Development of mRNA nanoparticles to produce chimeric antigen receptor - T cells to treat Diffuse Intrinsic Pontine Glioma

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Diffuse Intrinsic Pontine Glioma (DIPG) is a fatal paediatric brain cancer which remains incurable. The tumour develops in the pons and is protected from circulating therapeutics by the blood brain barrier. Therefore, novel therapeutics that can cross the blood brain barrier and selectively target DIPG cancer cells are needed. Chimeric antigen receptor (CAR)-modified T cells are a type of immunotherapy whereby T cells are genetically modified ex vivo to identify and kill cancer cells via CAR recognition of specific antigens on cancer cells, such as GD2 in brain cancer cells[1]. In addition, CAR T cells, similar to normal T cells, are able to infiltrate the brain and have been shown to successfully suppress the growth of brain tumours[2]. However, conventional CAR T cells cause toxicity due to uncontrolled responses and their ex vivo production process is expensive and lengthy. In an attempt to elude these limitations, we propose to investigate the most recent advances in mRNA therapy and nanomedicine to generate CAR T cells directly in vivo and in a low toxic and controlled manner.

To achieve this, we synthesised different lipid PEGylated nanoparticles (LNPs) encapsulating GD2 CAR-encoding mRNA using a microfluidics approach. To target mRNA delivery to T cells, LNPs were mixed with a T cell targeting CD3-PEG Bispecific Antibody (BsAb). Ability of mRNA-LNPs to transfect T cells to express CAR was evaluated in vitro by flow cytometry. Response and killing ability of transfected T cells was evaluated against GD2+ patient derived DIPG (Luciferase modified) neurosphere cultures, measuring cytokine release and cancer cell viability via luminescence analysis.

T cell transfection with our LNPs resulted in >85% T cells expressing GD2-CAR at 24h, followed by a gradual decline (30% T cells remaining CAR+) by 7 days post-transfection. CAR+ T cells reduced viability of DIPG neurospheres by >80% whereas normal T cells had no impact on survival. Upon exposure to DIPG neurospheres, CAR+ T cells secreted high concentrations of cytotoxic cytokines, including IFN-y and TNFa. We demonstrate our LNPs effectively generate CAR T cells, exhibiting potent anticancer effects against DIPG neurospheres in vitro. In follow-up studies we are determining the capacity of LNPs to transfect T cells *in vivo* to express GD2-CAR and will evaluate the in vivo efficacy of GD2+ T cells on reducing DIPG progression.

References:

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