment in vivo showed that AMPs are effectively delivered to LN where endogenous APCs are decorated to promote the activity of murine CAR-T cells.

Conclusions In vitro, AMP modification of CAR-binding peptide mimotopes induces activation, cytotoxicity, and effector functions of CAR-T cells. These AMP-peptides effectively accumulate in LN and boost CAR-T activation and expansion in vivo. This platform can potentially be utilized as a mechanism to expand and functionally enhance CAR-T cells in vivo for blood and solid tumors.

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Ethics Approval All animal experiments in this study were performed in accordance with the approval of IACUC Protocol CR-0039.

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