

Supplementary data

Novel T cells with improved in vivo anti-tumor activity generated by RNA electroporation

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Running title: Generating novel T cells by RNA electroporation to improve cancer treatment

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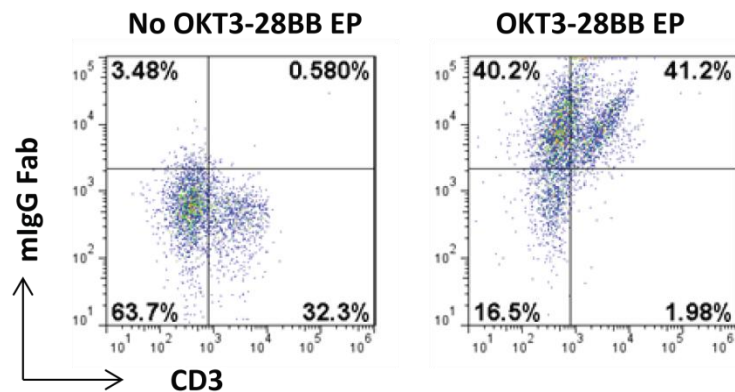
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MALPVTALLPLALLLHAARPQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQR
 ←
PGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDH

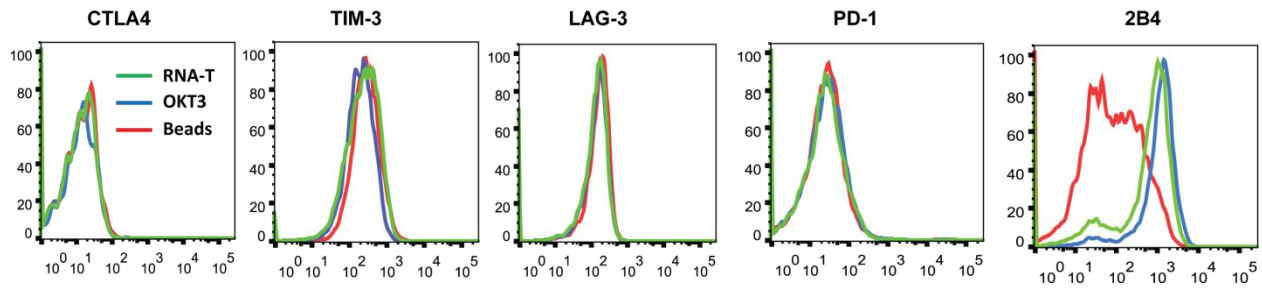
YCLDYWGQGTTLTVSSSGGGGGSGGGGGSGGGGSIVLTQSPAIMSASPGEKVTMTCSASSSVSY
 _____ **OKT3 scFv** _____
MNWYQQKSGTSPKRWIYDTSKLAGVPAHFRGSGSGTSSYSLTISGMEAEDAATYYCQQWSS

NPFTFGSGTKLEINRAAA **TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD**
 _____> | | <_____ **CD28 Hinge** _____> |
FWVLVVGGLACYSLLVTVAFIIFWY **RSKRSLLLHSDYMNMTPRRPGPTRKHYPYAPPRD**
 |<_____ **CD28 transmembrane** _____>|<_____ **CD28** _____>|
FAAYRSKRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
 _____>|<_____ **4-1BB** _____>|

Supplemental Figure 1. Sequence of OKT3-28BB. The sequence is composed of following parts as indicated: OKT3 Single-chain variable fragment (OKT3 scFv), hinge from CD28 (CD28 Hinge), transmembrane from CD28 (CD28 transmembrane), cytoplasmic region of human CD28 (CD28) and cytoplasmic region of human 4-1BB (4-1BB).



Supplemental Figure 2. OKT3-28BB expression of RNA electroporated PBMCs. PBMCs from a normal donor were electroporated with OKT3-28BB RNA (OKT3-28BB EP) and OKT3-28BB expression was detected using an antibody against murine IgG Fab (mIgG Fab) 18h post RNA electroporation. PBMCs that were not electroporated with OKT3-28BB RNA (No OKT3-28BB EP) was used as control.



Supplementary Figure 3. Expression of exhaustion markers. Expression of the exhaustion markers CTLA4, TIM-3, LAG-3, PD-1 and 2B4 were detected respectively for the T cells generated by OKT3-28BBZ RNA electroporation (RNA-T), OKT3/IL-2 stimulation (OKT3) or CD3/CD28 DynaBead stimulation (Beads) 10 days after stimulation.