ALETA BIOTHERAPEUTICS

#160

Evaluation and development of dual and triple antigen targeting CAR-T Engager proteins for Her2-positive CNS metastases and solid tumors.

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Introduction

The successful development of CAR-T cells for the treatment of solid tumors requires that key hurdles be overcome.

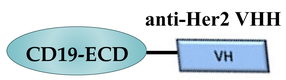
1) CAR T cells must expand substantially and then persist in the patient.
2) the CAR T cell population must retain cytotoxic activity and resist exhaustion and immunosuppression, a quality we can define as 'fitness'.
3) CAR T cell therapy must completely eliminate tumors in order to avoid relapses; in many indications this requires multi-antigen targeting.

We built a novel platform for repurposing CAR T cells that target CD19 (CAR-CD19 T cells). We create CAR-T Engager proteins that encode redirect the CAR-CD19 domain to any antigen of interest: each CAR-T Engagercontains the CD19 extracellular domain linked to one or more anti-tumor antigen binders such as llama VHHs:

Figure 1. Technology Overview

Building a CAR-T engager

- **Design & characterize Engager proteins built from modules: the CD19 extracellular domain (ECD) and at least one antigen binding domain**
- **An example with one antigen-binding domain**



- **We can then clone the Engager protein downstream of a CAR-CD19 sequence in a lentiviral construct:**

- **Transduce primary T cells, monitor expression, secretion and activity:**

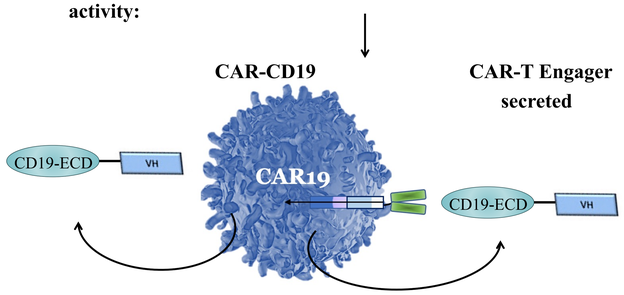


Figure 2. The Engager is secreted by the CAR-CD19 T cell and is functional: for example CAR-CD19 T cells that secrete the anti-Her2 Engager are cytotoxic against CD19-positive Nalm6 cells and also against Her2-positive SKOV3 cells.

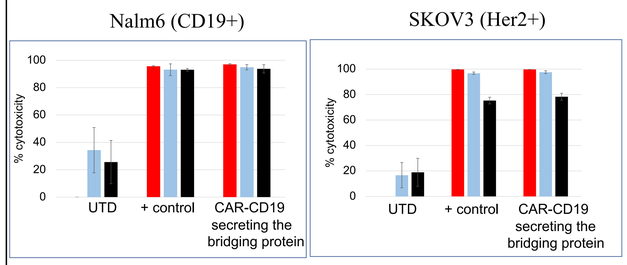


Figure 3. Serial re-stimulation assay demonstrates the proliferation and fitness benefit of "seeing" B-cells, which solid tumor cells do not provide: CAR-CD19 T cells that secrete the bridging protein expand in the presence of Raji B cells.

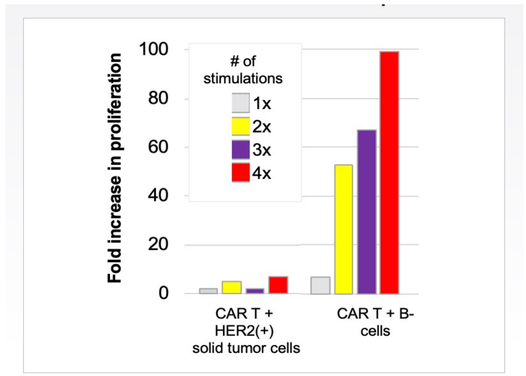


Figure 4. The functional consequence is seen in cell-number matched cytotoxicity experiments: T cell cytotoxic activity is enhanced in CAR T cells that have encounted B cells, and is reduced by encounter with solid tumor cells.

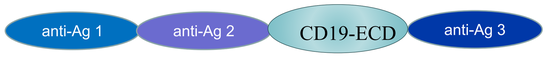
Re-stimulation cell	Raji	Raji	SKOV3	SKOV3
Target cell	JeKo-1	SKOV3	JeKo-1	SKOV3
Cytotoxicity	100%	94%	22%	18%

CONCLUSION 1: Interaction of CAR T cells with an immune cell - a B cell line - provides proliferative and fitness benefits.

Table 1. Optimizing antigen targeting of CNS cancers and Her2+ metastatic breast cancer: multiple antigens to target.

Examples	Patients	Antigens Expressed
Breast cancer CNS metastatic lesions	• adult	Her2, B7H3, MUC-1, EGFR
Glioblastoma / astrocytoma	• pediatric and adult	Her2, B7H3, IL13Ra2, EGFR
Medulloblastoma	• rare pediatric cancer • very rare in adults	Her2, B7H3, B7H6, EGFR
Ependymoma	• rare pediatric cancer • very rare in adults	Her2, B7H3, B7H6, EGFR
Meningioma	• pediatric and adult	Her2, B7H3, IL13Ra2, EGFR

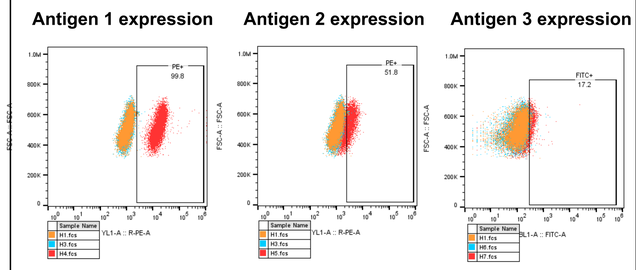
Table 2. Binding data across a triple antigen CAR T Engager. A triple antigen binding Engager protein was constructed and purified.



ELISA assays		Captured with			
		anti-CD19	antigen 1	antigen 2	antigen 3
Detected with	antigen 1	25 pM	x	20 pM	56 pM
	antigen 2	110 pM	86 pM	x	40 pM
	antigen 3	120 pM	300 pM	40 pM	x

The triple Engager can bind each individual antigen independently of binding to the other two antigens or to the CAR T Engager protein (CD19).

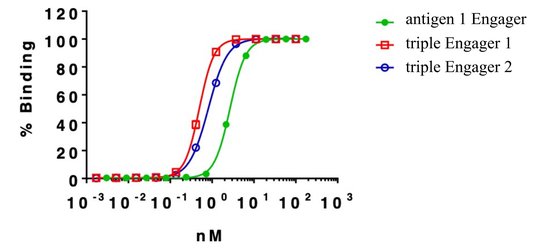
Figure 5. Target antigen expression on an epithelial carcinoma cell line. These three antigens cover many tumor types.



In breast carcinomas, these different antigens can be upregulated in response to therapy, eg. Antigen 3 is upregulated in response to targeting of Antigen 1.

The goal is two-fold: counter antigen heterogeneity with multi-antigen targeting and prevent resistance mediated by antigen upregulation.

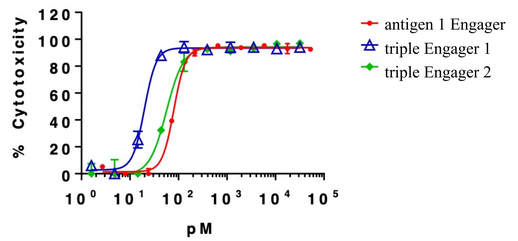
Figure 6. Binding of triple engager and single engagers to target cells that express all three antigens.



	Antigen 1 Engager	Triple Engager - 1	Triple Engager - 2
EC_{50}	2.7 nM	0.5 nM	0.8 nM

Different triple Engagers show enhanced binding to antigen-expressing cells.

Figure 7. The triple Engagers mediate cytotoxicity in the presence of anti-CD19 CAR T cells.



	Antigen 1 Engager	Triple Engager - 1	Triple Engager - 2
IC_{50}	80 pM	20 pM	50 pM

Triple Engager - 1 shows enhanced cytotoxicity against a triple antigen-expressing cell.

CONCLUSION 2 and Next Steps.

- We have made a variety of double and triple antigen CAR T Engagers for diverse malignancies including B cell cancers, AML and solid tumors.
- Here we are illustrating a novel set of triple antigen-binding CAR T Engagers that can target a variety of antigens on CNS primary tumors, and on metastases the arise from Breast Cancer, Lung Cancer and CRC.
- We see independent antigen binding, improved multi-antigen binding and enhanced cytotoxicity using Triple Engagers.
- We are now adding other functional domains to counter immunosuppression and provide cytokine support.
- Our goal is to advance our internal and partnered programs using these novel Engager designs.