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Supplemental Information

Development and Evaluation of an Optimal Human Single-Chain Variable Fragment-Derived BCMA-Targeted CAR T Cell Vector

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Supplemental Methods

Affinity for human BCMA.

Anti-BCMA scFvs were cloned into IgG1 format and affinities determined via BiaCore. Human scFvs were converted to chimeric murine IgG1 full length antibodies (mIgG1) by fusing the light chain variable region of the scFv with the murine light chain constant region (lambda or kappa, accordingly) and fusing the heavy chain variable region of the scFv with murine IgG1 heavy chain constant region. Both light chain and heavy chain were expressed under CMV promoter in separate plasmids and co-transfected to 293F cells for transient expression of the full length chimeric mIgG1 antibody. The binding affinities toward the BCMA antigen were determined via Biacore X100 (GE Healthcare; Stamford, CT). Briefly, scFv clones were loaded onto a CM5 chip conjugated with the mouse antibody capture kit (GE Healthcare, Cat#BR100838) according to the manufacturer's protocol. To begin, the mIgG1 antibody was run over the surface of the chip at a concentration of 5µg/mL for 120 seconds. Afterwards, the binding kinetics of the antigen was analyzed by single-cycle kinetics from 0.6 to 10µg/mL. Each concentration was subjected to 90 seconds of association and disassociation respectively. To regenerate the chip surface, a glycine-HCl, pH 1.7 regeneration solution was run over the chip according to manufacturers instructions. The binding constants were obtained using the 1:1 binding site model (Biacore X-100 evaluation software).

Co-culture with primary human cells. CAR T cells were co-cultured 1:1 (E:T ratio) with 50,000 isolated primary normal human cells (ScienCell; Carlsbad, CA) in a U bottom 96 well plate in triplicate. Co-culture was incubated for 18 hours, and supernatant was collected and frozen at -80^{o}C until analysis. INF-gamma detection was performed using the Milliplex MAP Human

Cytokine/Chemokine, Premixed 13 Plex kit (Millipore-Sigma; Darmstadt, Germany), and the Luminex FlexMAP 3D system (Luminex Corp; Austin, TX).

Supplemental Table Legend

Table S1. Affinity for human BCMA

scFvs were cloned into mIgG1 format and affinities determined via BiaCore. Of affinities available, all were in the single digit nanomolar range.

Supplemental Figure Legends

Figure S1. Human B cell derived scFv phage display library screening

A library consisting of $6x10^{10}$ human scFvs was panned with recombinant BCMA ECD-IgG1 Fc protein and subsequently validated on BCMA expressing 3T3 aAPCs; scFvs were then sequenced. Cell surface binding to human MM cell lines confirmed results of previous screen.

Figure S2. BCMA(171) CAR T cells are not activated in co-culture with primary cells from essential normal tissues.

(A) Vectors containing either (1) BCMA-targeting scFv's with C-terminal mCherry; or (2) TNFR and immunoglobulin (Ig) superfamily members with C-terminal GFP were transiently transfected into different populations of HEK293s cells. Potential scFv/target antigen interaction was identified as a double positive signal by automated flow cytometry. Peaks labeled 'B' indicate interactions with BCMA+ controls. Unlike scFv clone 130 which interacted with BCMA and two isoforms of SIRP-Beta1 (S, S-III), all other scFvs, including clone 125 (shown) and 171 (Figure 5), interacted only with BCMA. (B) BCMA(171)/4-1BBz CAR T cells or donor matched, untransduced T cells, were co-cultured 1:1 with isolated primary cells from a variety of essential normal tissues, or OPM2 human MM cell line (positive control). IFN was measured from the supernatant after an 18h co-culture by luminex assay. Only after co-culture with OPM2 cells, and not primary normal cells analyzed demonstrated relevant IFN release, indicating CAR T cell activation.

Table S1

Protein	KD (BiaCore)
αBCMA-183 mIgG1	1.2 nM
αBCMA-171 mIgG1	4.8 nM
αBCMA-137 mIgG1	5.7 nM
αBCMA-130 mIgG1	NA
αBCMA-125 mIgG1	8.1 nM

Figure S1

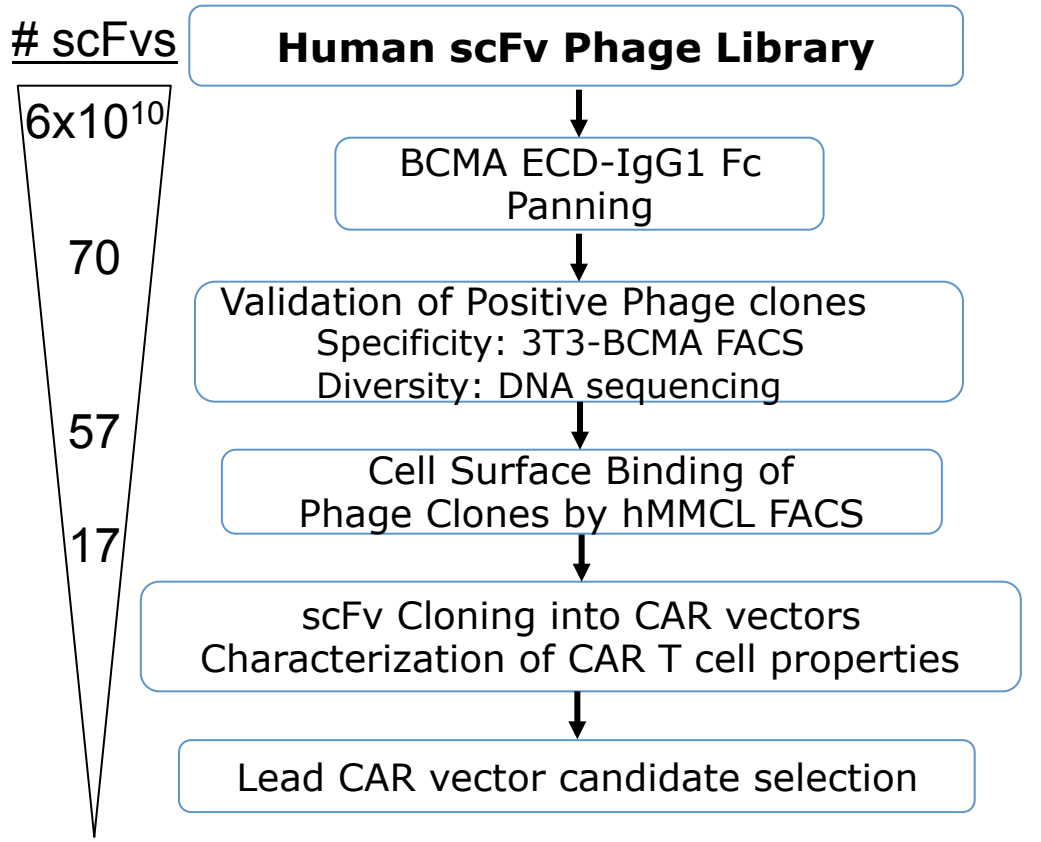
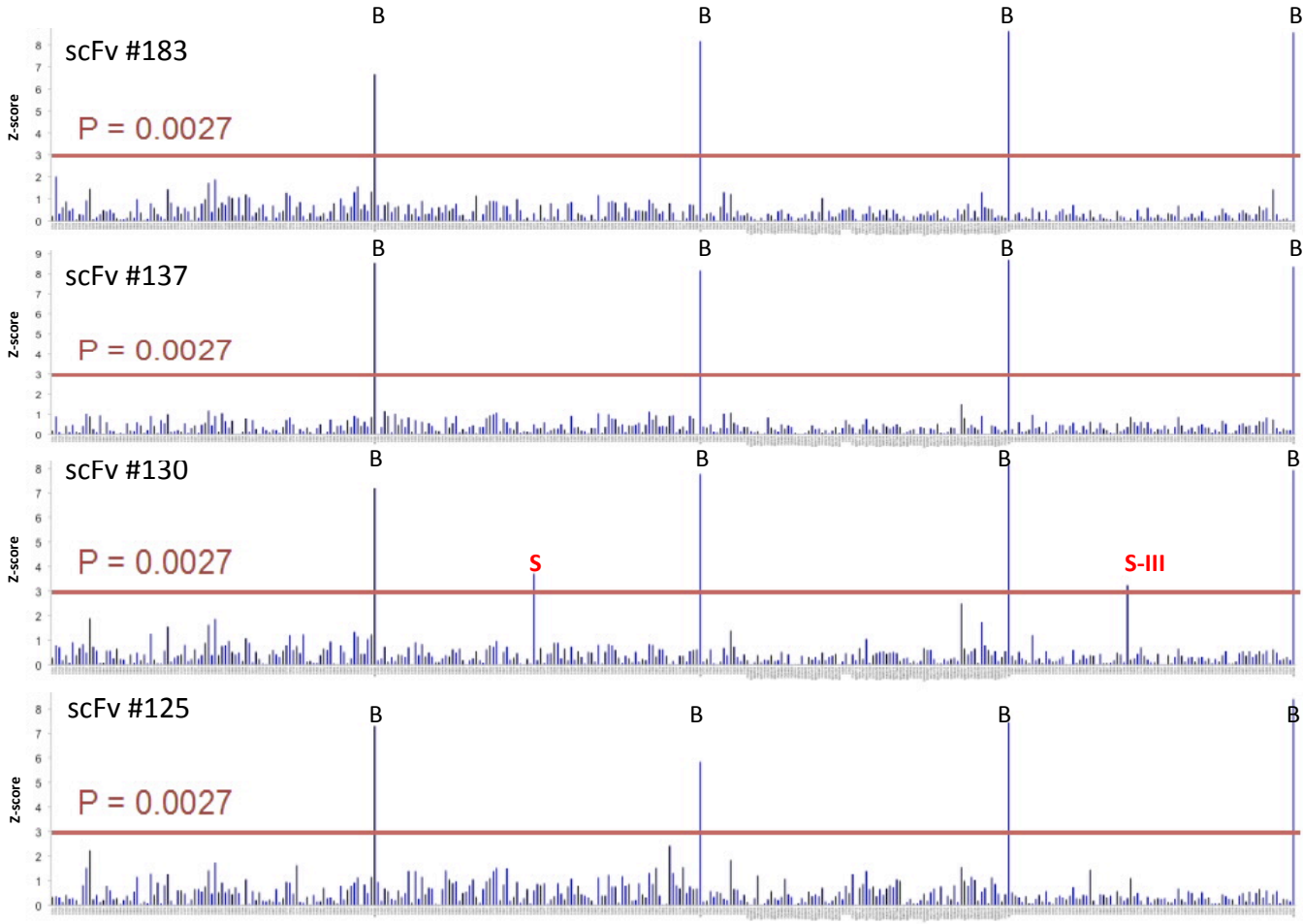
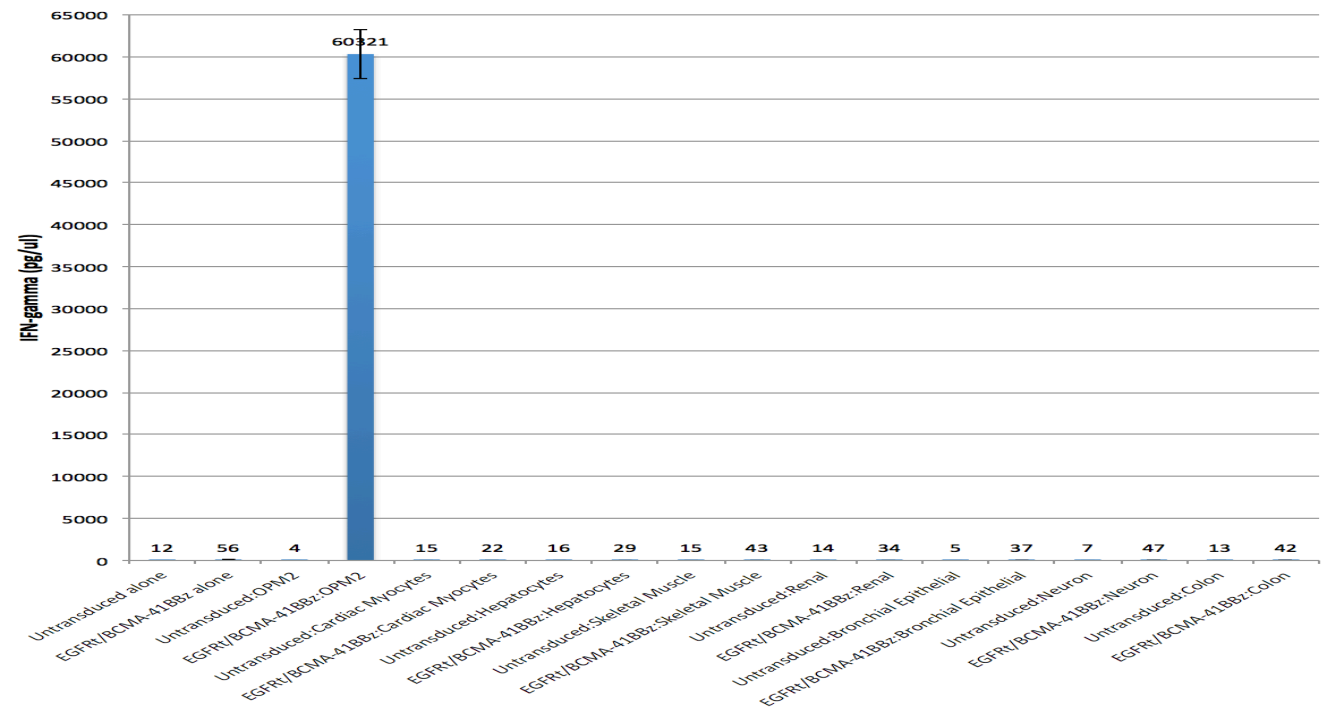

scFvs
6x10^10
70
57
17
Human scFv Phage Library
BCMA ECD-IgG1 Fc Panning
Validation of Positive Phage clones
Specificity: 3T3-BCMA FACS
Diversity: DNA sequencing
Cell Surface Binding of Phage Clones by hMMCL FACS
scFv Cloning into CAR vectors
Characterization of CAR T cell properties
Lead CAR vector candidate selection

Figure S2

A


scFv #183
P = 0.0027
scFv #137
P = 0.0027
scFv #130
P = 0.0027
S
S-III
scFv #125
P = 0.0027
Z-score
B

B


IFN-gamma (pg/ul)
60321
12
56
4
15
22
16
29
15
43
14
34
5
37
7
47
13
42
Untransduced alone
EGFRt/BCMA-41BBz alone
Untransduced:OPM2
EGFRt/BCMA-41BBz:OPM2
Untransduced:Cardiac Myocytes
EGFRt/BCMA-41BBz:Cardiac Myocytes
Untransduced:Hepatocytes
EGFRt/BCMA-41BBz:Hepatocytes
Untransduced:Skeletal Muscle
EGFRt/BCMA-41BBz:Skeletal Muscle
Untransduced:Renal
EGFRt/BCMA-41BBz:Renal
Untransduced:Bronchial Epithelial
EGFRt/BCMA-41BBz:Bronchial Epithelial
Untransduced:Neuron
EGFRt/BCMA-41BBz:Neuron
Untransduced:Colon
EGFRt/BCMA-41BBz:Colon