Development of mRNA nanoparticles to produce CAR T cells to treat Diffuse Intrinsic Pontine Glioma

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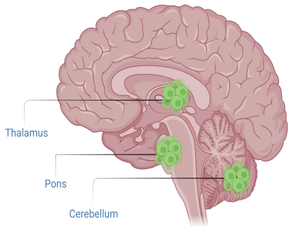
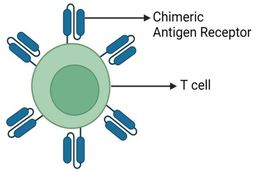
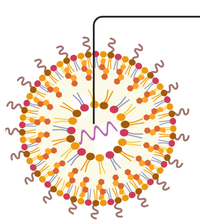
INTRODUCTION

Diffuse Intrinsic Pontine Glioma (DIPG) is a fatal paediatric brain cancer located in the pons with no cure. Novel treatment strategies are urgently needed.





Chimeric antigen receptor (CAR)-modified T cells engineered *ex vivo* show promise in suppressing brain tumours yet applicability in DIPG is hindered by off-target toxicities and high production cost.



To overcome these challenges, we propose to combine advances in **mRNA technology and nanomedicine** to generate CAR T cells directly *in vivo.*

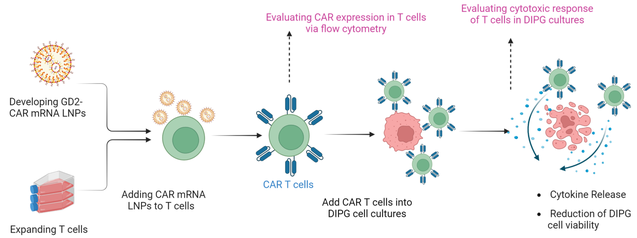
This approach aims to produce CAR T cells in a controlled and low-toxic manner thus overcoming the main disadvantages of conventional CAR-T cell therapy.

AIM

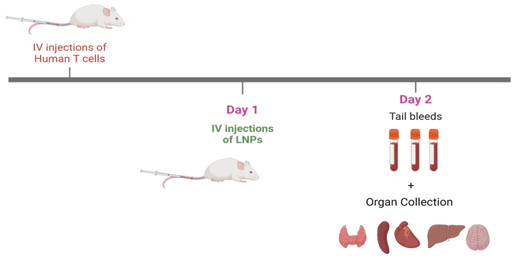
Investigate the use of injectable LNPs to deliver mRNA encoding CARs to circulating T cells *in vivo,* reprogramming these cells to recognise and suppress DIPG tumour growth.

METHODS

We synthesised lipid-based nanoparticles (LNPs) encapsulating mRNA encoding a GD2-targeting CAR, using microfluidics, and mixed these LNP with a T cell-targeting antibody for selective delivery to T cells.



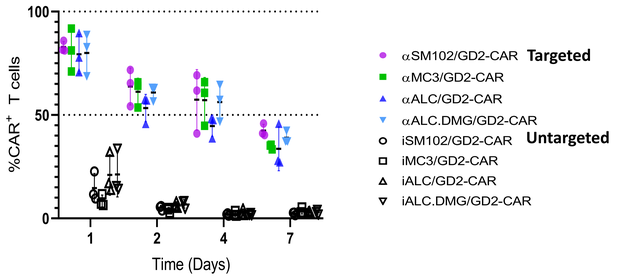
CAR expression in T cells was examined *in vitro* by flow cytometry, while their cytotoxicity against GD2+ DIPG neurospheres was demonstrated through cytokine release and luminescence-based viability assays.



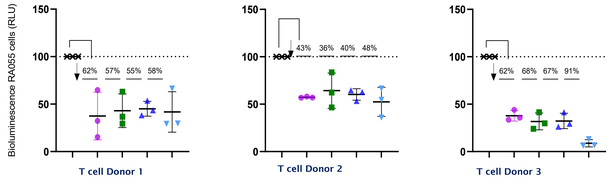
The capacity of LNPs to transfect T cells *in vivo* to express GD2-CAR was studied in humanised mice.

RESULTS

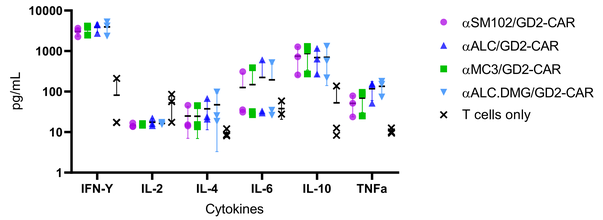
CAR expression in T cells was highest (>85% CAR+ T cells) 24h after exposure to mRNA-LNPs, followed by a gradual decline. 30% T cells remained CAR+ 7 days post-exposure.



CAR+ T cells reduced viability of DIPG neurospheres by >50% whereas normal T cells had no effect.

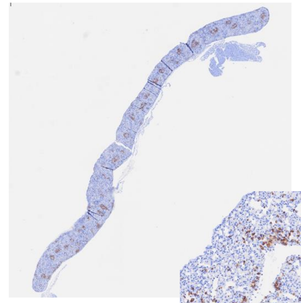


Upon exposure to DIPG neurospheres, CAR+ T cells secreted high concentrations of cytotoxic cytokines, including IFN-γ and TNF-α.

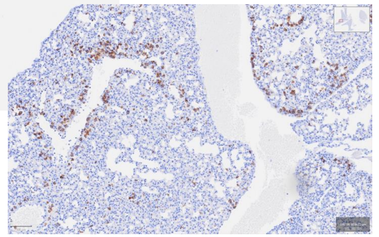


In vivo studies showed targeted LNP injections induced T cell migration to the spleen and other organs, with remaining T cells in circulation expressing GD2-CAR.

Unpublished data; please do not post



Mouse Spleen in a mice injected with targeted particles



Mouse Lung in a mice injected with targeted particles

CONCLUSION

This strategy offers the possibility to generate CAR expressing T cells using mRNA Lipid nanoparticles. Future studies will investigate the tumour targeting and antitumor potential of *in vivo* made GD2-CAR T cells in orthotopic mouse models of DIPG.

ACKNOWLEDGEMENTS

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