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From evolutionary selection to the possibility of design: Engineering CAR-T cell states beyond nature

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CAR-T cell therapy has transformed the treatment of hematological malignancies, yet its broader therapeutic potential remains constrained by a fundamental problem: T cell exhaustion. Persistent antigen stimulation progressively impairs T cell proliferation, persistence, and cytotoxicity while driving cells into a transcriptionally and epigenetically encoded exhaustion program. The central challenge is therefore to engineer T cell states that sustain antitumor function under prolonged antigen stimulation. One strategy has been to manipulate transcription factors (TFs) that naturally regulate T cell differentiation and function. The AP-1 family provides a prominent example. In 2019, Lynn and colleagues reported that exhausted CAR-T cells exhibited increased chromatin accessibility at AP-1-associated regulatory elements and that enforced c-Jun expression could partially counteract this dysfunctional state.[1] This work supported AP-1 modulation to improve CAR-T cell function and contributed to the development of LYL797, a clinical-stage ROR1-targeted CAR-T therapy incorporating c-Jun overexpression.[2] The new study by Takacsi-Nagy and colleagues in *Cell* takes this concept one step further.[3] Rather than asking which natural TF should be modulated, the authors ask a more ambitious question: what if the optimal TFs for a therapeutic T cell do not exist in nature?

BEYOND THE NATURAL REPERTOIRE

Takacsi-Nagy et al. developed DESynR (domain engineered via synthesis and recombination), an evolution-inspired strategy that deconstructs TF families into N-terminal effector domains (NEDs), DNA-binding domains (DBDs), and C-terminal effector domains (CEDs), and recombines these modules into synthetic architectures. Starting from 24 natural AP-1 family members, the authors generated 13,824 synthetic TFs and screened them directly in primary human CAR-T cells. Selected factors induced broad transcriptional and epigenetic remodeling, generating unconventional T cell states with enhanced persistence, effector function, and cytotoxicity alongside a dampened exhaustion program. Notably, synthetic factors largely shared regulatory elements with natural AP-1 counterparts rather than establishing a new binding landscape. Thus, DESynR appears to alter how existing regulatory elements are interpreted, shifting the engineering problem from TF abundance to domain organization. Its downstream effects on dimerization, cofactor engagement, and chromatin interactions remain proposed mechanisms, rather than independently engineered variables. Beyond AP-1, DESynR libraries from ETS and forkhead box (FOX) families also outperformed natural counterparts in proliferation/persistence screens, supporting broader applicability. Furthermore, high-performing architectures incorporated domains from parental TFs with low endogenous expression in T cells. For example, although *FOXR1* is expressed at very low levels across T cell subsets, its N-terminal domain was present in 80 of 129 enriched DESynR FOX factors. A representative *FOXR1*-N-containing construct, *FOXR1-FOXO3-FOXJ1*, improved proliferation under repetitive stimulation and modestly enhanced CAR-T cell cytotoxicity, unlike natural *FOXR1*, revealing latent functional potential that could not be predicted from expression of the intact natural factor.

Conceptually, DESynR shifts the basic unit of transcriptional engineering from the intact TF to its constituent functional domains. This parallels the evolution of CAR engineering, in which antigen-binding, hinge, transmembrane, costimulatory, and signaling modules can be independently recombined. DESynR extends this modular logic downstream of the receptor to the regulatory machinery that determines T cell state.

This broader ambition to construct regulatory systems beyond their natural configurations is well established in synthetic biology. Synthetic gene circuits, programmable and orthogonal TFs, and engineered regulatory elements have long been used to impose artificial gene expression programs and cellular states. DESynR builds on this foundation but follows a distinct design principle: rather than assembling predefined regulatory circuits or engineered TF–promoter pairs, it systematically recombines naturally evolved TF domains and uses phenotypic screening to identify functional architectures directly in primary human T cells. Its distinctive contribution therefore lies in coupling large-scale domain recombination with functional selection to explore TF architectures beyond those represented in the natural repertoire.

WHEN EVOLUTION AND THERAPEUTIC GOALS DIVERGE

Among the strongest-performing synthetic regulators was *JUN-FOS-BATF*, which enhanced CAR-T cell function under repeated antigen stimulation while promoting features associated with persistence and maintained cytotoxicity. These effects were observed across multiple CAR specificities and T cell subsets, supporting the broader relevance of the architecture. Importantly, *JUN-FOS-BATF* outperformed not only each of its parental AP-1 TFs individually, but also their combined overexpression, indicating that its advantage arose from the synthetic architecture rather than simply from increasing the abundance of the same natural components. More provocatively, such an architecture would have been difficult to predict from the natural AP-1 repertoire. Evolutionary pressures favored organismal and reproductive fitness rather than the specific demands of CAR-T therapy, including persistence under repeated stimulation, resistance to tumor-induced dysfunction, and survival within an immunosuppressive tumor microenvironment. A TF architecture favored by natural selection therefore need not be optimal for therapeutic engineering. DESynR addresses this gap by treating naturally derived TF domains as modular building blocks for constructing synthetic regulatory architectures optimized for therapeutic function.

FROM GENE SELECTION TO CELL-STATE DESIGN

This concept becomes particularly important when DESynR is considered alongside previous efforts to manipulate transcriptional regulators such as c-Jun, FOXO1, FOXP1, KLF2, and BACH2.[4] The case of BACH2 is instructive: BACH2 restrains terminal differentiation by competing with Jun-family AP-1 factors for the same enhancer elements, and its downregulation upon activation frees those sites for AP-1 occupancy. DESynR's central mechanistic finding—that synthetic AP-1 factors reshape pre-occupied regulatory sites rather than establish novel binding—suggests a broader principle: therapeutic control may not require access to new regulatory territory, but rather a new way of interpreting the existing landscape. DESynR achieves this by redesigning the factor itself rather than tuning competition among natural factors. Previous approaches follow a gene-centric paradigm: identify a natural regulator and modulate its activity. DESynR asks a different question: can a regulatory architecture be designed to integrate multiple desirable properties simultaneously? This is particularly relevant to CAR-T therapy because CAR-T cells must remain cytotoxic without becoming terminally differentiated, proliferate without losing functional competence, and persist without entering exhaustion. The screen itself illustrates this tension directly: some of the strongest persistence-promoting architectures (e.g., *MAFF-ATF3-BATF2*) developed a pronounced cytotoxicity defect under prolonged stimulation, whereas *JUN-FOS-BATF* was selected for further development because it retained cytotoxicity while extending persistence. Furthermore, selected DESynR factors also opened chromatin regions that were poorly accessible in control T cells and induced transcriptional programs normally associated with non-T cell lineages, demonstrating that synthetic regulators can access

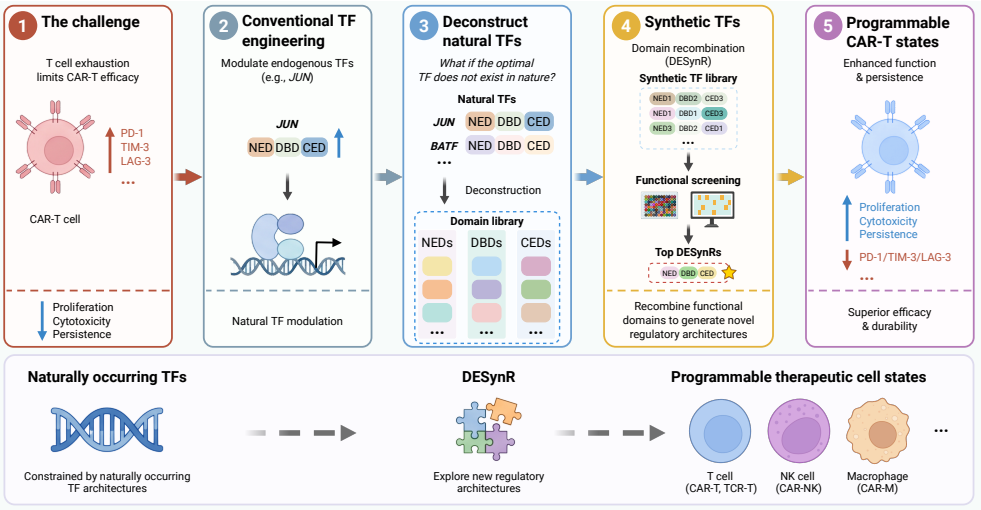


Figure 1. From natural transcription factor engineering to programmable therapeutic cell states Chronic antigen stimulation drives CAR-T cells toward exhaustion, limiting proliferation, cytotoxicity, and persistence. Conventional approaches primarily focused on modulating naturally occurring TFs, such as c-Jun, to improve CAR-T cell fitness. In contrast, DESynR adopts a modular approach by deconstructing natural TFs into functional domains, including N-terminal effector domains (NEDs), DNA-binding domains (DBDs), and C-terminal effector domains (CEDs). These domains are recombined to generate libraries of synthetic TFs, followed by functional screening to identify high-performing candidates. By creating novel regulatory architectures, DESynR expands the accessible regulatory space and enables the engineering of CAR-T cell states with enhanced proliferation, cytotoxicity, and persistence and reduced exhaustion. Beyond CAR-T cells, this strategy may offer opportunities to program other therapeutic immune cells, including CAR-NK cells and CAR-macrophages. Created with BioRender.com.

states beyond conventional T cell differentiation programs. Cell engineering may therefore aim to create new composite states tailored to therapeutic demands. In this framework, CAR-T engineering progresses from receptor design, to signaling optimization, to manipulation of transcriptional and epigenetic programs, and ultimately to the design of the regulatory architecture that controls cellular state itself.

THE TRANSLATIONAL BOUNDARY

The conceptual novelty of DESynR should nevertheless be balanced against substantial translational challenges. First, the current evidence remains preclinical. Although synthetic regulators were successfully implemented in human T cells, therapeutic activity was evaluated primarily in immunodeficient mouse models, and whether these engineered states retain their advantages in more physiologically relevant settings remains unclear. Second, the regulatory plasticity created by synthetic TFs is inherently a double-edged sword. Although characterized DESynR factors predominantly occupied established regulatory sites, unintended genomic interactions across the broader synthetic library have not been systematically excluded, and the long-term transcriptional consequences, stability, and safety of sustained expression remain incompletely defined. Third, coordinated expression of a CAR and a synthetic TF adds manufacturing complexity, including increased vector payload and additional demands on expression control, potency testing, product characterization, and lot-to-lot comparability. The biologically strongest construct may therefore not be the most clinically tractable. Fourth, the full-library screens primarily selected for proliferation and persistence under repetitive antigen challenge, whereas other properties, including stemness, effector function, cytotoxicity, and in vivo performance, were examined only in smaller follow-up panels. Other therapeutically relevant dimensions, such as metabolic fitness and trafficking or homing capacity, were not comprehensively assessed. This single-axis selection strategy may therefore overlook architectures with a more balanced therapeutic phenotype, while most synthetic constructs remain functionally unresolved beyond the primary screening axis. Finally, the generalizability of individual DESynR regulators across CAR architectures, antigen targets, tumor contexts, and T cell subsets remains to be established. Intellectual-property and commercial interests should also be considered when interpreting the pace and direction of clinical development.

ENGINEERING BEYOND EVOLUTION

The broader significance of DESynR may therefore lie less in any single synthetic TF than in the paradigm it establishes (Figure 1). DESynR proposes a more ambitious alternative: natural sequences can be treated as starting points rather than endpoints. For CAR-T therapy, this shift may be particularly consequential because the demands placed on engineered T cells differ from those shaping physiological T cell responses, and the optimal therapeutic state need not already exist within the natural T cell repertoire. The field may thus be moving from selecting natural regulatory programs to designing synthetic cellular states. DESynR provides an important proof of principle—already extended beyond AP-1 to the ETS and FOX families—that protein architectures can be systematically recombined and functionally screened to explore regulatory configurations beyond those represented in nature. The next frontier of CAR-T engineering may not be the discovery of another naturally favorable TF, but the deliberate construction of regulatory architectures—and ultimately cellular states tailored to the demands of therapy.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT (OpenAI) to improve the language and readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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AUTHOR CONTRIBUTIONS

Xuebing Leng drafted and revised the manuscript. Taidou Hu conceived the commentary and critically revised the manuscript. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.