116 DEVELOPMENT OF LOGIC GATED CAR-NK CELLS FOR THE TREATMENT OF SOLID TUMORS

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Background CAR-based therapies have transformed the treatment of several cancers, but this progress has not translated into solid tumors. One challenge of CAR-mediated therapies for solid tumors is the lack of specific tumor-associated antigens (TAA's) that are only expressed on cancer cells and not on healthy cells, thereby posing a risk for on-target off-tumor toxicities. This presents a unique opportunity to use Logic Gates to expand the universe of cancer targets that may be treated with CAR-based cell therapies. CEA, a widely expressed tumor antigen, found in >90% of colorectal cancer (CRC), is also expressed in healthy gastrointestinal and lung epithelial cells. Clinical experience targeting CEA resulted in severe dose-limiting toxicities,[1,2] highlighting the need for healthy tissue protection. Logic-gated gene circuits can prevent off-tumor toxicities by pairing a CEA activating-CAR (aCAR) with an inhibitory-CAR (iCAR) that recognizes a safety antigen (SA) uniquely expressed in healthy epithelial cells.

Methods We developed a bioinformatics-driven antigen paired discovery platform using single-cell transcriptomics to discover and prioritize TAA's and pair them with SA's that are selectively expressed on the membrane of healthy cells. TAA's and SA's were validated in primary cancer and healthy tissue samples using IHC. We constructed aCAR/iCAR gene circuits and tested their function in NK cells.

Results Our bioinformatics platform identified VSIG2 to be co-expressed with CEA in healthy gastrointestinal and lung epithelial cells. IHC confirmed the expression of VSIG2 on the membrane of healthy colon (N=72 samples) and lung (N=24 samples) epithelial cells.Using our Design-Build-Test-Learn platform, we screened >250 CAR constructs targeting CEA. CAR-NK cells were generated and tested for anti-tumor activity against CRC CEA+ cells and lead candidates were selected based on NK cell performance. A single dose of CEA-CAR-NK cells had anti-tumor activity in a human CRC xenograft model, reducing tumor burden in >33% of the treated mice. We identified iCARs with different intracellular domains derived from native domains containing immunoreceptor tyrosine-based inhibitory motifs. These iCARs suppressed >50% of aCAR-mediated killing ($p<0.05$) and significantly reduced TNFa secretion ($p<0.0005$) in a SA-specific manner.

Conclusions We are developing Logic-Gated CAR-NK cell therapies aimed at reducing on-target off-tumor toxicities, to spare healthy cells in a SA-dependent manner. SENTI-401 will focus on targeting CEA+ CRC tumors with a NOT gate that recognizes the SA VSIG2 in the colon and lungs.

REFERENCES

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