Abstract 928

**GENERATION OF MULTI-FUNCTIONAL AND TUMORICIDAL NKT-LIKE ANTIGEN-SPECIFIC T CELLS**

1Jaehyung Park*, 2Jeff Bellinder, 2Tisha San Miguel, 3Neal Lawrence, 1Atif Azam Khan, 1Nathan Lavoy, 1Chris Tompkins, 2Brianna Schoen, 1Charles River Laboratories, Bothell, WA, USA; 2Charles River Laboratories, Northridge, CA, USA

**Background**
Natural Killer T cells (NKTs) offer great promise for cancer immunotherapy. However, NKTs are extremely rare within human peripheral blood, posing difficulties to isolation and *ex vivo* expansion. Moreover, native NKTs may develop immunosuppressive activity within the tumor microenvironment.1 For these reasons, *in vitro* generated CD56+ T cells (NKT-like cells) have emerged as an alternative to native NKTs.2 However, as CD56+ T cells are *in vitro* generated from precursors (e.g., PBMCs) mostly using non-antigen-specific stimulants (e.g., IL-15, IFN-γ, PMA/Ionomycin, or PHA), tumor killing capacity of those induced CD56+ T cells has been limited to non-antigen specific killing pathway. Moreover, questions remain unanswered regarding how best to enhance productivity and cytotoxic capacity of CD56+ T cells.

We demonstrate *in vitro* generation of antigen-specific CD56+ T cells from human antigen-specific T cell populations (ASTCs) with a comparison of phenotype and cytotoxic functions between *in vitro* derived NKT-like cells and NKTs isolated from human apheresis.

**Methods**
During *in vitro* production of ASTCs (CD3+CD8+), wherein antigen specificity is created by TCR stimulation with a single antigen peptide (MART-1, ELAGIGILTV), a subset of CD56+ T cells was generated. CD56+ and CD56- T cell subsets were isolated using immunomagnetic separation. Phenotypic and functional characterization of CD56+ and CD56- populations from ASTCs were compared to NKTs directly isolated from apheresis.

Results MART-1-specific ASTCs were *in vitro* generated through scaled-up production to produce at least 700 million cells from a single donor batch and CD56+ T cell population increased from 4.49% in starting apheresis to 43% out of total ASTCs. Interestingly, both CD56+ and CD56- subsets exhibited similar MART-1 tetramer positivity of 88%. During co-culture of ASTCs with antigen peptide-loaded T2 cells, CD56+ subset induced greater T2 cell death than the CD56- subset, while greater IFN-γ secretion was detected from the CD56- subset (figure 1). In tumor cell killing assay with MeWo cells, all three effector cell populations (total MART-1-specific ASTCs, CD56+, CD56- cells) induced tumor killing efficiently, with up to 50% cell death observed with a 10:1 effector to target ratio (figure 2).

Conclusions Antigen-specific CD56+ T cells were generated and confirmed to have cytotoxic potential comparable to CD56- T cells in antigen-specific and MHC-restricted manner. To further understand generation and function of NKT-like cells, we are investigating generation of CD56+ T cell subset during production of ASTCs specific to additional antigens, and their cytotoxic functions in non-antigen specific and MHC-unrestricted manner.

**REFERENCES**

Ethics Approval This study was approved by HemaCare – Biorepository Protocol 001, Collection or Procurement of Samples from Healthy Participants Utilizing United States (US), European Union (EU) and Other International Criteria (Pro00034695).
Abstract 928 Figure 2  CD56+ and CD56- T cells from antigen-specific T cell development have comparable tumoricidal capacity. Target cells (MeWo tumor cell line expressing HLA-A*02:01) were pre-plated at 20,000 cells/well. Next day, effector cells (MART-1-specific ASTCs, HLA-A*02:01-restricted) were separated into CD56+ and CD56- cells and added to wells of target cells at 1:1, 5:1, or 10:1 effector:target ratios in triplicate. After 24 hours co-culture, T cells were washed from the adherent tumor cells using PBS. Remaining viable tumor cells were then assessed using Cell Titer Glo assay. Cytotoxicity was calculated as \[1-(\text{experimental RLU}/\text{target only RLU})\times100\], where RLU stands for relative luminescent units.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0928