A powerful, precise targeting system controlled by tumor deletions transforms CEA and MSLN CAR-T cells into tumor-selective agents

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Summary and key points

- We are addressing the problem of tumor-specific targets
- To gain selectivity, exploit tumor gene loss with a dual-receptor system called Tmod™
- The robustness and modularity of the Tmod system is illustrated by Tmod constructs directed against the tumor-associated antigens CEA and MSLN
- The Tmod platform can be readily extended to more cancer patients

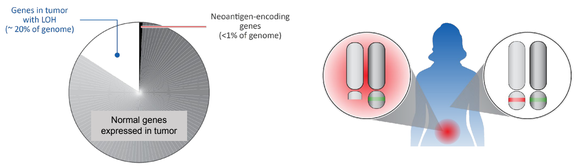
Background

Solid tumors comprise >90% of cancers. Carcinoembryonic antigen (CEA) and mesothelin (MSLN) are classic tumor-associated antigens that are expressed in many solid tumors including the majority of lung, colorectal and pancreatic cancers. However, both CEA and MSLN 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

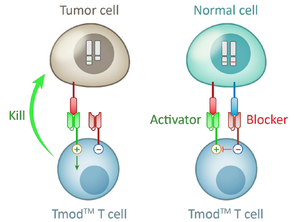
We have developed a safety mechanism to protect normal tissues without abrogating sensitivity of cytotoxic T cells directed at CEA(+) or MSLN(+) tumors in a subset of patients with defined loss of heterozygosity (LOH) in their tumors. This dual-receptor (Tmod™) system exploits common LOH at the HLA locus 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 CEA- or MSLN-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 CEA or MSLN. 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.

Figure 1A. Loss of heterozygosity (LOH): A new (or rejuvenated) source of targets



The supreme challenge in oncology: Discrimination between tumor and normal tissue. LOH is a large target opportunity for all-or-none tumor vs. normal discrimination via the Tmod blocker. Patients with germline heterozygosity of A*02 and clonal LOH of A*02 in their tumors (red bar on chromosome on the right) are selected.

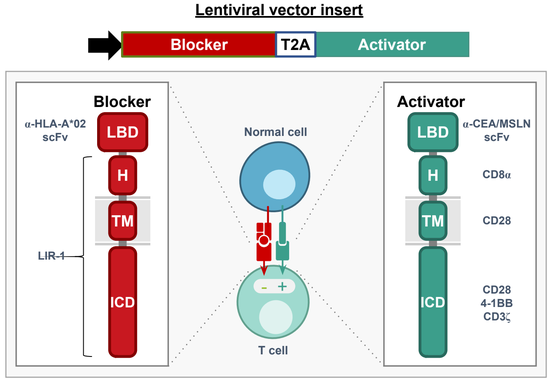
Figure 1B. The Tmod system can exploit LOH in tumors



Engineered T cells kill tumors but spare normal cells by leveraging loss of genes in tumors. In the case of CEA and MSLN Tmod, the activator antigen is CEA or MSLN; the blocker antigen is HLA-A*02.

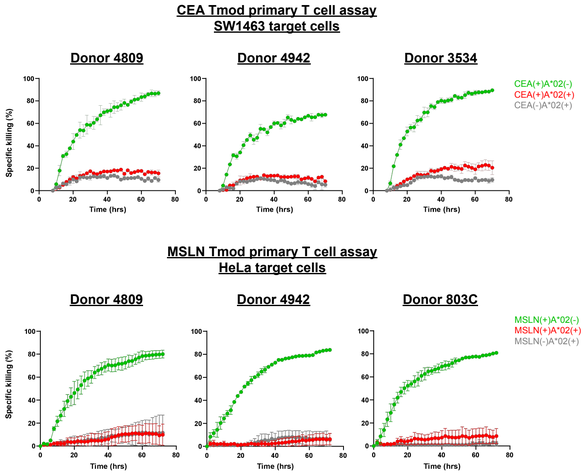
Results

Figure 2. Tmod approach to achieve selective cytotoxicity with two targets



Molecular composition of CEA- and MSLN-targeted Tmod constructs. The activator and blocker receptors are co-expressed in a single construct and the encoded fusion protein is cleaved in the cell to generate the activator and blocker.

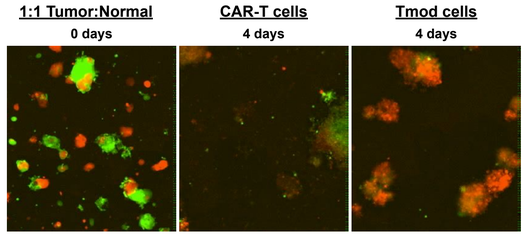
Figure 3. The CEA and MSLN Tmod constructs function in primary T cells to govern tumor vs. normal cytotoxicity



Cytotoxicity data of CEA and MSLN Tmod constructs from three A*02(-) donors in cell lines with native expression of CEA or MSLN and A*02. Raw data are plotted without background (i.e. untransduced T cell killing) subtraction. Tumor and normal target cells are SW1463 (E:T = 3:1) and HeLa (0.5:1) with or without genetic modifications as shown in the key.

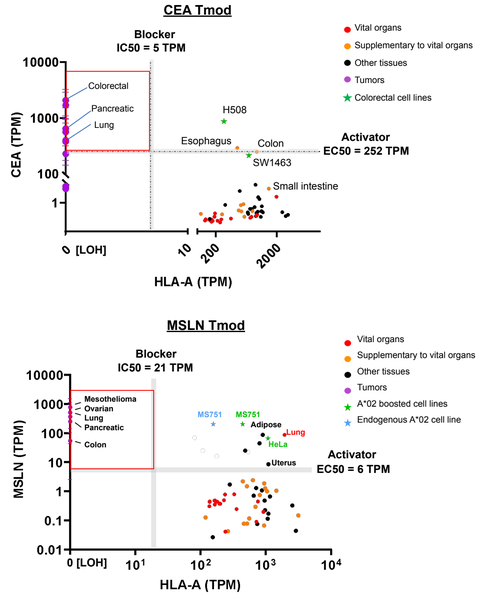
Results (cont.)

Figure 4. Tmod primary T cells selectively kill tumor cells mixed with normal cells: CEA example.



Tumor cells are H508 CEA(+)A*02(-) cells that stably express GFP (green). Normal cells are H508 CEA(+)A*02(+) cells that stably express RFP (red). T cells were from A*02(+) donor D12333.

Figure 5. Quantitative pharmacology of Tmod platform: CEA and MSLN Tmod have excellent tumor vs. normal selectivity



CEA and MSLN Tmod are predicted to kill tumors while protecting normal tissues. Data representation requires standard curves (not shown) that relate surface protein level to RNA-Seq value for cell lines and tissues. Purple points represent tumor types with HLA-A expression set at 0 TPM to account for selection of A*02(-) tumors by LOH. Tumor data from TCGA database; Normal tissue data from GTEx database.

Results (cont.)

Figure 6. Robust activity: CEA and MSLN Tmod selectively kill tumors in vivo

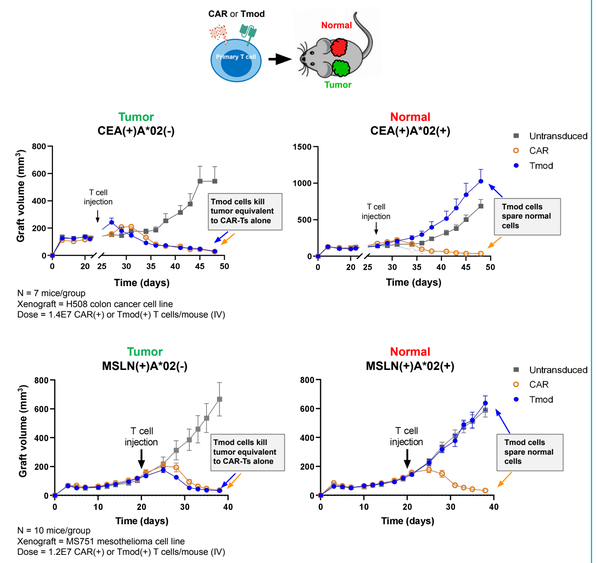
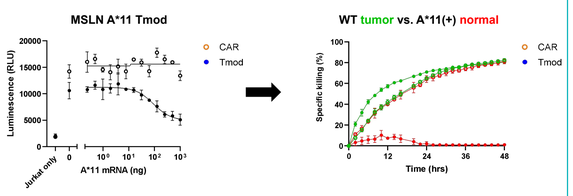


Figure 7. The Tmod platform is modular and can be extended to more patients by pairing different activators and blockers: MSLN/A*11 example



Jurkat cells expressing MSLN CAR and A*11 blocker constructs were blocked in the presence of increasing blocker antigen on endogenous MSLN(+) HeLa target cells. MSLN CAR A*11 Tmod cytotoxicity in primary T cells with MSLN(+)A*11(-) tumor or MSLN(+)A*11(+) normal HeLa target cells shows selective blocking in the presence of blocker antigen. E:T = 0.8:1.

References

1. Hamburger *et al.*, Engineered T cells directed at tumors with defined allelic loss. *Mol Immunol* 2020
2. Hwang *et al.*, Targeting loss of heterozygosity for cancer-specific immunotherapy. *Proc Natl Acad Sci* 2021

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