Background CD137 (4-1BB) is an immune costimulatory receptor that has been recognized for its potential as an immunotherapy drug target in cancer alongside checkpoint inhibitors. We have developed a new class of modular synthetic drugs, termed Bicycle® tumor-targeted immune cell agonists (Bicycle® TICAs), which are multifunctional molecules comprised of constrained bicyclic peptides. The first molecule of this class, BT7480, a Nectin-4-dependent CD137 agonist, entered clinical trials in 2021. Preclinical data demonstrates that BT7480 induces highly potent, tumor localized CD137 agonism leading to tumor regressions and immunogenic memory in a syngeneic mouse model.

Methods In vivo pharmacodynamic responses to treatment with CD137 Bicycle® TICAs in a huCD137 MC38 syngeneic tumor mouse model were evaluated using mRNA in situ hybridization (ISH), NanoString, and scRNA sequencing.

Results NanoString RNA profiling of tumors excised from mice treated with BT7480 at multiple timepoints revealed a two-step process—an early and rapid increase of genes relating to myeloid cell activation that precedes the upregulation of cytotoxicity-related genes. These data indicate that a wider reprogramming of the immune microenvironment beyond T cells may occur early after BT7480 administration. These pharmacodynamic changes were also observed following treatment with an EphA2/CD137 Bicycle TICA® (BT7455), indicating a common feature of targeted CD137 Bicycle TICAs.

To better understand the cellular mechanism of action of CD137 Bicycle® TICAs, we used mRNA ISH to attempt to identify the source of Ccl17 and Ccl22 mRNAs, which ruled out Cd3 and Itgam mRNA positive cells as the source of the chemokine expression. Depletion of CD8+ T cells from the mice prior to CD137 Bicycle TICA® treatment did not alter the early increase in gene expression of distinct chemokines, further indicating the involvement of other immune cell types than just effector T cells. To pinpoint the cells responsible for the early burst of signaling activity, we next turned to single cell RNA sequencing of CD137 Bicycle TICA®-treated tumors, which highlighted the involvement of other cell populations such as dendritic cells in the early steps of CD137-driven anti-tumor immunity by CD137 Bicycle® TICAs.

Conclusions Altogether, these data highlight that CD137 Bicycle® TICAs like BT7480 and BT7455 impact immune cell populations beyond T cells. This suggests that abundant T cell infiltrates may not be required for Bicycle® CD137 agonists to initiate tumor rejection, which broadens the patient population that may benefit from CD137 Bicycle TICA® treatment to also include myeloid-rich tumors with lower T cell infiltration ("colder" tumors).

REFERENCES