BT7480, a novel fully synthetic tumor-targeted immune cell agonist (TICA™) induces tumor localized CD137 agonism

POSTER# 5552

Kristen Hurov, Punit Upadhyaya, Johanna Lahdenranta, Jessica Kublin, Jun Ma, Elizabeth Repash, Marianna Kleyman, Julia Kristensson, Liuhong Chen, Eric Haines, Carly Campbell, Sailaja Battula, Stephen Blakemore, Kevin McDonnell, Phil Brandish, Phil Jeffrey, and Nicholas Keen

ABSTRACT

- 4-1BB (CD137/TNFRSF9) is a costimulatory molecule belonging to the TNF receptor superfamily that is expressed on activated T and NK cells.
- Despite compelling preclinical data, 4-1BB agonistic antibodies have been hampered by failure to delineate hepatotoxicity from efficacy in the clinic [1,2]. Next generation strategies are focused on bispecific approaches aimed at promoting target-mediated clustering of 4-1BB to limit systemic and liver toxicities [3,4].
- *Bicycles*® are fully synthetic, constrained bicyclic peptides that have high affinity and selectivity to their targets. We incorporated *Bicycle* binders specific for tumor antigens into multifunctional molecules with 4-1BB binding *Bicycles*. We termed these Tumor-targeted Immune Cell Agonists (TICAs). Unlike traditional biologic approaches, the small size (~4-8 kDa) and tunable pharmacokinetic (PK) parameters of *Bicycle* TICAs enable superior tumor penetration and allow exploration into the relationship between pulsatile dosing and 4-1BB activation while de-risking hepatoxicity concerns due to a differentiated renal elimination mechanism combined with a tumor-localized immune response.
- BT7480 is a TICA that activates 4-1BB by targeting the highly expressed tumor cell antigen Nectin-4 and demonstrates extremely potent 4-1BB agonism in primary human PBMC/tumor cell co-culture assays.
- Treatment of tumors expressing Nectin-4 with BT7480 in immune competent mouse models led to increased T cell infiltration and a cytotoxic gene signature.
- BT7480 induced complete regressions and resistance to re-challenge with intermittent dosing and the established immunologic memory was dependent on cytotoxic T cells.
- In non-human primates (NHPs), BT7480 exhibited dose linear exposure and is well tolerated up to 10 mg/kg. Liver enzymes and cytokines were not significantly altered by BT7480 in these healthy NHPs.

INTRODUCTION

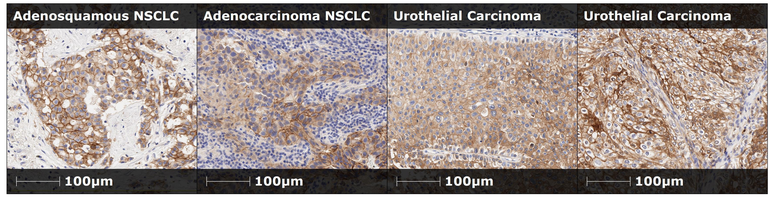

Figure 1: Nectin-4/PVRL4 is a cell adhesion molecule that is highly expressed in multiple tumor types, including non-small cell lung cancer (NSCLC) and urothelial (bladder) cancer. Representative images using a proprietary Nectin-4 IHC assay demonstrate clear Nectin-4 tumor cell membrane staining, with minimal background staining in non-tumor tissue.

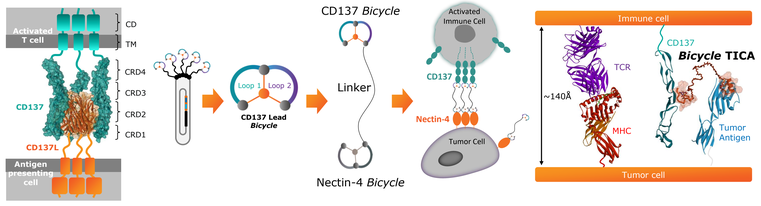

Figure 2: CD137 is a highly validated immunotherapy target that is expressed on T and NK cells, as well as several other immune cells. CD137 requires trimerization and clustering for its activation. Phage screening, affinity maturation, and chemical optimization resulted in the lead CD137-binding *Bicycle* that was then linked to a Nectin-4-binding *Bicycle*. Further chemical optimization yielded the development candidate BT7480. TICAs enable optimum spacing at an immune synapse as compared to the spacing formed through interaction of the TCR and MHC.

RESULTS

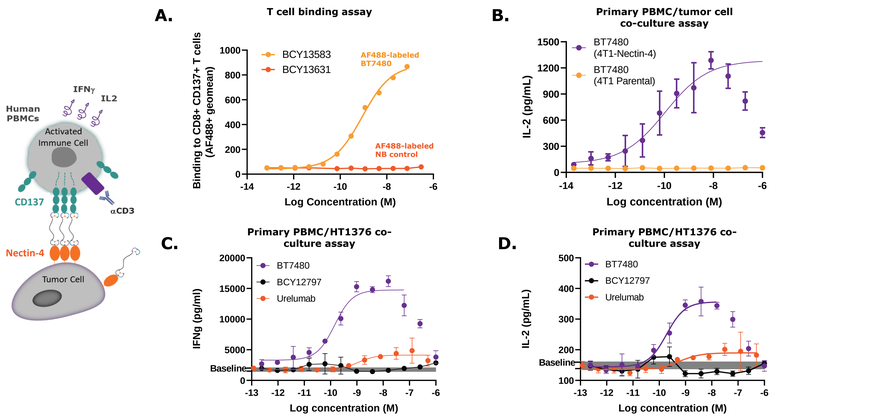

Figure 3: BT7480 bound specifically to CD137-expressing T cells and led to a Nectin-4-dependent increase in cytokine release in primary PBMC/tumor cell co-culture assays. A) BT7480 bound to primary T cells that express CD137. Human PBMCs were stimulated with anti-CD3 to induce CD137 expression. BCY13583 (Alexa Fluor(AF)-488-tagged BT7480) binding to CD137+ T cells was monitored using flow cytometry. Binding to CD137-negative cells was not detected. BCY13631, a non-binding analog of AF488-tagged BT7480, did not bind to T cells. B) BT7480 activity in a primary immune cell assay was Nectin-4 dependent. Human PBMCs were stimulated with anti-CD3 and co-cultured with mouse 4T1 cells or 4T1 cells that were engineered to overexpress Nectin-4 and IL-2 levels in the media were measured at 48 h by Luminex (n=3, +/-SD). C) and D) BT7480 led to increased cytokine release in a co-culture assay using human PBMCs and the human urothelial cancer cell line, HT1376 (n=3, +/-SD). A non-binding analog of BT7480, BCY12797, was not active. The cytokine release induced by Urelumab (Creative BioLabs) is shown for comparison. Similar results were observed with multiple independent PBMC donors.

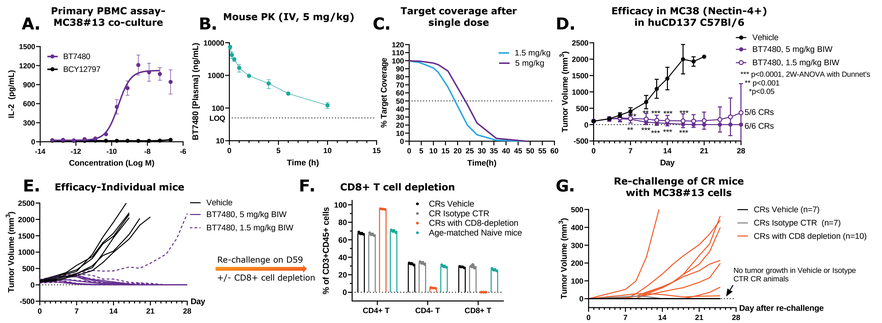

Figure 4: Intermittent dosing of BT7480 led to robust anti-tumor activity and resistance to re-challenge in a syngeneic mouse model. A) BT7480 activity in a human PBMC assay when in co-culture with MC38 cells that were engineered to express Nectin-4 (MC38#13). B) Plasma PK in CD1 mouse after a single IV dose of 5 mg/kg. C) With a 5 mg/kg dose, BT7480 can maintain at least 50% target coverage for ~25 hours. D) In syngeneic mice carrying MC38#13 tumors, BT7480 led to complete responses (CRs) in 11/12 mice dosed BIW. E) Efficacy plots for individual animals from D. F) CD8+ T cells in CR mice were depleted prior to re-challenge, as indicated by flow cytometry. G) Complete responder (CR) mice (with or w/o CD8-depletion) were re-implanted with MC38#13 cells. All tumors were rejected in animals w/o CD8-depletion, indicating an established memory response. Memory response was dependent on the presence of CD8+ T cells since most tumors (8/10) grew in CD8-depleted mice.

RESULTS

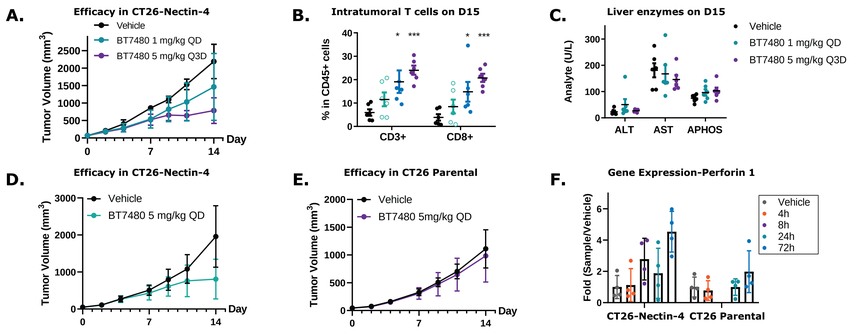

Figure 5: BT7480 led to increased tumor-infiltrating CD8+ T cells, and Nectin-4-dependent efficacy and gene expression changes. A) Intermittent IP dosing of BT7480 led to anti-tumor activity in a syngeneic mouse model carrying a CT26-Nectin-4-expressing tumor. B) Immune cell populations in the tumor were measured by flow cytometry at the end of study (day 15). C) Liver enzymes were measured on day 15; no significant changes were observed. D) and E) In a dual flank study, CT26 parental and CT26-Nectin-4-expressing tumors were implanted into the same mouse. Anti-tumor activity was observed in the tumor expressing Nectin-4 (D), but not in the parental tumor (E). F) Increased expression of a cytotoxic gene signature including the pore forming cytolytic gene, *Perforin 1*, was observed in response to BT7480 specifically in the tumor expressing Nectin-4. Gene expression in tumors was measured by Nanostring (n=4, +/-SD).

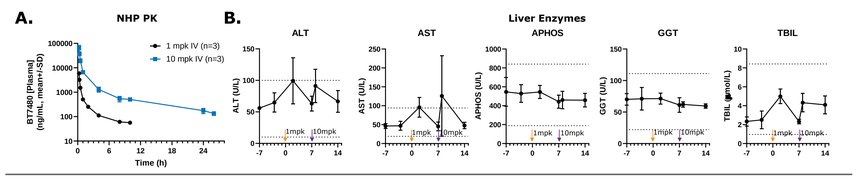

Figure 6: BT7480 exhibited dose linear exposure and is well tolerated in NHPs up to 10 mg/kg. A) Animals (n=3 +/- SD) were dosed 1 mg/kg IV on Day 0 and 10 mg/kg on Day 7. Exposures at 10 mg/kg are higher than those predicted to be required for a human efficacious dose. B) Clinical chemistry panel indicated that liver enzymes were generally well within the normal range (indicated by the dotted horizontal lines). The AST level in 1 out of 3 animals rose just above the normal range after the 10 mg/kg dose was administered and quickly recovered. Circulating cytokines were also monitored at 1h and 24h post-dose and were not significantly elevated in response to BT7480.

CONCLUSIONS/SUMMARY

- BT7480 is a Nectin-4/CD137 TICA that represents a new generation of chemically synthetic tumor antigen targeted 4-1BB agonists.
- BT7480 led to highly potent Nectin-4 dependent activity in vitro and in vivo, including complete responses and anti-tumor immunity in preclinical syngeneic mouse models.
- BT7480 was well tolerated in NHP at exposures above that of the predicted efficacious dose in humans. Further IND-enabling safety studies are ongoing.

References: [1] Segal et al, *Clin Cancer Res* 23(8): 1929-36 (2017); [2] Chester et al, *Blood* 131(1): 49-57 (2018); [3] Hinner et al, Clin Cancer Res 25(19): 5878-89 (2019); [4] Claus et al, *Sci Transl Med* 11(496): eaav5989 (2019)

Questions? Contact maren.killackey@bicycletx.com

Bicycle Therapeutics
4 Hartwell Place, Lexington, MA 02421
www.bicycletherapeutics.com