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TRANSLATION IN BRIEF

UBER CAR

As researchers tinker with combinations of antibody fragments and accessory molecules to optimize chimeric antigen receptor (CAR) T cells, a study from Michel Sadelain's group at Memorial Sloan Kettering Cancer Center has shown that adding a costimulatory molecule to sit alongside the CAR produces greater potency and persistency in killing tumor cells than fusing multiple domains to create ever more complex CAR molecules. The new format could yield a third-generation product that prevents T cell exhaustion, lasts longer than earlier iterations, and has greater potential for making inroads into solid tumors.

Sadelain is director of the center for cell engineering and head of the gene transfer and gene expression laboratory at MSKCC and a scientific co-founder of Juno Therapeutics Inc. (NASDAQ:JUNO).

First-generation CAR molecules involved an extracellular domain derived from an anti-CD19 antibody and an intracellular signaling domain from CD3, and were succeeded by second-generation constructs that amplified T cell activation by adding a costimulatory domain, often derived from CD28 or tumor necrosis factor receptor superfamily member 9 (4-1BB; TNFRSF9; CD137).

Sadelain told BioCentury that he wanted to combine the two domains into a single therapy because the CD28 domain was more effective at boosting the initial T cell response, whereas the 4-1BB domain was more effective at prolonging T cell persistence, which compensated over time for the lower tumor-killing potency it imparted.

His team systematically tested the effect of different combinations of costimulatory signals in CAR T cells, either by adding extra costimulatory domains to create triple-fusion CARs or expressing a separate full-length costimulatory molecule alongside a second-generation CAR.

The combinations contained double fusions of an anti-CD19 extracellular domain with either a CD28 cytoplasmic domain or a 4-1BB cytoplasmic domain, or a triple fusion of all three domains. As an alternative, full-length 4-1BB or CD80 cassettes were co-expressed on T cells containing either the anti-CD19/CD28 or anti-CD19/4-1BB double-fusion CARs.

In a mouse xenograft model of CD19-positive leukemia, the optimal combination was produced by cells that co-expressed the full-length 4-1BB with the anti-CD19/CD28 CAR, which produced the highest rate of tumor killing and persisted the longest of all the formats tested. In addition, that combination decreased expression of programmed cell death 1 (PD-1; PDCD1; CD279) and other T cell exhaustion markers compared with the other combinations.

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> Michel Sadelain, Memorial Sloan Kettering

Sadelain's team also discovered that the T cells containing the optimal combination expressed type I interferon response genes and secreted interferon β (IFNβ) after attacking the tumors, which suggested the cells induced innate and adaptive responses against the tumor. Results were published in *Cancer Cell*.

Sadelain told BioCentury, "These CAR T cells have a potent antitumor profile, good expansion properties, good persistence properties, less exhaustion markers, and on top of that they make IFNβ, which we think will be very useful based on the known antitumor effects of IFNβ against a range of tumors, including solid tumors."

He added: "Because the cells have all these attributes, we anticipate smaller T cell doses may be effective."

Sadelain said the team has filed patents on the methods, initiated manufacturing of the cells, and plans to test the next-generation CAR T cells in the clinic in 2016. He added the team is in discussions with an undisclosed biotech to co-develop the therapy. *Zhao, Z., et al. "Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells." Cancer Cell (2015)*

— Stephen Parmley

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