Dual Camelid Nanobody Tri-Specific Killer Engager Molecules to Target Metastatic Castration-Resistant Prostate Cancers with Improved Cytokine Safety Profiles

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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 a tri-specific killer engager molecule with an IL-15 linker that joins a camelid nanobody CD16 that targets NK cells and a camelid nanobody B7-H3 that targets B7-H3 on tumor cells. Novel therapeutic strategies are needed to mitigate toxicities of conventional T cell therapies.

Methods: A camelid nanobody specific for human B7-H3 (camB7-H3) TriKE has been previously validated and presented using eukaryotic systems. Prokaryotic production and isolation were completed by Cytovance (Oklahoma City, OK) under GMP conditions. GMP-grade camB7-H3 TriKE was validated against benchmarked research-grade standard produced in the lab using CD107a degranulation and interferon gamma production by flow cytometry, flow cytometry-based proliferation assays, and EC50 curves were generated. A bispecific T cell antibod (bsAb)y using the OKT3 anti-CD3 clone and camB7-H3 was produced and validated for cytokine production benchmarking. Cytokine (IL-2Ra, IL-2, IL-6, and IFN-gamma) production by NK cells co-cultured with engager and target was evaluated using commercial ELISA kits. Finally, in-use testing was completed using standard insulin syringes. 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 and produced nearly identical EC50 curves for NK cell activation and proliferation. Cytokine analysis by ELISA noted a nearly 10-fold decrease in cytokine release mediators using camB7-H3 TriKE compared to camB7-H3 bsAb. In-use studies demonstrated no significant loss due to protein binding for up to 4 days with product stability at up to 37C for similar time frames.

Conclusions: GMP production of camB7-H3 TriKE has been completed by a qualified contractor and clinical stability studies are pending. We anticipate submission of an Investigational New Drug application to the FDA in the 4th quarter of 2025 for consideration of a phase Ia/Ib basket clinical trial with a prostate cancer specific arm and expansion cohort. Anticipated first patient dosing is April 2026. Patients are anticipated to receive up to 5 doses of subcutaneously-administered camB7-H3 TriKE per week in a fast-track dose-escalation model. camB7-H3 TriKE GMP product exhibits nearly identical activity and specificity as our proof-of-concept research-grade product. In use testing shows preliminary feasibility for camB7-H3 TriKE to be stored at 4C for up to 4 days, potentially facilitating outpatient self-administration once initial target dose and safety doses have been cleared.

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.

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