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CAR19 T cells secreting antigen-retargeting fusion proteins have remarkable potency against diverse tumor types

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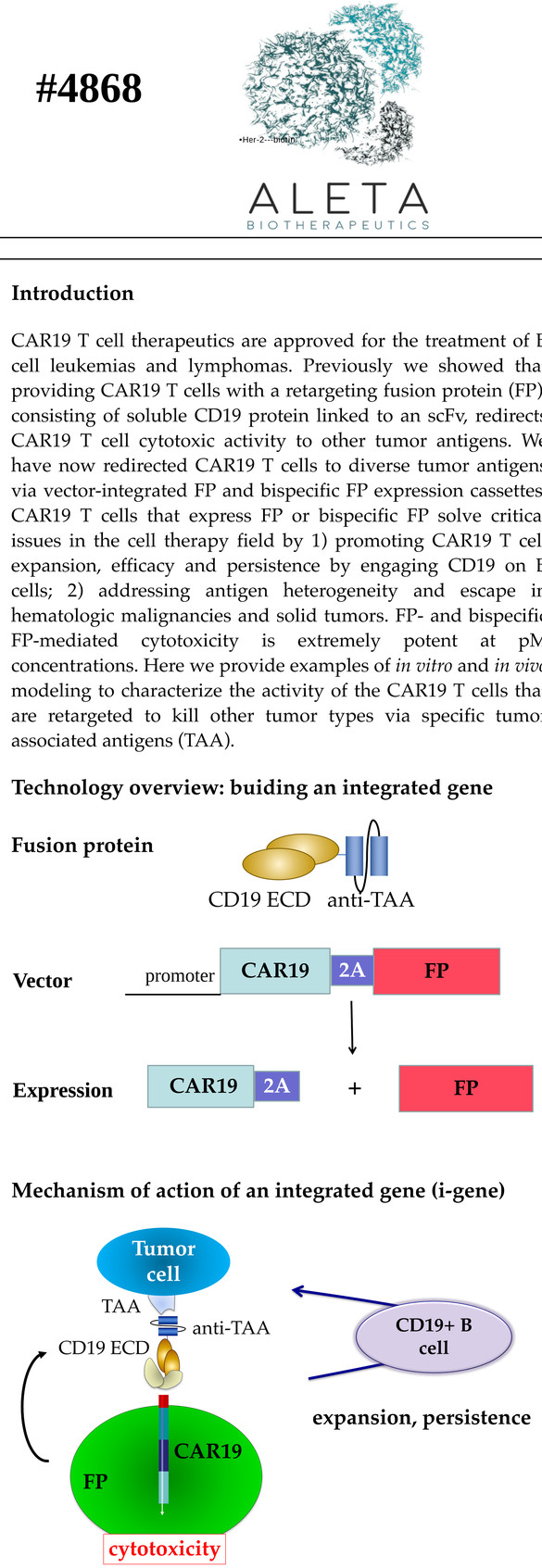
Introduction

CAR19 T cell therapeutics are approved for the treatment of B cell leukemias and lymphomas. Previously we showed that providing CAR19 T cells with a retargeting fusion protein (FP), consisting of soluble CD19 protein linked to an scFv, redirects CAR19 T cell cytotoxic activity to other tumor antigens. We have now redirected CAR19 T cells to diverse tumor antigens via vector-integrated FP and bispecific FP expression cassettes. CAR19 T cells that express FP or bispecific FP solve critical issues in the cell therapy field by 1) promoting CAR19 T cell expansion, efficacy and persistence by engaging CD19 on B cells; 2) addressing antigen heterogeneity and escape in hematologic malignancies and solid tumors. FP- and bispecific FP-mediated cytotoxicity is extremely potent at pM concentrations. Here we provide examples of *in vitro* and *in vivo* modeling to characterize the activity of the CAR19 T cells that are retargeted to kill other tumor types via specific tumor associated antigens (TAA).

Technology overview: buiding an integrated gene



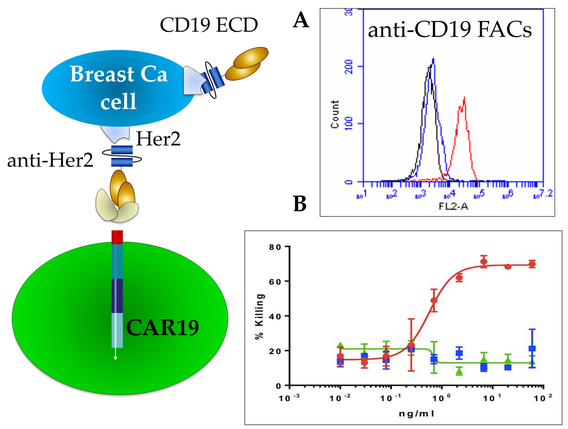
Experimental Procedures

A CAR19 construct was created in a lentiviral vector having an MCSV promoter. The CAR consists of an anti-CD19 scFv, stalk, TM and CD28 and/or 4-1BB cytoplasmic signaling domains, and the CD3e cytoplasmic signaling domain. FP and bispecific FP cassettes were designed to encode the extracellular domain of the CD19 protein, followed by an scFv or VHH to one (FP) or two (bispecific) antigens, separated from the CAR sequence by a P2A cleavage site.

Results

I - Flow cytometry and binding analyses demonstrated FP and bispecific-FP-mediated bridging between target tumor cells and CAR19 T cells.

Figure 1 – the CD19-anti-Her2 FP bridges to Her2+ cells and mediates cytotoxicity. a) demonstrates binding of the CD19-anti-Her2 FP to Skov3 ovarian cancer cells (Her2-positive/CD19-negative) by flow cytometry. The binding affinity was determined to have an EC_{50} of 2nM (not shown). b) shows Skov3 cytotoxicity in relation to increasing concentration of the CD19-anti-Her2 scFv FP, but not control FPs, in the presence of CAR19 T cells. 50% cytotoxicity is achieved with 10pM CD19-anti-Her2 FP.

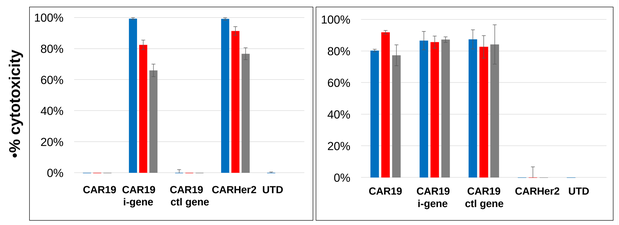


II – Primary human T cells transduced with a lentiviral construct expression encoding CAR19 and the CD19-anti-Her2 scFv FP are cytotoxic to Her2+ cells and to CD19+ cells.

Figure 2. The CAR19 T cell carrying the CD19-anti-Her2 scFv i-gene (CR19/i-gene) has robust cytotoxic activity a) CAR19/i-gene mediates Her2-positive Skov3 cell cytotoxicity comparable to CARHer2; controls do not. b) all CAR19s kill CD19+ Nalm6 cells, including CAR19/i-gene T cells. c) CAR19/i-gene cells mediate Her2 / CD19 serial killing.

d) CAR19/i-gene cells expand in vivo in the presence of Raji cells. e) CAR19 i-gene cells infiltrate Her2+ Skov3 cells in vivo.

a. Skov3 cells (Her2+) **b. Nalm6 cells (CD19+)**

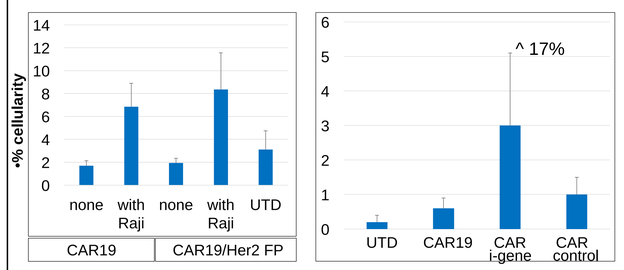


c. Serial killing

1st target cell	2nd target cell	CAR-19-i-gene	CAR-19-ctl gene
Nalm6-CD19+	Nalm6-CD19+	+	+
Nalm6-CD19+	Skov3-Her2+	+	-
Skov3-Her2+	Nalm6-CD19+	+	-
Skov3-Her2+	Skov3-Her2+	+	-

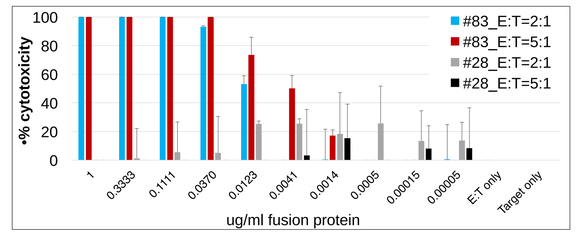
Pilot activity *in vivo* using Raji (D) and Skov3 (E) cells are antigenic targets

d: Raji **e: Skov3**



III. Diverse programs illustrate the potency and flexibility of the retargeting platform.

Figure 3. The CAR19 T cell carrying the CD19-anti-CD20 scFv i-gene (CR19/i-gene) has robust cytotoxic activity



•#83: CD19 – anti-CD20 scFv
•#28: CD19 control

EC_{50} (50% killed)" 150pM ~ 8ng/ml on K562-CD20 cells

Table 1. CD19-FPs provide robust cytotoxicity across diverse antigens and indications

FP	binding EC_{50} (FACS)	cytotoxicity EC_{50}	target cell	indication
CD19-anti-Her2	2nM	10pM	SKOV3 OvCa	solid tumors
CD19-anti-Clec12a	2nM	10pM	U937	AML
CD19-anti-CD20	20nM	150pM	293-CD20	NHL
CD19-anti-BCMA	70nM	750pM	H929 MM	MM
CD19-anti-ROR1	1nM	50pM	786-O RCC	solid tumors

Table 2. CD19-bispecific FPs can target multiple antigens, independently or synergistically

bispecific IMPACT	cytotoxicity EC_{50}	indication
CD19-anti-Her2-anti-EGFR	< 1pM	POC
CD19-anti-CD20-anti-CD22	tbd	NHL, ALL
CD19-anti-Her2-anti-ROR1	tbd	gastric cancer and other solid tumors

Near term path forward

- Aleta is advancing multiple programs based on robust CAR19/i-gene characterization
- The CAR19/CD19-anti-Her2 i-gene CAR T cells and the CAR19/CD19-anti-CD20 i-gene CAR-T cells have advanced to *in vivo* efficacy modeling
- The CAR19/CD19-anti-Her2 i-gene vector and the CAR19/CD19-anti-CD20 i-gene vector are in GMP-developable systems for IND enablement
- The CAR19/CD19-anti-CD20-anti-CD22 i-gene program is in lead optimization
- The CAR19/CD19-anti-Clec21a program is in lead optimization