257 NOT GATE GENE CIRCUITS EXPAND THE RANGE OF TUMOR-ASSOCIATED ANTIGENS ADDRESSABLE BY CAR-NK AND -T CELL THERAPIES

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Background Chimeric antigen receptors (CARs) can direct immune cells, such as T or NK cells, to kill cancer cells expressing a tumor-associated antigen (TAA). TAA expression on healthy tissues can lead to on-target/off-tumor toxicity (e.g., CD19 CARs can cause B cell aplasia). Furthermore, application of CARs to solid tumors can be lethal because TAAs are frequently expressed on healthy tissues of vital organs. NOT gate CARs address this issue by detecting protective antigens (PAs) on healthy cells and inhibiting killing against them, thus potentially mitigating on-target/off-tumor toxicity.

Methods We developed NOT-gated inhibitory CARs (iCARs) to complement activating CARs (aCARs) against FLT3, a TAA for acute myeloid leukemia whose development has been hampered by significant off-tumor toxicity against HSCs in preclinical studies, and against aCEA, a TAA for colorectal cancer that causes dose-limiting off-tumor toxicity against healthy colon epithelium. To discover candidate PAs for these TAAs, we implemented a multi-step bioinformatics pipeline leveraging multiple microarray, bulk RNAseq, scRNAseq, and IHC datasets. We confirmed PA expression on at-risk cell types using flow cytometry (FLT3) or IHC of tissue arrays (CEA). NK or T cells were transduced with anti-TAA aCARs and anti-PA iCARs, and their ability to kill TAA^+ cells but spare TAA^+PA^+ cells was assessed in vitro and in vivo.

Results We discovered that EMCN was expressed on up to 76% of HSCs, validating its use as a PA to protect healthy HSCs from FLT3 CAR-mediated toxicity. FLT3 aCAR-NK cells co-expressing an EMCN iCAR demonstrated significantly lower toxicity against HSCs compared to FLT3 aCAR-NK cells and did not negatively impact HSC colony formation. These NOT-gated FLT3 aCAR-NK cells extended mouse survival in a leukemia model while enriching PA^+ target cells to 70–90% in mixed cell model in vivo. We identified VSIG2 as a PA to protect normal colon epithelial cells from CEA aCAR-mediated toxicity. VSIG2 was expressed on 80% of normal CEA^+ colon epithelial cells by IHC. CEA aCAR-NK or -T cells co-expressing VSIG2 iCARs reduced killing of CEA^+VSIG2^+ target cells by up to 70–98% relative to CEA^+ target cells in vitro.

Conclusions We successfully validated examples of NOT gated cell therapies for both liquid tumors and solid tumors, both in vitro and in vivo, in both NK cells and T cells, using multiple novel clinically relevant TAA/PA combinations. NOT gates therefore may expand the range of cancers treatable by CAR immune cells by mitigating on-target/off-tumor toxicity.

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