

Naive-biased *in vivo* CAR-T cell therapy

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***In vivo* CAR-T therapy offers a scalable, off-the-shelf alternative to complex *ex vivo* CAR-T manufacturing, yet the field struggles to achieve efficient T-cell transduction in the human body without inducing systemic T-cell activation-induced toxicity. In a study published in *Vita*, Ma and colleagues demonstrate that AAV6-M2, a capsid variant targeting CD62L⁺ naive T cells, achieves high transduction of T cells with durable CAR expression, reverses pathology in a humanized model of systemic lupus erythematosus, and exhibits markedly reduced liver tropism compared to wild-type AAV6.**

Ex vivo CAR-T cell therapy has transformed the treatment of hematologic malignancies and autoimmune diseases, achieving remarkable complete remission rates in refractory B-cell leukemias and enabling drug-free remissions in systemic lupus erythematosus (SLE)^{1,2}. However, both autologous and allogeneic CAR-T therapies face substantial translational barriers. Autologous manufacturing requires individualized leukapheresis, multi-week *ex vivo* expansion in specialized GMP facilities, and treatment costs in the hundreds of thousands of dollars per patient³. Allogeneic strategies circumvent personalized manufacturing but introduce risks of graft-versus-host disease and exhibit limited *in vivo* persistence. Critically, both modalities require lymphodepleting chemotherapy prior to cell infusion, thereby restricting patient eligibility and limiting institutional accessibility⁴. These constraints underscore an urgent need for simplified CAR-T generation strategies that bypass *ex vivo* manipulation while preserving therapeutic efficacy.

In vivo CAR-T therapy represents a paradigm shift addressing these limitations. By delivering CAR-encoding vectors directly to patients' circulating T cells, this strategy eliminates *ex vivo* manipulation and converts cellular immunotherapy into an "off-the-shelf" gene therapy product⁵. Manufacturing complexity and costs are substantially reduced, treatment timelines compress from weeks to hours, and lymphodepletion becomes unnecessary — preserving patients' intact immune systems.

Current *in vivo* CAR-T strategies employ viral and non-viral delivery platforms, each with critical limitations⁶. Non-viral vectors (e.g., lipid nanoparticles, polymeric nanoparticles) offer scalable manufacturing but produce transient CAR expression (days) and trigger innate immune responses and potential antibody-mediated rejection, which constrain the frequency and feasibility of repeat administrations. Lentiviral vectors with T cell-targeting ligands achieve reasonable transduction and durable expression through genomic integration, yet pose insertional mutagenesis risks and critically, require broad T cell activation for efficient transduction — triggering cytokine release syndrome, organ toxicity, and hemophagocytosis.

In this issue of *Vita*, Ma and colleagues report the development of an *in vivo* CAR-T delivery platform based on engineered adeno-associated viral (AAV) vectors⁷. AAV vectors offer compelling advantages for *in vivo* gene delivery: episomal persistence minimizes insertional mutagenesis risk; multiple FDA-approved therapies (Luxturna, Zolgensma, Hemgenix) establish a clinical safety precedent; and compact packaging capacity accommodates CAR constructs⁸. However, traditional AAV serotypes exhibit poor tropism for T cells, limiting their application for *in vivo* CAR-T generation. Ma and colleagues overcome this barrier through AI-guided directed evolution of AAV6 capsid VP3 libraries, identifying AAV6-M2, a variant that enables efficient, safe, and durable *in vivo* CAR-T therapy.

This study demonstrates four critical translational innovations (Fig. 1). First, the AI-guided capsid engineering platform establishes a generalizable pipeline for developing tissue- and cell-specific AAV variants. Systematic screening of VP3 mutant libraries enables the generation of vectors targeting any immune cell subset or organ system beyond T cells, representing a scalable framework for precision gene delivery. Second, AAV6-M2 selectively transduces CD62L⁺ naive and central memory T cells, populations with superior proliferative capacity and self-renewal potential⁹. These engineered cells generate functional CAR-T products that sustain therapeutic levels for 6 weeks in humanized mouse models, representing the first successful *in vivo* reprogramming of quiescent human T cell subsets. Third, AAV6-M2 achieves high transduction efficiency in primary human T cells without requiring scFv-mediated targeting or T cell activation, thereby circumventing activation-associated toxicities inherent to conventional delivery systems. Fourth, capsid surface charge engineering confers cross-species hepatic de-targeting, reducing liver tropism by > 100-fold in both mice and cynomolgus macaques compared to wild-type AAV6. This modification significantly mitigates hepatotoxicity risk while enhancing vector bioavailability at target lymphoid compartments.

In humanized SLE models, single-dose AAV6-M2 administration demonstrates profound disease-modifying efficacy, reversing lupus nephritis and systemic pathology. These findings validate therapeutic potential for autoimmune disease intervention and collectively position AAV6-M2 as a clinically viable off-the-shelf *in vivo* CAR-T platform, warranting human trials to assess safety, immunogenicity, optimal dosing, and therapeutic durability across diverse disease contexts.

Despite these advances, several translational challenges warrant further investigation. A fundamental limitation across all *in vivo* CAR delivery platforms is achieving T cell-selective transduction. The absence of truly T cell-specific surface receptors creates risk of off-target toxicity, wherein even low-

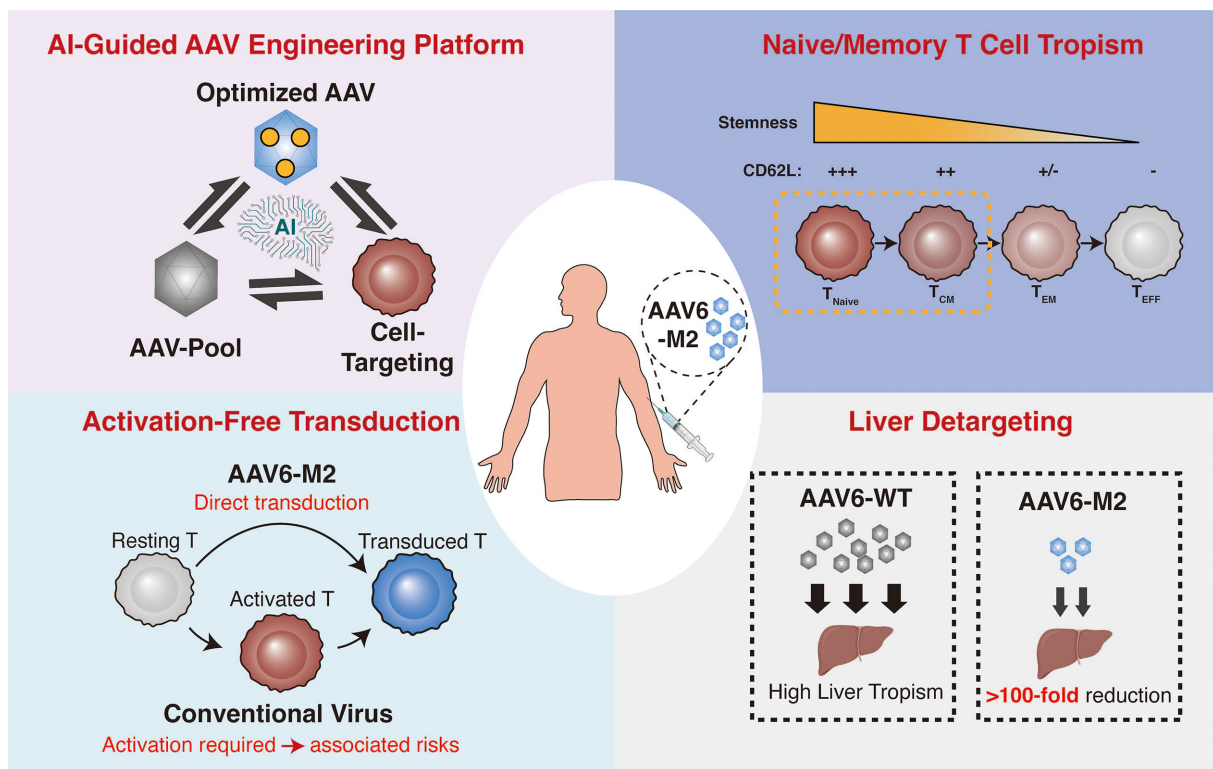


Fig. 1 AI-guided engineering of AAV6-M2 enables activation-free *in vivo* CAR-T therapy through naive T cell targeting. AI-guided capsid optimization platform (top left) generates AAV6-M2 with preferential tropism for CD62L-expressed naive and central memory T cells (top right), enabling direct transduction of resting T cells without activation-associated toxicity (bottom left) and > 100-fold reduction in liver tropism compared to wild-type AAV6 (bottom right). This figure was created in part using BioRender.

level transduction of non-T cells expressing the targeting ligand could trigger unintended immune dysregulation or functional impairment. For AAV6-M2, CD62L expression extends beyond T cells to include NK cells, monocytes, and B cell subsets, necessitating additional safeguards. Incorporation of T cell-restricted promoters represents one strategy to confine CAR expression exclusively to T lineages, mitigating off-target risk while preserving broad T cell transduction. A second challenge involves therapeutic durability and redosing constraints. Recent studies suggest that certain genetically driven forms of SLE require sustained CAR-T persistence for long-term disease control, whereas transient B cell depletion by CAR-T therapy can lead to disease relapse in these SLE cases¹⁰. However, episomal AAV genomes dilute with cell division and preexisting anti-capsid antibodies preclude repeat dosing in seropositive individuals. An AAV system utilizing compact genome editors such as AsCas12f for site-specific CAR integration offers a potential solution. This approach combines AAV6-M2's *in vivo* delivery efficiency with stable chromosomal insertion to achieve heritable, dilution-resistant expression. Beyond AAV6-M2 itself, this study provides a conceptual blueprint for alternative *in vivo* T cell engineering strategies: CD62L nanobody-conjugated lipid nanoparticles, alternative naive/memory markers (CCR7, IL-7R α), or other capsid variants may leverage similar principles to preferentially target quiescent T cells, collectively advancing the development of next-generation off-the-shelf cellular immunotherapies.

COMPETING INTERESTS

The author declares no competing interests.

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ADDITIONAL INFORMATION

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