CAR T cells specific for CD19 can be redirected to kill CD19 negative tumors

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1 - Introduction

Remarkable progress has been made in the treatment of relapsed/refractory Acute Lymphocytic Leukemia and Non-Hodgkin Lymphoma with CAR-CD19 T cells. In contrast, progress against CD19-negative hematological cancers and solid tumors has been limited. Intensive efforts to optimize cellular therapeutics for better efficacy include provision of cytokine support and countering immuno-suppression. However, lack of sufficient antigen is a significant additional hurdle that CAR-T therapeutics for solid tumors must overcome. We present a novel strategy to utilize CD19 for sustained antigen presentation in order to promote cellular therapeutic expansion, efficacy and persistence. The strategy, called IMPACT™ (Integrated Modules of Pimtnize Adoptive Cell Therapy), employs a methodology that is modular in design and can be applied to diverse antigens and tumor types, yet retains the well-established advantages of CAR T cells directed to CD19.

2 - Technology Overview

IMPACT™ fusion proteins are created by cloning the extracellular domain (ECD) of a CAR T cell target protein (e.g. CD19) to an scFv that recognizes a second target protein. The system is modular: diverse ECD-scFv fusion proteins have been designed and expressed. In one iteration, this fusion protein is purified and utilized in conjunction with an existing CAR T cell, e.g. a CD19-anti-Her2 fusion protein is purified and added to a culture of CAR19 T cells and Her2+ tumor cells, creating a bridge that triggers CAR19 T cell cytotoxic activity. In another iteration, the CAR19 T cells express the CD19-anti-Her2 fusion protein, and this expression is sufficient to create the “cytotoxic bridge” as shown in Figure 1. The normal B cell pools ensures expansion and persistence of the CAR19 T cells in vivo.

3 - Purified IMPACT™ fusion proteins

We cloned the human CD19 ECD (two IgC domains) and the scFv derived from the anti-Her2 mAb trastuzumab into a lentiviral expression system for transfection then purification by affinity chromatography and SEC (Lake Pharma). Monomeric protein was used in our experiments; this monomer is stable in solution. The fusion protein carries a C-terminal His tag. Control proteins (CD19 ECD-His and CD22 ECD 1-3 trastuzumab scFv) were also created.

4 - Construction and characterization of CAR19 T cells

The scFv from anti-CD19 mAb FMC63 was cloned in frame with a FLAG-tagged linker, the CD28 transmembrane sequence and the cytoplasmic domains of CD28, 4-1BB and CD32; The FLAG tag was encoded to facilitate detection of the CAR by FACS analysis following transduction into primary human T cells (Figure 3). The CAR19 T cells were tested for cytokotic activity in a cell killing assay using B cell lymphoma lines (ATCC). One example is shown in Figure 3.

5 - Fusion protein mediated cytotoxicity

The ability of the fusion proteins to bridge to CAR19 cells and mediate killing of Her2+ SKOV-3 tumor cells was evaluated. First we performed a titration.

6 - CAR19 with the IMPACT™ fusion protein encoded as an integrated gene

The development candidates are being constructed as integrated genes (i-genes) using lentiviral vectors and packaging systems. A prototype schematic is shown here:

In Closing:

We conclude that IMPACT™ fusion proteins mediate redirected tumor cell killing at very low concentrations, are not ‘shut-down’ by the soluble protein, and can be successfully secreted from CAR T cells. The first in vivo study using purified fusion proteins, CAR19 cells and SKOV-3 tumors is in progress.

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