

June 17, 2013

SCIENCE SPOTLIGHT

# Souped-Up Cars Drive T-Cell Killing Of ROR1-Positive Tumors

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Adoptive T-cell therapy harnesses the power of the immune system to target and kill tumor cells. Normally, cytotoxic CD8 T-cells are activated to lyse target cells by the T-cell receptor binding small protein fragments (antigens) presented at the cell surface by major histocompatibility (MHC) molecules. T-cells from cancer patients can be genetically modified to recognize molecules on cancer cells without relying on MHC presentation, which can be disrupted in cancer cells, by gene transfer of chimeric antigen receptors (CARs). These modified T-cells are then expanded *ex vivo*, and transferred back into patients to eradicate tumors. Identifying CARs that target multiple cancers without recognizing normal tissues can increase the use of adoptive T-cell therapy.

CARs are synthetic receptors built to link the single-chain variable fragment (scFV) of a monoclonal antibody to intracellular signaling domains, including the T-cell receptor CD3 $\zeta$  chain that mediates T-cell activation and cytotoxicity when bound to a target cell (see figure). In previous studies, Dr. Michael Hudecek and colleagues in the laboratory of Dr. Stanley Riddell (Clinical Research Division) engineered and validated a CAR made from a monoclonal antibody that recognizes the cell surface receptor tyrosine kinase-like orphan receptor 1 (ROR1) (Hudecek *et al.*, 2010). ROR1 is expressed on a variety of B-cell malignancies, and subsets of some solid tumors, including breast, colon, lung, and kidney tumors. ROR1 functions in oncogenic signaling to promote tumor cell survival in epithelial tumors. Importantly, ROR1 is not expressed on vital organs, except adipose and pancreatic tissue, which reduces potential toxicities from killing of normal cells. In the original study, the ROR1-CAR transgene was delivered by lentivirus into purified CD8 T-cells from healthy human donors. These modified T-cells both recognized and killed ROR1-positive tumor B-cells, derived from chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) patients.

Extending these results, researchers in the current study modified the spacer region of the ROR1-CAR to improve T-cell activation. Previous studies had determined that, depending on the region of the protein the antibody fragment bound, different spacer lengths allowed more flexibility for the modified T-cells to attach and kill target cells. Since the antibody fragment recognizes a distal region of the ROR1 protein, the authors hypothesized that shorter spacer sequences would enhance killing. Indeed, they found that truncating the spacer length, from the original long hinge (229 amino acids)

to intermediate (119 amino acids) and short hinges (12 amino acids) progressively increased cytolytic activity to ROR1-positive cells.

CAR activity can influence proliferation and survival of the T-cells, which correlates with anti-tumor activity of adoptive T-cell therapy. The researchers found that the shortest hinge caused the greatest increase in T-cell proliferation after engagement of ROR1 on tumor cells. Importantly, this increased proliferation did not result in activation-induced cell death of the T-cells. The researchers also looked at the ability of the cells to produce cytokines (IFN- $\gamma$ , TNF- $\alpha$ , and IL-2) important for T-cell activity after target recognition, and they again found that the shortest hinge resulted in the highest amount of cytokine production.

Hudecek *et al.* then replaced the scFV antibody fragment with a higher affinity antibody that also recognized the distal region of the ROR1 protein. This higher affinity ROR1-CAR paired with a short spacer increased T-cell proliferation and cytokine production after tumor cell binding. The efficacy of this optimized ROR1-CAR was compared to CD19-CAR. CD19 is also a cell surface marker found on subsets of malignant B-cells, and clinical trials have shown efficacy of CD19-CAR T-cell therapy in human patients. T-cells with either CAR were equally effective at killing target cells expressing both CD19 and ROR1, and similar cytokine levels were produced. The modified T-cells were compared for anti-tumor activity *in vivo* in a mouse model of systemic mantle cell lymphoma. ROR1-CAR T-cells had comparable activity to CD19-CAR T-cells after a single dose.

Finally, the researchers showed that ROR1-CAR T-cells were effective against breast or kidney epithelial tumor cells that express ROR1. Notably, there was an increase in cytokine secretion and proliferation for ROR1-CAR T-cells against a breast cancer cell line compared to the lymphoma cell lines. The presence of abundant ligands for NKG2D, a costimulatory molecule on T-cells, and blocking antibodies demonstrated that the NKG2D pathway provided costimulation and activation of ROR1-CAR T-cells against breast cancer cell lines. Whether this co-stimulation improves adoptive T-cell therapy *in vivo* remains to be determined.

These studies show that an optimized ROR1-CAR is effective in driving T-cell killing of B cell lymphomas *in vivo* and epithelial tumors *in vitro*. The expression of CD19 on normal B-cells increases the toxicity of CD19-CAR T-cell therapy. The low level of expression of ROR1 on normal tissues may make ROR-CAR T-cell therapy less toxic, but requires additional studies. Clinical trials in humans can then evaluate the potential for adoptive T-cell therapy against ROR1.

[Hudecek M, Lupo Stanghellini MT, Kosasih PL, Sommermeyer D, Jensen M, Rader C, Riddell S.](#) 2013. Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-

specific chimeric antigen receptor T-cells. *Clinical Cancer Research*. Epub ahead of print, doi: 10.1158/1078-0432.CCR-13-0330

Also see: [Hudecek M, Schmitt TM, Baskar S, Lupo-Stanghellini MT, Nishida T, Yamamoto TN, Bleakley M, Turtle CJ, Chang WC, Greisman HA, Wood B, Maloney DG, Jensen MC, Rader C, Riddell SR](#). 2010. The B-cell tumor-associated antigen ROR1 can be targeted with T cells modified to express a ROR1-specific chimeric antigen receptor. *Blood* 116:4532-41.

[Turtle CJ, Hudecek M, Jensen MC, Riddell SR](#). 2012. Engineered T cells for anti-cancer therapy. *Current Opinion Immunology*24:633-9

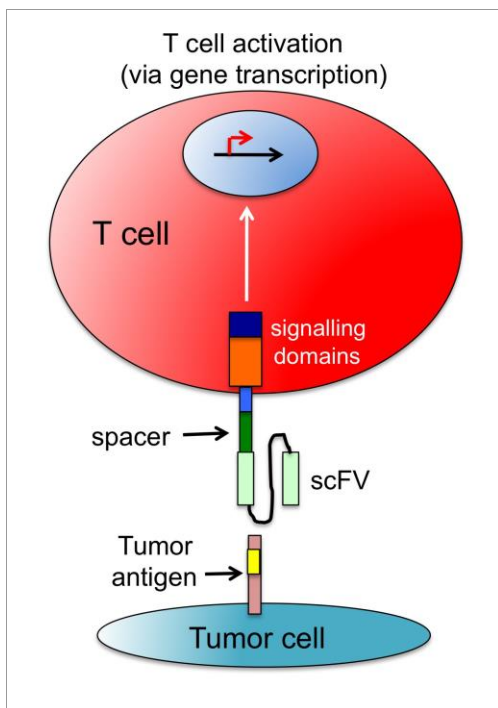


Image adapted from Turtle et al., 2012.

Chimeric antigen receptors (CARs) are designed to allow T-cell recognition and killing of tumor cells. CARs can be modified by changing the binding affinity of the antibody fragment (scFV) to the tumor cell surface marker, the spacer length, or the intracellular costimulatory domains to increase T-cell killing of tumor cells.