Therapeutics & Vaccines

Nonviral Generation of Genome-Edited CAR T Cells

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a method to generate genome-edited T cells including CAR T cells using site-specific genome editing where the editing machinery consists only of proteins and nucleic acids without any viral vectors.

Overview

Use of viral vectors for chimeric antigen receptor (CAR) T cell manufacturing constitutes a bottleneck in the supply chain for biomanufacturing and can be problematic due to: (1) batch-to-batch variability, (2) use of xenogeneic components during manufacturing of viral vectors, and (3) the high random integration of viral elements into the human genome. The poorly specified integration of the CAR transgene can lead to heterogeneous expression that can be readily silenced, in part by host cell recognition of viral genetic elements. Ideally, CAR T cells could be made via a process that does not involve using viruses to incorporate the CAR gene into the genome of T cells.

While genome editing has been used to generate CART cells with a site-specific integration of the CAR, these methods rely on transduction of the T cells with AAVs. To date, such methods to generate CAR T cells have shown limited to no activity in solid tumors. Needed are new methods for generating genetically modified T cells, including CAR T cells, that would lead to measurable efficacy against either hematologic malignancies or solid tumors.

The Invention

UW-Madison researchers have developed nonviral, ex vivo methods of site-specifically inserting a transgene containing a CAR gene into a T cell genome. This is achieved by introducing into a population of unmodified T cells a Cas9 ribonucleoprotein and a nonviral double-stranded homology-directed repair (HDR) template, to provide genome-edited T cells. The Cas9 ribonucleoprotein includes a Cas9 protein and a guide RNA that directs double-stranded DNA cleavage of a cleavage site in a T cell expressed gene. The nonviral double-stranded HDR template comprises the synthetic DNA sequence flanked by homology arms that are complementary to sequences on both sides of the cleavage site in the T cell expressed gene. The transgene is specifically integrated into the cleavage site of the T cell expressed gene created by the Cas9 ribonucleoprotein in the genome-edited T cells, and the cells are then cultured.

Applications

- Biomanufacturing of T cells

Key Benefits

- Eliminates the need for viral incorporation of the CAR construct into T cells
- May be used to generate CAR T cells active against both hematological malignancies and solid tumors

- Reduces the risk of incorrect insertion and may improve success rates of CAR T cells (through disruption of TRAC and production of more active CAR T cells).

Stage of Development

The researchers have demonstrated that nonviral CRISPR-CAR T cells are efficiently manufactured in 9 days and exhibit decreased detrimental signaling and exhaustion before encountering their target antigen. They have also shown that nonviral TRAC-CAR T cells potently upregulate cytotoxic transcriptional programs and kill target-antigen positive human cancer cells in vitro within co-culture assays. These nonviral TRAC-CAR T cells successfully cause tumor regression in vivo within human xenograft cancer models in mice at comparable efficiency to state-of-the-art, viral CAR T cells. The cells can be manufactured in a xeno-free manner and have high potential to simplify and advance CART cell manufacturing by elimination of viral vectors.

Additional Information

For More Information About the Inventors

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Tech Fields

- Therapeutics & Vaccines : Oncology

For current licensing status, please contact Andy DeTienne at adetienne@warf.org or 608-960-9857