Introduction

Wide-spread adoption of autologous cell therapies continues to be challenged by manufacturing difficulties, availability, safety, consistency and cost of production. In order to overcome many of these challenges, we have developed an alternative approach employing allogeneic gamma delta (Vδ1) T cells to create a universal T cell therapy for the treatment of cancers. Vδ1 T cells are a minor lymphocyte population in the circulation but can be found in larger numbers in tissues where they exhibit potent immune monitoring, anti-viral, anti-tumor and anti-microbial functions. Vδ1 T cells can recognize pathogen stressed and transformed target cells in an HLA independent fashion and represent a functional "bridge" between innate and adaptive immunity thereby facilitating activation in an allogenic setting without the concern of Graft versus Host Disease (GVHD) that challenges development of allogeneic T cells.

Here we present a Vδ1 CAR T cell product targeting CD20 antigen for the treatment of B cell malignancies. Vδ1 T cells are the predominant tissue-associated Vδ1 T cell subset in humans and are thought to recognize signals of cellular stress, viral infection and inflammation. In our process, Vδ1 T cells were selected and expanded from peripheral blood of normal healthy donors and engineered with a 2nd generation CAR construct (4-1BB) to create an allogeneic CAR T cell product. The level of expansion, purity and retention of excellent anti-tumor potency following CAR support creation of a substantial number of doses (~1000 production run) of well characterized, uniform product that is available in an "off-the-shelf" manner. CAR engineered Vδ1 T cells perform substantially similar to the allogeneic gamma delta T cells and potentiates the multi-recceptor targeting of lymphoma cell lines.

We have demonstrated that in response to target cells, Vδ1 CAR T cells secrete effecter cytokines, induce apoptosis and clear lymphoma cells in vitro, and these functions can be further potentiated by providing exogenous cytokines to support proliferation. When tested in vivo, Vδ1 CAR T cells demonstrated potent antitumor activity in both disseminated and subcutaneous models of B cell lymphoma without xenogeneic GVHD. We show that the 4-1BB co-stimulatory domain significantly improves the persistence of Vδ1 CAR T cells, and additional engineering may further optimize the cell product. Overall, these data show selectively expanded Vδ1 T cells represent a unique, safe and effective platform for therapeutic intervention in various cancers which warrants further clinical investigation of this 4-20 targeted Vδ1 CAR T cell product drug candidate.

Manufacturing of Allogeneic V61 CAR T Cells

• Robust activation using a proprietary Vδ1 TCR monomeric antibody
• Viral transduction using v-retroviral vector to introduce CAR transgene
• Large scale perfusion bioreactor based expansion
• Excellent post cryopreservation viability and function
• ~1000 doses per batch; available "off-the-shelf"
• cGMP compliant manufacturing

Healthy normal donors provide reliable and scalable source of PBMC starting material
• Donor screening identifies optimal donors that provide robust expansion, transduction and end of culture purity of V61 CD20 T CAR T cells

Robust Cytotoxicity on B Cell Lymphoma Lines

Short-term cytotoxicity: Unengaged V61 T cells show varying level of cytotoxicity on B cell lymphoma lines but not normal allogeneic B cells. Addition of 10000 CD20 CAR expressing V61 T cells to Jurkat target cells at 50:1 E:T ratio can result in 72% lysis, whereas no significant lysis is seen with control T cells. All donors tested have been treated with Cytodex 3 beads and show elevated cytotoxicity as compared to untreated donors.

• Vδ1 CAR T cells show robust cytotoxic activity on CD20+ B cell lymphoma lines including BCL-6+ Will-2 B cell lymphoma lines
• Unengaged V61 T cells show varying degree of innate cytotoxicity on wide range of cancer cell lines (no killing of Normal allo B cells; introduction of CAR potentiates killing

Summary

• Healthy donor-derived CD20 CAR-engineered V61 cells can be consistently manufactured to create an "off-the-shelf" immune cell product
• Vδ1 CAR CD20 T cells display robust anti-tumor activity against a range of CD20 expressing tumor lines in vitro and demonstrate potent effecter cytokine production and upregulation of activation markers (e.g. CD69)
• Vδ1 CAR T cell expand in response to target cell engagement and expansion is augmented by exogenous homoeostatic cytokines, such as IL-2 or IL-15, both in vitro and in vivo
• Vδ1 CAR T cells show robust in vivo activity in disseminated and s.c. models of B cell lymphomas
• Vδ1 CAR T cells do not induce GVHD in murine models
• Large-scale cGMP-compliant manufacture of healthy donor-derived V61 CAR CD20 T cells is expected to provide a safe and effective therapeutic option for patients with CD20 expressing malignancies; clinical studies are anticipated to start in early 2020