A POWERFUL, PRECISE TARGETING SYSTEM CONTROLLED BY TUMOR DELETIONS TRANSFORMS CEA AND MSLN CAR-T CELLS INTO TUMOR-SELECTIVE AGENTS

Agnes Hamburger, Han Xu, Yuta Ando, Grace Asuelime, Kristian Bolanos-Ibarra, Mark Daris, Kiran Deshmukh, Breanna DiAndreth, Fernando Fisher, Grant Gabrelow, Maria Imun, David Ju, Wen-Hua Lee, Chuck Li, Edwin Liu, Aaron Martin, Michele Mcelvain, Jee-Young Mock, Daniel Nampe, Martin Naradikian, Mark Sandberg, Sanam Shafaattalab, Shruti Sharma, Talar Tokatlian, Dora Toledo-Warshaviak, Xueyin Wang, Lu-Min Wong, Alexander Kamb*. A2 Biotherapeutics, Agoura Hills, CA, USA

Background Mesothelin (MSLN) and carcinoembryonic antigen (CEA) are classic tumor-associated antigens that are expressed in many solid tumors including the majority of lung, colorectal and pancreatic cancers. However, both MSLN and CEA are also expressed in vital normal organs. This normal expression creates risk of serious inflammation for CEA- or MSLN-directed therapeutics. To date all active CEA- or MSLN-targeted investigational therapeutics have been toxic when administered systemically.

Methods We have developed a safety mechanism to protect normal tissues without abrogating sensitivity of cytotoxic T cells directed at MSLN(+) or CEA(+) tumors in a subset of patients with defined loss of heterozygosity (LOH) in their tumors (figure 1). This dual-receptor (Tmod< sup >TM</sup>) system exploits common LOH at the HLA locus in cancer cells, allowing T cells to recognize the difference between tumor and normal tissue.^{1 2} T cells engineered with specific Tmod constructs contain: (i) a MSLN- or CEA-activated CAR; and, (ii) an inhibitory receptor gated by HLA-A*02. HLA-A*02 binding blocks T cell cytotoxicity, even in the presence of MSLN or CEA. The Tmod system is designed to treat heterozygous HLA class I patients, selected for HLA LOH. When HLA-A*02 is absent from tumors selected for LOH, the CARs are predicted to mediate potent killing of the A*02(-) malignant cells.

Results The Tmod system robustly protects surrogate normal cells even in mixed-cell populations in vitro while mediating robust cytotoxicity of tumor cells in xenograft models (see example in figure 2). The MSLN CAR can also be paired with other blockers, supporting scalability of the approach to patients beyond HLA-A*02 heterozygotes.

Abstract 122 Figure 1 Illustration of the Tmod T cell engaging with tumor cells with somatic loss of HLA-A*02 and with normal cells.

Abstract 122 Figure 2 Bioluminescence measurements show the average difference between the size of the MSLN(+)A*02(+) 'normal' graft compared to the MSLN(+)A*02(-) tumor graft on the two flanks of mice after T cell infusion. Both tumor and normal grafts are destroyed by CAR-Ts (CAR-3 and M5 benchmark) while the MSLN Tmod cells kill the tumor but not the normal graft.

Conclusions The Tmod mechanism may provide an alternative route to leverage solid-tumor antigens such as MSLN and CEA in safer, more effective ways than previously possible.

REFERENCES

- Hamburger AE, DiAndreth B, Cui J, et al. Engineered T cells directed at tumors with defined allelic loss. *Mol Immunol* 2020;**128**:298–310.
- Hwang MS, Mog BJ, Douglass J, et al. Targeting loss of heterozygosity for cancer-specific immunotherapy. *Proc Natl Acad Sci U S A* 2021;**118**(12): e2022410118.

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