Redirecting Natural Killer Cells to Target B7-H3 Using Tri-specific Killer Engagers or Secreted Camelid Nanobody Engagers and Chimeric Antigen Receptors

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Background: B7-H3 (CD276) has gained significant clinical interest as a pan-tumor target antigen for development of various immuno-oncology agents. Due to its expression a variety of cancers and minimal expression on normal tissues, B7-H3 is an ideal tumor antigen target. To effectively target B7-H3 we developed bi-specific natural killer cell engager (BiKE) consisting of an anti-CD16 camelid nanobody NK cell engager and a camelid (cam) anti-B7-H3 nanobody to target tumors to be secreted by expanded natural killer (NK) cells or a chimeric antigen receptor (CAR) using camB7-H3. Separately, we have developed a tri-specific killer engager molecule with an IL-15 linker that is currently undergoing GMP-production and pending submission of an Investigational New Drug application to the FDA.

Methods: A camelid nanobody specific for human B7-H3 (camB7-H3) has been previously validated and presented. BiKE was cloned into a specialized lentiviral vector for NK cell transduction. A camB7-H3 CAR construct with varying linker lengths and signaling domains were transduced into NK cell lines. TriKE was produced using a prokaryotic system and tested for efficacy compared to prior standard lots. TriKE, secreted BiKE and CAR function will be evaluated with flow cytometry-based functional assays evaluating CD107a and IFNg by flow cytometry, IncuCyte 3D spheroids, and xCelligence impedance assays. Levels of secreted BiKE will be quantified using an in-house ELISA. Normal tissue cross-reactivity studies are ongoing using immunohistochemistry.

Results: Engineering lots of B7-H3 TriKE demonstrated similar efficacy to our in-house produced reference lots. Secreted BiKE production by expanded NK cells is currently underway. Lentivirus has been successfully produced and NK cells will be transduced in the coming weeks. We anticipate that transduced NK cells with secrete sufficient BiKE to activate NK cells and lead to target prostate cancer cell line destruction. B7-H3 CAR resulted in NK cell degranulation and target cytotoxicity, but to varying levels depending on CAR structure and target B7-H3 expression.

Conclusions: GMP production of TriKE is ongoing with an anticipated submission to the FDA in the first quarter of 2025 for consideration of a phase I/II basket clinical trial with a prostate cancer specific arm and expansion cohort. Additionally, we have successfully produced and validated the specificity and function of camelid nanobody based BiKE and CAR targeting the pan-tumor antigen B7-H3, facilitating additional delivery methods. Further validation of novel delivery methods to facilitate effector cell secretion of BiKE or CAR-NK development is currently underway and undergoing optimization. We anticipate that tuning of lentiviral dose and CAR characteristics will be required for optimization of efficacy against prostate cancer cells.

Disclosures: Felices and Miller receive research support and stock and, with the University of Minnesota, are shared owners of the TriKE technology licensed by the University to GT Biopharma, Inc. This relationship has been reviewed and managed by the University of Minnesota in accordance with its conflict-of-interest policies.

Miller receives research funding from GT Biopharma. Miller and Felices receive consultancy from GT Biopharma. Zorko has received travel funding from Caris Life Sciences and Telix Pharmaceuticals and consulting fees from Bayer and Dendreon.

NAZ is supported by a DoD Early Investigator Award (Award Number W81XWH-22-1-0242), The Randy Shaver Community Cancer Fund, the Department of Urology Philanthropic Fund, and a 2022 Prostate Cancer Foundation Young Investigator Award.