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Commentary

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The use of targeted LNP/mRNA technology to generate functional, transient CAR T cells and treat cardiac injury *in vivo*

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Rurik *et al.*[1] published in *Science*, provide results from an elegant proof-of-concept study that modified messenger RNA (mRNA) encapsulated in targeted lipid nanoparticles (LNPs) can be delivered intravenously to produce functional engineered T cells *in vivo*. Specifically, they generated transient anti-fibrotic chimeric antigen receptor (CAR) T cells *in vivo* by delivering modified mRNA in CD5 T cell-targeted LNPs. They tested the efficacy of the approach by injecting CD5-targeted LNPs into mice with Ang II/PE induced heart failure. Results showed efficient delivery of modified mRNA encoding the CAR to T lymphocytes, which produced transient yet effective CAR T cells *in vivo*. Then, by producing CAR T cells that were anti-fibrotic, they showed reduced fibrosis and restored cardiac function after Ang II/PE injury in mice.

WHAT ARE CAR T CELLS?

CAR T cells are genetically engineered to produce an artificial T cell receptor, giving the T cells the ability to target a specific protein. The receptors are chimeric because they combine both antigen-binding and T cell activating functions into a single receptor.

CAR T cells were originally developed for use in cancer immunotherapy, where T cells are harvested from the patient, and are genetically altered to express a specific CAR, which targets an antigen that is present on the surface of tumours but not on healthy cells. They are then infused back into the patient to target and destroy the cancer cells. CAR-T cells have shown astonishing results in the treatment of mostly relapsed or refractory (r/r) haematological malignancies, with the first CAR T cell therapies being FDA-approved in 2017, and there are now 5 approved CAR T therapies. Over 500 clinical trials analysing CAR T cells for the treatment of cancer are currently underway worldwide[2].

The Epstein group have previously demonstrated that adoptive transfer of CAR T cells that target and eliminate activated fibroblasts resulted in a significant reduction in cardiac fibrosis and restoration of function after injury in mice[3]. However, the indefinite persistence of infused CAR T cells could pose a risk with future diseases. Rurik *et al.*[1] have set out to leverage the power of nucleoside-modified mRNA technology to develop a transient anti-fibrotic CAR T therapeutic *in vivo*.

WHAT ARE LNPs?

LNPs are spherical vesicles made of ionisable lipids, which are positively charged at low pH and neutral at physiological pH (reducing potential toxic effects). Due to their size and properties, LNPs are taken up by cells via endocytosis. Then because of the ionizability of the lipids at low pH, this enables endosomal escape, which permits the release of the cargo into the cytoplasm. Moreover, LNPs usually contain a helper lipid to promote cell binding, cholesterol to fill the gaps between the lipids, and polyethylene glycol for stability[4].

Over the last few years, LNPs have been formulated and optimised for nucleic acid delivery. Once in the body, mRNA-loaded LNPs are endocytosed by various cell types and shortly after cellular uptake, the mRNA escapes the endosome, releasing the mRNA into the cytoplasm, where it is transiently transcribed before degrading[5].

Most recently, LNP-mRNA technology has underlined recent successes in the rapid development of BioNTech/Pfizer's BNT162b2 and Moderna's mRNA-1273 COVID-19 vaccines. However, before the COVID-19 vaccines, Patisiran, a lipid nanoparticle-based short interfering RNA (siRNA) drug for the treatment of polyneuropathies induced by hereditary transthyretin amyloidosis, was the first clinically approved RNAi therapeutic[6]. Therefore, LNP-mRNA technology holds great promise for therapeutic strategies to treat various diseases.

Targeting antibodies can be decorated on the surface of LNPs to direct uptake (and mRNA expression) to specific cell types. Rurik *et al.*[1] (2022) hypothesised that an LNP directed to T lymphocytes could deliver sufficient mRNAs to produce functional CAR T cells *in vivo* [Figure 1]. As mRNA is restricted to the cytoplasm and is incapable of genomic integration, intrinsically unstable, and diluted during cell division, these CAR T cells would be, by design, transient[1]. This study was carried out in a mouse model.

First, Rurik *et al.*[1] generated modified nucleoside-containing mRNA encoding a CAR designed against fibroblast activation protein (FAP) (a marker of activated fibroblasts) and packaged it in CD5 T Cells-targeted LNPs (referred to as "targeting antibody/LNP-mRNA cargo" or CD5/LNPFAPCAR) [Figure 1]. They showed that *in vitro*, CD5-targeted LNPs delivered their mRNA cargo to most T cells (~80%) in culture, which expressed FAPCAR. These LNP-generated CAR T cells were able to kill FAP-expressing target cells (~50% efficiency) in a dose-dependent manner *in vitro*, similar to virally engineered FAPCAR T cells. Similar gene transfer was found for human T cells *in vitro*.

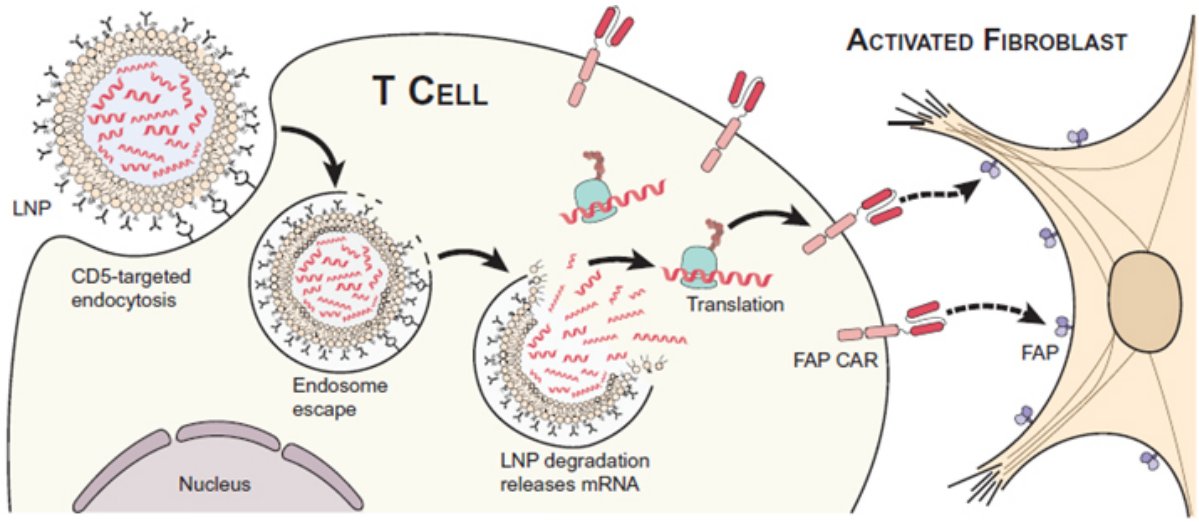

Figure 1. The molecular events showing the generation of transient FAPCAR T cells using CD5-targeted LNPs. Adapted from Rurik *et al.*[1]. LNPs: Lipid nanoparticles.

Second, they assessed whether CD5-targeted LNP mRNA could also efficiently reprogram T cells *in vivo*. Mice were intravenously injected with CD5/LNPs containing luciferase mRNA (CD5/LNP-Luc), and abundant luciferase activity was found to be expressed in splenic T cells. In another experiment, CD5/LNPs loaded with mRNA encoding Cre recombinase (CD5/LNP-Cre) and injected into Ai6 Cre-reporter mice (Rosa26CAG-LSL-ZsGreen) showed genetic recombination (ZsGreen expression) specifically in CD3+ T cells (both CD4+ and CD8+ subsets) (~80%) but not in CD3- (non-T) cells (~15%) (mainly representing B cells, dendritic cells, and macrophages).

Third, they showed that in the Ang II/PE induced heart failure mouse model, which results in increased cardiac fibrosis, targeted LNPs delivered FAPCAR mRNA (CD5/LNP-FAPCAR) to T cells at an efficiency of 20% increase in FAPCAR+ splenic T cells, 48 hours after LNP injection. Importantly, no FAPCAR expression was found in splenic T cells 1 week after injection, demonstrating the transient nature of FAPCAR expression in this *in vivo* model. Using live imaging confocal microscopy, it was shown in AngII/PE injured FAPCAR T cell-treated mice, that FAPCAR T cells trogocytose FAP from activated cardiac fibroblasts and return FAP to the spleen. These findings support the production of functional FAPCAR T cells *in situ*.

Finally, it was shown that CD5/LNP-FAPCAR treatment produced FAPCAR T cells (~58%), which accumulated within regions of the injured heart occupied by FAP+ fibroblasts. CD5/LNP-FAPCAR treated Ang II/PE induced heart failure mice showed improved cardiac function and decreased interstitial fibrosis. The findings of in-vivo produced, transient FAPCAR T cells were consistent with the groups previous studies using adoptive transferred viral FAPCAR T cells[3].

The findings of Rurik *et al.*[1] utilising modified mRNA therapeutics by targeting LNPs to specific cell types are likely to have far-reaching applications. The generation of engineered T cells *in vivo* using mRNA, is attractive because of the transient nature of the produced CAR T cells. Although not unequivocally demonstrated in the study, the lack of CAR T cell persistence in the body in the long-term will limit the off-target and toxic effects of this therapeutic approach. As pointed out by the authors, unlike patients with cancer, those suffering from disorders like fibrotic heart failure may not require a complete elimination of

pathologic cells (activated fibroblasts) but may symptomatically benefit from an overall reduction in the burden of disease. Of note, cardiac fibrosis was decreased by almost half (~4.5% to 2.5%) following CD5/LNP-FAPCAR-treatment in Ang II/PE induced heart failure mice. A 'partial elimination' approach would be relevant to heart failure and disease, considering the multi-faceted role of different cell types, including immune cells, in governing disease mechanisms and remodelling processes. Moreover, the transient nature of mRNA-based therapeutics makes it desirable for applications that are directed towards cell reprogramming or genome editing. Also, targeted LNP/mRNA technology allows for precise dosing and re-dosing if necessary.

How ageing would affect the efficacy of modified mRNA therapeutics and the generation of engineered T cells *in vivo* are important questions. There is overall downregulation of immune responsiveness with ageing and concomitant with this is a moderate rise in circulating inflammatory mediators - inflammaging. An aged immune system drives senescence and the systemic ageing of all solid organs, including the heart[7,8]. Furthermore, old age impairs the ability of the heart to repair and regenerate[9].

Future studies should focus on clarifying the transient nature of the *in vivo* produced engineered CAR T cells, any off-target effects, optimisation of dosing strategy, LNP composition, the role of ageing and targeting of particular cell types and disease. The impact of targeted LNP/mRNA therapeutic approaches is likely to generate 'off-the-shelf' products which are scalable, easy to apply, and cost-effective to prevent and treat a variety of diseases, including heart disease and failure.

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Not applicable.

Consent for publication

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REFERENCES

1. Rurik JG, Tombácz I, Yadegari A, et al. CAR T cells produced in vivo to treat cardiac injury. *Science* 2022;375:91-6. DOI PubMed
2. Albinger N, Hartmann J, Ullrich E. Current status and perspective of CAR-T and CAR-NK cell therapy trials in Germany. *Gene Ther* 2021;28:513-27. DOI PubMed PMC
3. Aghajanian H, Kimura T, Rurik JG, et al. Targeting cardiac fibrosis with engineered T cells. *Nature* 2019;573:430-3. DOI PubMed PMC

4. Let's talk about lipid nanoparticles. *Nat Rev Mater* 2021;6:99-99. DOI
5. Weissman D. mRNA transcript therapy. *Expert Rev Vaccines* 2015;14:265-81. DOI PubMed
6. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. *N Engl J Med* 2018;379:11-21. DOI PubMed
7. Prata LGPL, Ovsyannikova IG, Tchkonia T, Kirkland JL. Senescent cell clearance by the immune system: Emerging therapeutic opportunities. *Semin Immunol* 2018;40:101275. DOI PubMed PMC
8. Yousefzadeh MJ, Flores RR, Zhu Y, et al. An aged immune system drives senescence and ageing of solid organs. *Nature* 2021;594:100-5. DOI PubMed PMC
9. Lewis-McDougall FC, Ruchaya PJ, Domenjo-Vila E, et al. Aged-senescent cells contribute to impaired heart regeneration. *Aging Cell* 2019;18:e12931. DOI PubMed PMC