A targeted aptamer delivery system for glioblastoma immunotherapy

SCAN ME

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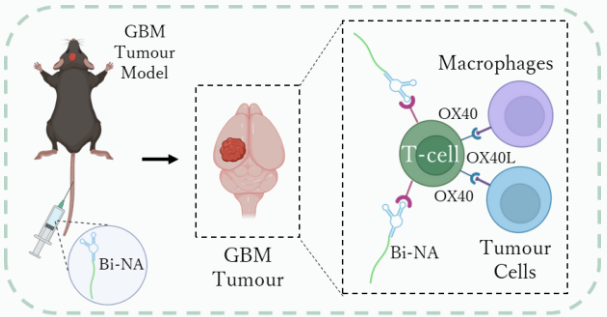
Introduction

- Glioblastoma (GB) remains immensely difficult to treat; most patients do not survive beyond 15 months after diagnosis.[1]
- The increasing number of approved nucleic acid (NA) therapeutics demonstrates their clinical potential.
- **Effective delivery of NA and minimising off-target effects remained challenging.**
- Aptamers are single-stranded oligonucleotides that bind strongly and specifically to diverse targets.[2] Aptamers can be designed as targeting ligands or delivery system, increasing efficacy and minimising side effects.
- Conjugating T-cell-specific aptamer to the NA could **guide the NA to the desired immune cells for GB treatment.**

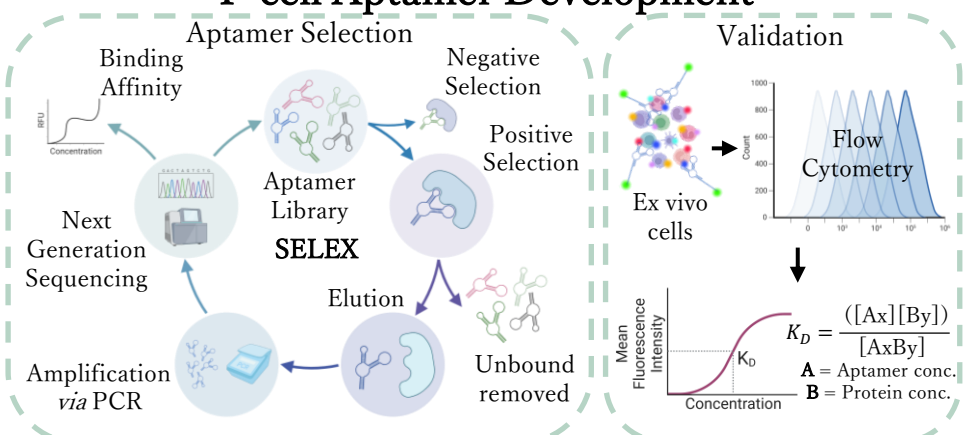
Aim

This research aims to create a therapeutic delivery system (**Bi-NA**) using novel T-cell targeting aptamer to improve the therapeutic effect of mRNA being delivered to T-cells in GB in vivo models.

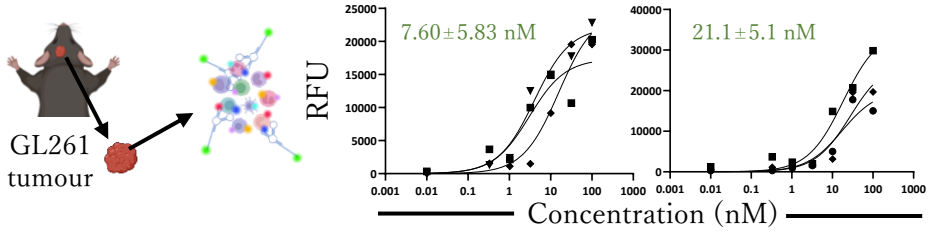


Delivering OX40 mRNA (mOX40) to T-cells using the **Bi-NA** system will overexpress this marker and **trigger an immune response** with the T-cells and their reciprocal APC.

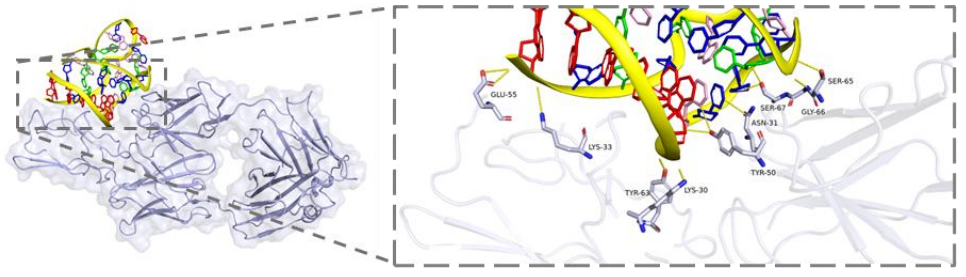
T-cell Aptamer Development



- Eight cycles of SELEX were completed on T-cell recombinant protein, using a randomised DNA library (30 nucleotides) followed by Next Generation Sequencing and sequence identification.
- The 3 top aptamer candidates were validated by in vitro and ex vivo binding affinity assays across both human and mouse T-cells.



- Selected aptamers have K_ds (binding affinity) ranging from **7.60 – 21.1 nM.**



- In silico computational docking of T-cell aptamer with there reciprocal receptor protein. Docking score: -278.05, confidence value: 0.9283.

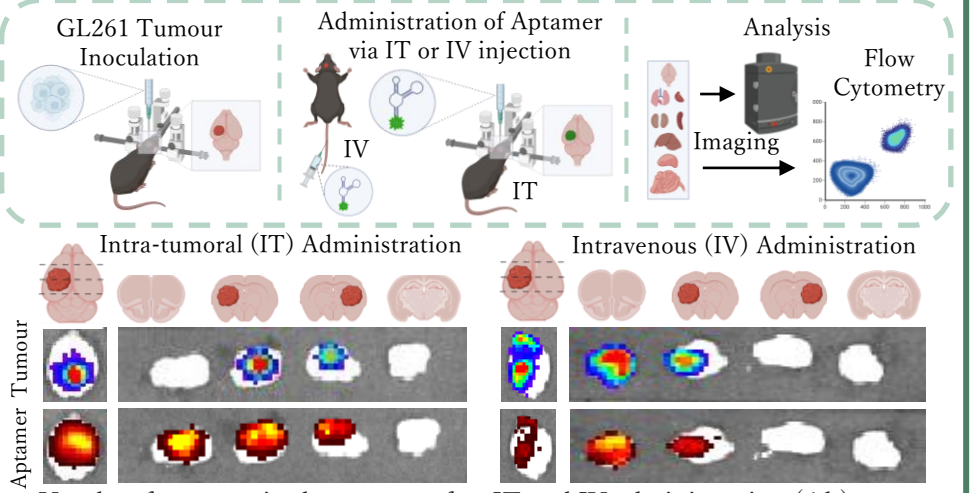
T-cell Activation

T-cell aptamer activates the T-cells to the same level as an activating antibody. Shown by ~**30% increase in CD69 expression** via flow cytometry in ex vivo splenocytes.

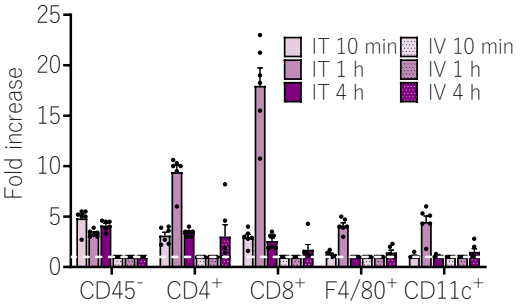
Future Work

- Validate the platform in humanised models and patient samples

Biodistribution of T-cell Aptamer in vivo

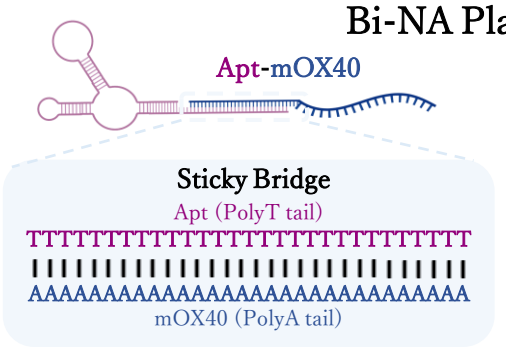


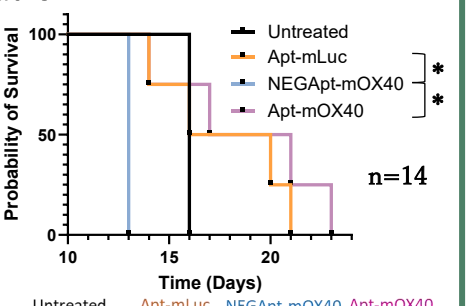
- Uptake of aptamer in the tumour after IT and IV administration (4 h).
- In vivo imaging results show **co-localisation between the aptamer and the tumour** with distribution to CD45⁺ cells and CD45⁻ tumour cells.



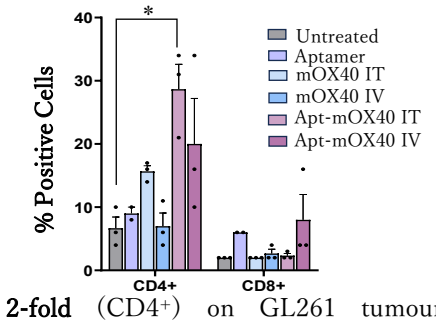
Flow cytometry data showing the uptake of Aptamer-ATTO647 relative to a negative aptamer.
Fold increase > 1 indicates **selectivity of the aptamer for the phenotype**

Bi-NA Platform

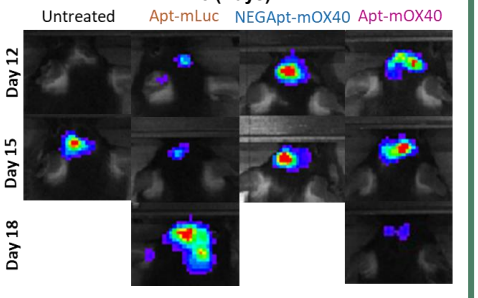




GL261 tumour bearing mice



2-fold (CD4⁺) on GL261 tumours, compared with non-targeted mOX40.[3]



Apt-mOX40 extended the survival of GL261 tumour bearing mice compared to the NEGApt-mOX40 from **13 days** to **19 days** (n=14).

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