Advancing mRNA-based nanomedicines for the in vivo reprogramming of circulating T cells to treat Diffuse Intrinsic Pontine Glioma

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Diffuse Intrinsic Pontine Glioma (DIPG) is a fatal paediatric brain cancer located in the pons, protected by the blood-brain barrier (BBB), which limits therapeutic options. Chimeric antigen receptor (CAR)-modified T cells, engineered ex vivo to target antigens like GD2 on cancer cells, show promise in suppressing brain tumours[1] yet applicability in DIPG is hindered by uncontrollable effects that can lead to unforeseen off-target toxicities, high production cost, and long delivery timeframes. To address these challenges, we propose utilizing advances in mRNA therapy and nanomedicine to generate CAR T cells directly in vivo. This approach aims to produce CAR T cells in a precisely controlled and low-toxic manner to enhance treatment efficacy whilst lowering risk of side effects, thus overcoming the main disadvantages of conventional CAR-T cell therapy.

To achieve this, we synthesized lipid-based nanoparticles (LNPs) encapsulating GD2 CAR-encoding mRNA using microfluidics and mixed them with a T cell-targeting antibody for selective delivery to T cells. CAR expression in T cells in vitro was confirmed by flow cytometry, while their cytotoxicity against GD2+ DIPG neurospheres was demonstrated through cytokine release and luminescence-based viability assays, showing effective cancer cell targeting and killing.

T cell transfection with our LNPs resulted *in vitro* in >85% T cells expressing GD2-CAR at 24h, followed by a gradual decline to 30% by day 7. CAR+ T cells reduced viability of DIPG neurospheres by >50% whereas normal T cells had no effect. Upon exposure to DIPG neurospheres, CAR+ T cells secreted high concentrations of cytotoxic cytokines, including IFN-γ and TNF-α demonstrating similar anticancer effects to conventional CAR-T cells. Moreover, we studied the capacity of LNPs to transfect T cells *in vivo* to express GD2-CAR in human T cell engrafted NSG mice. Targeted LNP injections induced T cell migration to the spleen and other organs, with 40-50% of remaining T cells in circulation expressing GD2-CAR. Future studies will delineate the events driving the T cell migration and investigate the tumour migration and antitumor potential of in vivo made GD2-CAR T cells in orthotopic mouse models of DIPG.

References:

[1] Mount, C. W., Majzner, R. G., Sundaresh, S., Arnold, E. P., Kadapakkam, M., Haile, S., ... & Mackall, C. L. (2018). Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M+ diffuse midline gliomas. Nature medicine, 24(5), 572-579