ALETA BIOTHERAPEUTICS

#133

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Development of novel CAR T engager proteins for cell therapies that target metastatic and primary CNS malignancies

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Background.

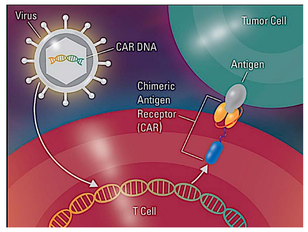
Treatment of solid tumors with cell therapeutics will require optimal T cell persistence, fitness, and trafficking. Heterogeneous solid tumors will also have to be attacked through multiple antigens simultaneously in order to prevent resistance linked to loss of antigen expression. Here we use chimeric antigen receptor (CAR) T cells and bridging proteins that act as CAR-T engagers to create an optimal platform for attacking solid tumors in the CNS.

CAR T cells only work when they bind to tumor antigens:

Cytotoxicity: Antigen encounter triggers the CAR T cell to secrete factors that kill the tumor cells.

Serial killing: Each CAR T cell can find and kill up to 1000 tumor cells.

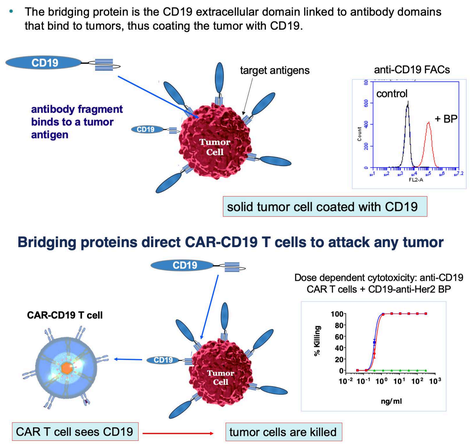
Proliferation: CAR T cells reproduce, creating clones of itself that amplify the anti-tumor activity.



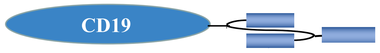
Persistence: The target tumor protein must be consistently expressed to support CAR T cell expansion and persistence in the patient.

Leveraging anti-CD19 CAR T cells to improve persistence:

- CAR-T cell persistence is a natural attribute of anti-CD19 CAR T cells that can expand and persist using normal B cells as an antigen source
- Further, CAR-T cell fitness is naturally enhanced by the interaction with normal B cells
- Anti-CD19 CAR CD19 T cells are known to traffic throughout the patient including to the CNS



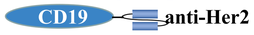
Bridging proteins are anti-CD19 CAR T Cell Engagers:



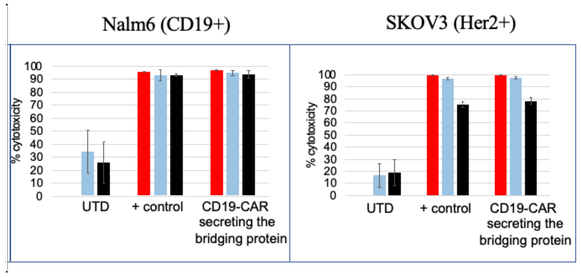
Built with -

- an anti-tumor antigen domain, eg. with an scFv or a llama VH
- two anti-tumor antigen domains eg. with a scFv and a VH or with two different VH
- other functions: add cytokines, immune checkpoint, extend half-life

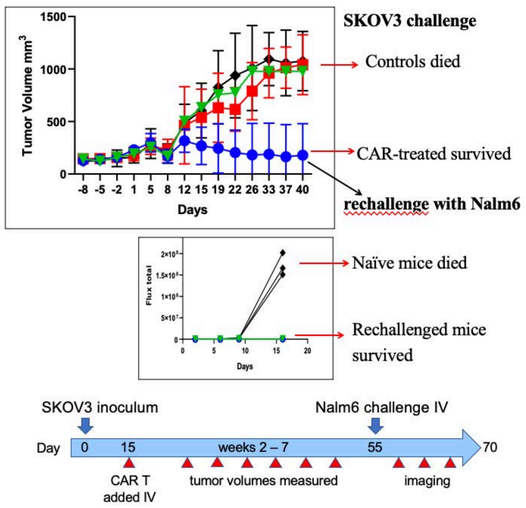
Example 1. A secreted CD19-anti-Her2 bridging protein for Her2-positive CNS metastases.



The bridging protein is secreted by anti-CD19 CAR T cells: these CAR T cells are active against CD19-positive Nalm6 cell and Her2-positive SKOV3 cells *in vitro*



These CAR T cells retain cytotoxic activity *in vivo*



Next step: GMP campaign and IND enablement

Example 2. A multi-antigen CAR T engager protein for CNS primary tumors.

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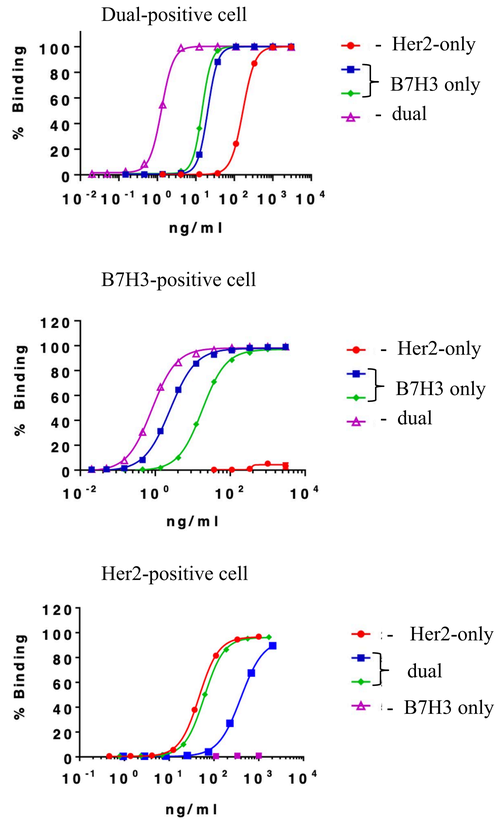
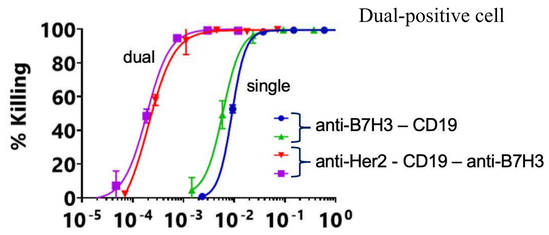
Cancers	Patients	Antigens
Glioblastoma / astrocytoma	pediatric & adult	Her2, B7H3, IL13Ra2, EGFR
Medulloblastoma	pediatric	Her2, B7H3, B7H6, EGFR
Ependymoma	pediatric	Her2, B7H3, B7H6, EGFR
Meningioma	pediatric & adult	Her2, B7H3, IL13Ra2, EGFR

The bridging protein binds to single and dual antigen-positive cells



Next step: clone into viral vector.

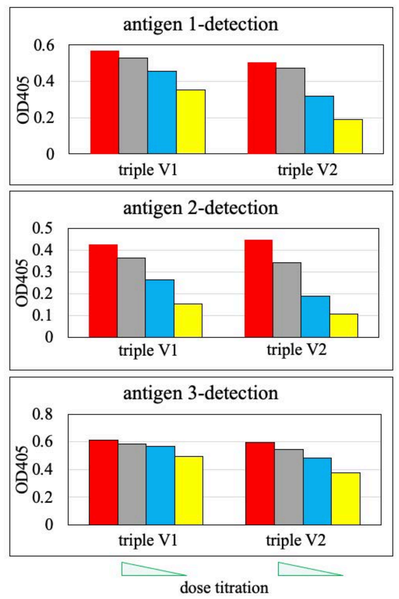
Cytotoxicity mediated by single and dual-antigen bridging proteins in the presence of anti-CD19 CAR T cells in vitro



	anti-B7H3 A	anti-B7H3 B	A plus anti-Her2	B plus anti-Her2
IC_{50} (ng/ml)	9	6	0.2	0.19
IC_{50} (pM)	202	105	2.4	2.6

IC_{50} at ~2.5 pM = 200 pgs/ml

Example 3. A tri-antigen CAR T engager protein for CNS primary tumors.



anti-CD19 antibody captures two versions of a tri-antigen bridging protein: all three antigens can bind

Next step: finalize antigen selection and nominate candidate.