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703. CELLULAR IMMUNOTHERAPIES OTHER THAN CAR-T CELLS: BASIC AND TRANSLATIONAL

Pre-clinical characterization and process development of a BCMA-directed CAR NK-cell therapy product for relapsed/refractory multiple myelomaCharles Yin^{1,2}, Angela Hamie², Janna Kozuska³, Carina Debes-Marun², Bee Tan², Pankaj Tailor², Michael Chu²¹University of Alberta, Laboratory Medicine and Pathology, Edmonton, Canada²University of Alberta, Medical Oncology, Edmonton, Canada³University of Alberta, Alberta Cell Therapy Manufacturing (ACTM), Edmonton, Canada

Abstract The burgeoning field of cellular therapy, in particular chimeric antigen receptor (CAR) T-cell therapy, has revolutionized the treatment of multiple myeloma. CAR T-cells directed against B-cell maturation antigen (BCMA) has proven effective in inducing responses and prolonging overall survival in myeloma, even in the multiply relapsed/refractory setting. However, CAR T-cells possess several inherent drawbacks that limits their clinical effectiveness. Patients exposed to multiple previous lines of chemo- and immune-therapy may have insufficient T-cell numbers for collection or have T-cells that possess an exhausted phenotype, leading to difficulties during CAR T-cell manufacturing and a suboptimal final product. CAR T-cell therapy is also associated with a number of potentially serious toxicities, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). CAR natural killer (NK)-cells are an emerging technology that overcomes many of the limitations inherited in CAR T-cells. Given NK-cells do not recognize and respond to non-self HLA molecules in the way T-cells do, they can be generated as an allogeneic, "off-the-shelf" product. CAR NK-cells also demonstrate an improved safety profile, with significantly reduced risk of CRS and ICANS as compared to CAR T-cells. In this study, we describe the characteristics and pre-clinical process development of a BCMA-directed CAR-NK cell product intended for use in patients with relapsed/refractory multiple myeloma.

Primary human NK-cells were readily isolated from healthy donor peripheral blood, buffy coats and cord blood with reasonable purity using either positive or negative selection strategies. A majority (>96%) of isolated NK-cells from donor peripheral blood or buffy coats were CD56dim/CD16+ cytotoxic NK-cells. Using commercially-available human NK-cell serum-free culture media, recombinant human IL2/IL15/IL21 cytokine mixture and soluble protein nanoparticle-bound anti-CD2 and anti-NKp46 antibodies we were able to achieve 30-40x expansion of NK cells within a 9-day period. We then introduced an anti-BCMA ScFV/41BB/CD3z CAR mRNA construct into both the NK92 cell line and primary human NK cells using a viral vector-free LNP delivery system. Transfection efficiencies with our LNP-based system were around 50-60% with excellent cell viability and persistent cell surface CAR expression for up to 5 days in culture. CAR NK-cells demonstrated markedly increased *in vitro* specific killing of BCMA-expression myeloma cell lines compared to non-transfected NK-cell controls in a dose-dependent manner. Preliminary work pre-clinical process development work has begun to scale up production of these CAR NK-cells using G-Rex® bioreactors in order to generate sufficient CAR NK-cells (800 million cells/dose) to support a phase Ib/II clinical trial in relapsed/refractory multiple myeloma.

In summary, we have shown that primary human NK-cells can be isolated, expanded and transfected to transiently express an anti-BCMA CAR construct. These anti-BCMA CAR NK-cells demonstrate excellent viability and *in vitro* specific killing. Importantly, we have deliberately employed feeder cell and viral vector-free methods in order to minimize the potential for biological contamination. We have also opted to use introduce our CAR construct as mRNA in order to avoid any risk of insertional mutagenesis and associated potential for secondary malignancies. These strategies are expected to lead to increased ease of clinical translation and faster time to regulatory approval. This study is expected to lay out the foundation for the development of an allogeneic, off-the-shelf CAR NK-cell product for relapsed/refractory multiple myeloma that is able to overcome to drawbacks of conventional CAR T-cell therapy.

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