CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNOTHERAPIES: TARGETING NEW FRONTIERS

SPOTLIGHT

COMMENTARY

# *In vivo* CAR therapy: quo vadis?

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*In vivo* CAR therapies are rapidly emerging as a strategy to address the scalability, access, and toxicity limitations associated with conventional *ex vivo* CAR-T cell products. This article reviews viral and LNP–RNA platform technologies, emerging clinical evidence, and key safety and regulatory considerations relative to established CAR-T and immune cell engager approaches. While early progress is encouraging, careful translational evaluation is essential to determine whether *in vivo* CAR strategies can ultimately meet or surpass current standards of care across oncology, autoimmunity, and other indications.

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Last decade witnessed the advent of engineered T cell therapies to the therapeutic armamentarium in oncology, through several approved autologous CAR and TCR-engineered cell products [1] and some exciting emerging evidence of CD19 and BCMA-directed CAR-T cell efficacy in B cell involved autoimmune disorders [2]. Amongst the lessons learned to date, especially with viral-engineered CAR-T cell products, the remarkable efficacy in certain B cell malignancies translating to cures in many patients stands out. Nevertheless, scalability, patient access limitations, and toxicities owing to utilization of lymphodepletion conditioning, difficult-to-predict or manage on target toxicities, and caveats related to utilization of genomically integrated viral vectors, ignited considerable interest in novel technologies. Notably, less than 30% of eligible patients with large B cell lymphoma (LBCL) receive CAR-T treatment, largely due to manufacturing constraints and rapid disease progression during the waiting period.

Hence, major efforts have been deployed during the last few years in bringing together the unprecedented potency afforded by CAR-T therapy with the scalability of engineered viral vectors and RNA-nanomedicines for direct *in vivo* engineering of immune cells [3]. *In vivo* CARs are being developed to overcome these limitations by shifting the manufacturing burden away from complex, patient-specific cell processing towards standard drug manufacturing, aiming for both off-the-shelf availability and applicability to outpatient setting thereby augmenting access. These translated into a rapidly expanding ecosystem of likely more than 35 biotechnology companies operating predominantly

in China and the US, with at least eight of them reaching a clinical stage. The two main technologies are based on engineered lentiviral vectors modified for selective cell targeting, and RNA formulated in lipid nanoparticles (LNPs) targeted mostly through antibody-based functionalization to enable select lymphocytes engineering – with key features, advantages and limitations described in detail elsewhere [3]. This remarkable progress catalyzed by the involvement of large pharmaceutical companies through strategic partnerships or merger acquisitions announced predominantly during the last two years will likely result in an even more rapid pace of expansion of this field.

Based on the work publicized to date, several themes are emerging. First, by utilizing optimized viral or LNP–RNA platforms, one can achieve a biologically and potentially clinically relevant level of CAR engineering of immune cells *in vivo* [4,5]. While this is limited for now to B cell lineage/plasma cell antigens in multiple myeloma and lupus respectively, the rapid kinetics of CAR-engineering and of the pharmacologic effect are notable. There is also emerging clinical proof of biology with a macrophage-tropic nanoformulation loaded with an anti-Trop2 CAR in mRNA format in solid tumors. Secondly, from a safety perspective, a profound pharmacologic effect is accompanied by toxicities – similar to conventional *ex vivo* engineered CAR-T cell products – that can be managed utilizing low dose corticosteroids and IL-6R blockade. In fact, pre-emptive utilization of low dose dexamethasone is quite prevalent for LNP–RNA platforms to preempt exaggerated acute phase response owing to infusion of nanoparticles. Third, it is feasible and safe to repeat infusions of LNP–RNA formulations over a limited interval, to generate sufficiently large and sustained CAR-T cell populations for a pharmacologic effect to occur. Fourth, utilization of non-human primates to guide the evaluation, optimization of individual platforms and even product candidates – both engineered lentiviral vectors and LNP–RNAs – has been critically enabling for successful clinical translation. Fifth, there are diverse development and regulatory options for industry sponsors based on ecosystems with different clinical entry bars (China: lower, Australia: intermediate, and North America: higher) and availability of global contract research and manufacturing organizations supporting nimble insourcing/outsourcing strategies. Finally, the rather boisterous advent of *in vivo* CAR-Technologies and the continuous progress with immune cell engagers, is shifting the attention of investors and large biopharma away from conventional *ex vivo* engineered immune cell products carrying a negative economic impact with respect to the latter.

But is this veritable 'gold rush' solidly supported by the evidence to date, or do we need more clinical experience with *in vivo* CARs in B cell disorders and across broader disease populations? One particular concern with *in vivo* genomically integrating CAR payloads relates to uncontrollable persistence or secondary expansion of functional CAR-expressing immune cells leading to protracted or recurring on target toxicities that may require up to 15 years patient monitoring (Table 1). More specifically, for lentiviral vectors, the concern of genotoxicity stems from the potential risk of insertional mutagenesis, as the CAR gene randomly inserts into the host cell genome at site that could activate oncogenes or disrupt tumor suppressor genes, events that may trigger or facilitate a secondary malignant process. Safety monitoring should include clonal tracking, integration-site analysis, and evaluation of unexpected cell phenotypes. Emerging genomic integrating technologies that afford targeted or preferential integration sites with insulating elements and/or that carry controllable CARs (with suicide/depletion tags; small-molecule based

inducible ON/OFF switches) may overcome some of these challenges. In turn, while dose-tunable and devoid of genotoxicity, LNP-based formulations with transiently expressed constructs off RNA payloads present a range of other challenges from engineering efficiency to liver-tropism and immunogenicity – that may preclude redosing as discussed below.

Depending on these factors, we could envision a lower entry bar for the genomic integrating vector technologies in oncology as compared to autoimmunity. Conversely, the tunability and transiency afforded by RNA based *in vivo* CAR platforms may be an advantage in indications associated with high safety bar (autoimmunity). Nevertheless, key questions need to be answered with the LNP–RNA platform, including possibility to yield desired levels of CAR cell engineering more reproducibly for a durable pharmacologic effect to occur. Utilization and optimization of the targeted LNP–RNA platform, and re-dosing without immunogenicity risks seem to be critically important; time will tell whether the technologies currently in early clinical development stage meet this bar. Over-arching questions related to the broader competitive landscape and include whether the current *in vivo* CAR technologies match the potency of currently available *ex vivo* viral-engineered CAR-T cell products benefiting from a highly optimized treatment regimen including pre-conditioning by lymphodepletion. To fully displace conventional CAR-T cell products, *in vivo* approaches must meet comparable or superior clinical performance bars. Hence, we anticipate that the first wave of *in vivo* products will likely be positioned in disease indications associated with a higher safety bar but lower threshold for efficacy. Methods to augment immune cell fitness *in vivo* may greatly enable this treatment modality in particular indications where this is a limiting factor. Another question is whether *in vivo* CAR therapies in any format may carry potency, dose tunability, or safety advantages over the rapidly evolving arena of recombinant immune cell engagers. In oncology indications, in earlier lines, definition of patients at high risk for relapsing post standard of care including conventional CAR-T cell intervention – for example through monitoring minimal residual disease (MRD) – would provide an opportunity to accelerate novel treatment modalities (e.g., *in vivo* CAR-Therapies, immune cell engagers) for consolidative/curative purpose especially, particularly if their safety profile is more favorable owing to lack of chemo-based lymphodepletion These questions are likely to influence the interest and resourcing behind life cycle management of current conventional CAR-T cell technologies versus *in vivo* CAR platforms and immune cell engagers, respectively. Despite scalability hurdles, a rapid sunset for *ex vivo* engineered CAR-T cell products may not occur unless these novel technologies match their clinical efficacy.

Based on the considerations discussed above and the novelty of this area, a comprehensive translational approach will be key to characterize in detail the strengths and shortcomings of *in vivo* CAR technologies currently deployed in clinic or nearing clinical stage. Together with other aspects such as manufacturing scalability and cost of goods, this will help guide product development towards registration or back to bench for additional optimizations. For integrating viral based vectors, it will be important to continue to drive towards achieving exquisite tissue and cell selectivity, avoiding off-target tissue or cell uptake and engineering. Genotoxicity could severely limit broad utilization of this emerging modality. For LNP–RNA approaches, minimizing liver tropism, undesired uptake by macrophages, and immunogenicity will be important to enable chronic therapies safely in an outpatient setting.

Particular attention needs to be dedicated to product immunogenicity

monitoring and mitigation planning. Innate responses, liver tropism, complement activation, and dsRNA-sensing of residual dsRNA or other immunogenic impurities may drive acute infusion reactions and transient laboratory changes (e.g., changes in liver enzymes). In addition, adaptive immune responses may include anti-viral vector or LNP formulation antibodies and/or anti-CAR construct immunity that may limit the treatment efficacy or re-dosing. Finally, pre-existing anti-PEG antibodies may pose challenges for select technologies.

Next-generation approaches for *in vivo* CAR engineering in particular, and immune system reprogramming in general, will need to integrate the strengths of currently explored modalities and dial out the liabilities (Table 1). Exquisitely precise payload delivery, gene modifications including CAR sequence insertion, and RNA-based gene writing, coupled with novel features allowing tunability and spatiotemporal control of expression, will likely catalyze an expansion of molecular and cellular targets, payload architectures, and clinical indications pursued. In oncology, there are two interesting paths: first, in indications with low burden or MRD, could a safe and tunable *in vivo* CAR approach achieve the pharmacological effect to eradicate all the residual cancer cells likely disseminated throughout the body? Second, in case of bulky tumoral processes, can one orchestrate through multifaceted *in vivo* immune system engineering, an attack that would drive both tumor resolution and pre-empt clonal evasion mechanisms? Would there be a need to co-target the stroma, reprogram the tumor microenvironment, block, or exploit in other ways the numerous immune checkpoints, or co-opt broader mechanisms to achieve a meaningfully deep and durable response? Can these be achieved by *in vivo* immune engineering utilizing a larger spectrum of tools?

In indications outside oncology such as autoimmunity, major opportunities will be afforded by exploiting the mechanism

## TABLE 1

Paving the way to ***in vivo*** engineering of the immune system.

<table>
<tr><th colspan="2">Current technologies</th><th>Next-generation technologies</th></tr>
<tr><td colspan="2">• Unknown clinical safety profile owing to integration of different technologies never explored together in clinic<br>• Uncertain competitive advantages over immune cell engagers or optimized cell therapies</td><td rowspan="2">• Vectors or formulations with exquisite tissue and cell selectivity, avoiding off target tissue / target cell uptake. This includes next-generation targeted LNPs and lentiviral vectors, improved for tissue and cell tropism<br>• Diversified categories of payloads (multiplexed CAR/TCRs, biological response modifiers, checkpoints, gene editing, gene writing systems)<br>• Payload designs allowing prolonged RNA payload expression, precise genomic integration and/or enhanced spatial-temporal control of expression<br>• Novel technologies with enhanced tunability achieving desired exposure over time without cellular, genomic sequelae or immune reactions<br>• Advances with analytical characterization, preclinical, clinical pharmacology analysis and regulatory environment, leading to streamlined development pathways.</td></tr>
<tr><td>Lentiviral vectors<br>• Relatively stochastic integration sites<br>• Potentially difficult monitoring for chronic or recurring toxicities<br>• Possible off-target cell transduction in cells such as macrophages or target cells<br>• Uncertain applicability to indications with ↑ safety bar (e.g. autoimmunity)<br>• Manufacturing hurdles (scale up, product characterization)</td><td>RNA–LNP Format<br>• Transfection limited by uptake, endo-lysosomal escape, translational capacity, or other mechanisms<br>• Transient engineering profile; unknown high potency, fitting indications with ↑ efficacy bar<br>• Possible off-target cell uptake; liver and myeloid cells tropism<br>• Potential immunogenicity; uncertainty whether it can be administered chronically</td></tr>
</table>

LNP: lipid nanoparticle. TCR: T cell receptor. CAR: chimeric antigen receptor.

of 'immune reset' aimed to impart durable, drug-free responses [2]. This generally involves the transient yet global (blood and tissues) elimination of all immune cells including autoreactive memory cells, followed by the regeneration of a normally functioning immune system largely devoid of pathogenic immune cells. This can result, at least in select disease indications, in a prolonged clinical benefit and even partial or complete recovery or organs functional capacity without requiring concomitant treatments. Nevertheless, as the field is nascent, major questions still need to be answered, including whether a transient yet global B cell depletion, for example, is sufficient to impart a drug-free durable response, especially in disease indications where predisposing genetic factors or other immune populations co-drive the pathogenesis. Hence, will it be possible to 'reset' other components of the immune system such as T cells and even innate immunity, and/or co-deploy counter-regulatory mechanisms such as T regulatory cells with applicability beyond autoimmune disorders and transplantation? Finally, will it be possible to correct, *in vivo*, genetic defects with strong phenotypic penetrance, which predispose subjects to serious immunological disorders? The therapeutic tools primarily exploited in oncology and autoimmune settings, could ultimately be applicable across a much broader realm of indications including regenerative medicine, heralding the age of this new treatment modality: *in vivo* engineering of cells and tissues.

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