Harnessing both the innate and adaptive immune system could increase the efficiency of current cancer immunotherapies and promote durable anti-tumor immunity. Invariant natural killer T (iNKT) cells are innate-like lymphocytes that bridge innate and adaptive immune responses and promote anti-cancer immunity. iNKT cells are activated and respond rapidly via multiple signals such as recognition of lipid antigens through the invariant T cell receptor (TCR), pro-inflammatory cytokines or recognition of stress ligands. Here we describe, AgenT-797, a novel allogeneic and "off-the-shelf" iNKT cell therapy, designed to promote effective anti-cancer immunity against a wide range of malignancies.

Methods
iNKT cells isolated from healthy donors were expanded by stimulation of the invariant TCR with alpha-Galactosylceramide (aGalCer) and cytokines using the AgenTus manufacturing protocol. The phenotype and functional activity of the expanded unmodified iNKT cells, AgenT-797, were characterized by flow cytometry. The cytotoxic potential of AgenT-797 was assessed in tumor co-culture assays against CD1d-expressing cancer cell lines. To further direct anti-tumor responses, iNKT cells were engineered to express Chimeric Antigen Receptors (CARs), and the cytotoxic potential assessed against antigen expressing cancer cells.

Background

AgenT-797, a novel allogenic and “off-the-shelf” iNKT cell therapy promotes effective tumor killing

Figure 1: iNKT cells can directly target tumors and can orchestrate the immune system for an enhanced tumor response – an ideal vehicle for cell therapy
iNKT cells can recognize CD1d expressing tumor cells via their invariant TCR, but also through their NKGD2 receptor and stress ligands. Upon recognition, iNKT cells can directly kill the tumor cells. On top of that, as potent producers of IFNγ, activated iNKT cells can recruit nearby effector cells to further enhance the anti-tumor activity. iNKT cells can also target CD1d expressing suppressive myeloid cells. IL12 produced by myeloid cells can activate iNKT cells, which in turn can activate effector NK and T cells.

Figure 2: AgenT-797 is manufactured by isolation of iNKT cells from peripheral blood of healthy donors and stimulation and expansion in vitro
A. Levels of circulating iNKT cells were measured in iNKT cells of healthy donors and manufacturing cut-off was set at 0.05% of CD3+ lymphocytes (left graph). From selected donors, iNKT cells were purified and cells expanded using AgenTus manufacturing protocol. At the end manufactured, purity of culture is 99% (Representative flow plots are shown in right)
B. Cytokines that were produced during expansion of iNKT cells were measured from supernatant cultures at the end of manufacturing. Both Th1 (IFNγ, GM-CSF, TNFa) and Th2L4.3, IL10 cytokines were produced.
C. Various activation and exhaustion markers were measured on AgenT-797 after manufacturing.

Figure 3: agenT-797 cells can target tumor cell lines in a TCR dependent and independent manner
A. TCR-dependent: AgenT-797 cells were challenged with aGalCer pulsed C1R-COD1 cell line (E:T = 1:1) and activation, target cell killing, and cytokine production was measured after 24 hours
B. TCR-independent: AgenT-797 cells were challenged with K562-COD1 cell line (E:T = 1:1 and 10:1) and activation, target cell killing and cytokine production was measured after 24 hours.
C. Expression of various activating NK cell receptors was measured by flow cytometry. Pink and blue line indicate individual donors.

Figure 4: iNKT cells can be engineered to express a chimeric antigen receptor (CAR) to redirect the anti-tumor activity
A. iNKT cells were transduced with a chimeric antigen receptor and expanded. Level of transduction efficiency was determined by flow cytometry. Non-transduced iNKT cells were used as control.
B. CAR-iNKT cells were challenged with a cell line expressing the target antigen at different E:T ratios. Activation of NT and CAR-iNKT cells were measured by 11BB-suppuration. Percent target cell killing was determined based on target cell alone wells.

Conclusions
- iNKT cells are an ideal cell therapy vehicle for its natural ability to target tumor cells as well as reshape the tumor microenvironment.
- AgenT-797 is manufactured by isolating iNKT cells from peripheral blood of healthy donors and expanded in vitro using AgenTus manufacturing protocol in less than 30 days.
- AGET-797 cells can produce both Th1 and Th2 cytokines upon stimulation
- AgenT-797 are cytotoxic against target tumor cells both in a TCR dependent and antigen independent manner
- iNKT cells can be engineered to express a CAR to further potentiate their anti-tumor potential

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