Nectin-4-dependent immune cell stimulation and anti-tumor efficacy by BT7480, a Nectin-4/CD137 *Bicycle*® tumor-targeted immune cell agonist (TICA™)

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ABSTRACT

- The costimulatory immune receptor CD137 (4-1BB/TNFRSF9) has been recognized for its potential as a drug target in cancer alongside checkpoint inhibitors, but this promise has not been realized for patients due to hepatic toxicity and limited efficacy of current biologic based therapies (1,2). Thus, alternative approaches to this important target are warranted. Next generation strategies are focused on bispecific approaches aimed at promoting target-mediated clustering of CD137 to limit systemic and liver toxicities (3,4).
- *Bicycles*® are small, structurally constrained peptides discovered via phage display and optimized using structure-driven design and medicinal chemistry approaches. We have applied this disruptive technology to the problem, identifying CD137 *Bicycles* and chemically linking these to tumor antigen binding *Bicycles* to generate multifunctional molecules that induce tumor antigen dependent, tumor localized agonism of CD137. We termed these Tumor-targeted Immune Cell Agonists (TICAs™) (5).
- BT7480 is a TICA that was designed to deliver highly potent CD137 agonism to Nectin-4 overexpressing tumor tissue. BT7480 binds potently to Nectin-4 expressing cells and engages CD137 expressed on immune cells *in trans*, leading to robust Nectin-4-dependent CD137 agonism in primary human PBMC/tumor cell co-culture assays. Treatment of Nectin-4 expressing tumors in immunocompetent mice with BT7480 induces complete tumor regressions and subsequent resistance to tumor re-challenge.
- BT7480 treatment leads to profound reprogramming of the tumor immune microenvironment including increased T cell infiltration and upregulation of a cytotoxic cell gene signature. We also observed an increase in the macrophage cell score and interestingly, time course evaluation revealed a unique mechanism of action – rapid activation of myeloid cells (<24h) concomitant with a pulse of chemokine and cytokine secretion peaking at 2-3 days, leading to a dramatic infiltration of cytotoxic T cells on days 4-6.

INTRODUCTION

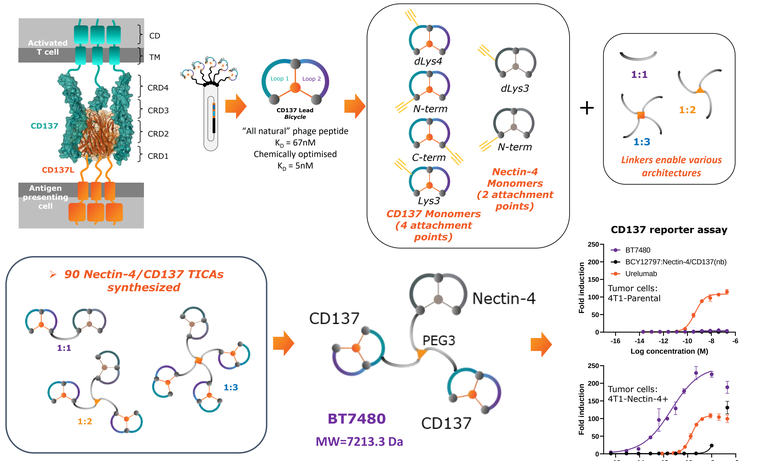

Figure 1: Path to identification of BT7480. CD137 is a highly validated immunotherapy target that is expressed on T and NK cells, as well as several other immune cells. CD137 requires trimerization and clustering for its activation. Phage screening, affinity maturation, and chemical optimization resulted in the lead CD137-binding *Bicycle* that was then linked to a Nectin-4-binding *Bicycle*. Nectin-4/PVRL4 is a cell adhesion molecule that is highly expressed in multiple tumor types. An extensive medicinal chemistry campaign and SAR investigation yielded the development candidate BT7480, which displayed potent Nectin-4 dependent CD137 agonism activity in a model system that co-cultured Jurkat-CD137 reporter cells with human tumor cells that express Nectin-4.

RESULTS

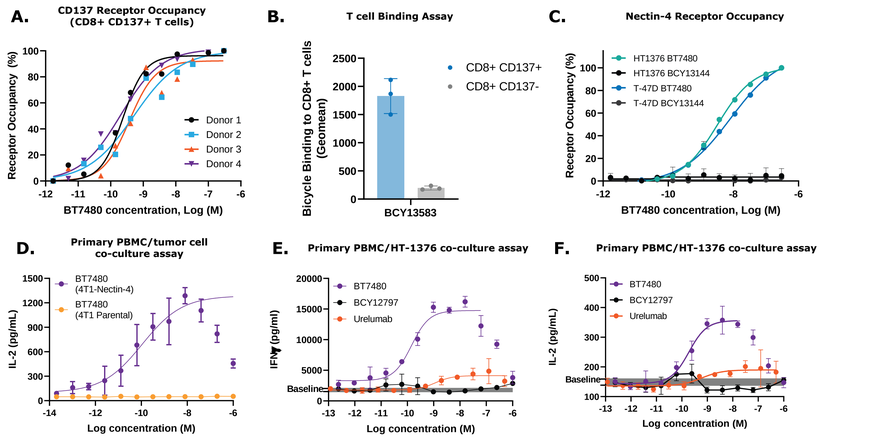

Figure 2: BT7480 bound to target-expressing cells and led to an increase in Nectin-4-dependent cytokine release in primary PBMC/tumor cell co-culture assays. (A) Human PBMCs (Donors 1-4) were stimulated with anti-CD3 and treated with BT7480. Unbound CD137 receptors and total CD137 receptors were detected by flow cytometry using an Alexa Fluor® (AF) 488-labeled CD137 *Bicycle* dimer (BCY15416) and a non-competitive CD137 mAb, respectively. Receptor occupancy (RO) was calculated as %RO=(1-MFI-Emin)/(Emax-Emin))*100. (B) AF488-tagged BT7480 (BCY13583) bound to CD8+CD137+ T cells, but not CD137- cells as monitored by flow cytometry. (C) Nectin-4-expressing tumor cells were treated with either BT7480 or BCY13144 (a non-Nectin-4 binding analogue of BT7480) and RO was determined by flow cytometry using a competitive Nectin-4 mAb (n=3, +/-SD). (D) 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 parental cells or 4T1-Nectin-4+ cells and IL-2 levels in the media were measured (n=3, +/-SD). (E-F) As in (D), except PBMCs were co-cultured with human Nectin-4-expressing HT-1376 cells. BT7480 led to increased IFNγ and IL-2 release whereas a non-CD137 binding analogue of BT7480, BCY12797, was not active. The cytokine release induced by anti-CD137 mAb (Urelumab) is shown for comparison. Similar results were observed with multiple independent PBMC donors.

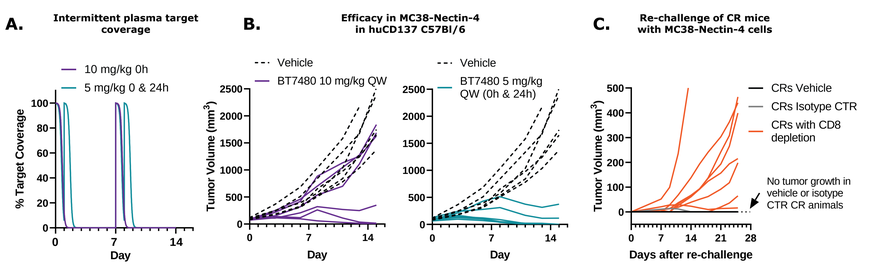

Figure 3: Intermittent dosing of BT7480 led to robust anti-tumor activity and resistance to re-challenge in a syngeneic mouse model. A) Weekly (QW) 5 mg/kg (0h & 24h) dosing of BT7480 maintains ≥20% target coverage for ~44 hours (simulated from PK data). A 5 mg/kg 0h and 24h dosing schedule mimics once weekly dosing. B) Weekly BT7480 exposure from 5 mg/kg (0 and 24h) dosing was maximally efficacious in MC38-Nectin-4 tumor model in huCD137 C57Bl/6 mice (n=6/cohort; vehicle vs. 10 mg/kg: p<0.05, vehicle vs. 5 mg/kg (0 & 24h): p<0.0001; mixed-effects analysis days 0–15). C) BT7480 complete responder (CR) mice from a similar study were re-implanted with MC38-Nectin-4 cells after treatment with CD8 cell depleting antibody or isotype control antibody. All tumors were rejected in animals without CD8 depletion (n=7 for CRs Vehicle and CRs Isotype), indicating an established memory response. Memory response was dependent on CD8+ T cells since most tumors (8/10) grew in CD8-depleted mice.

RESULTS

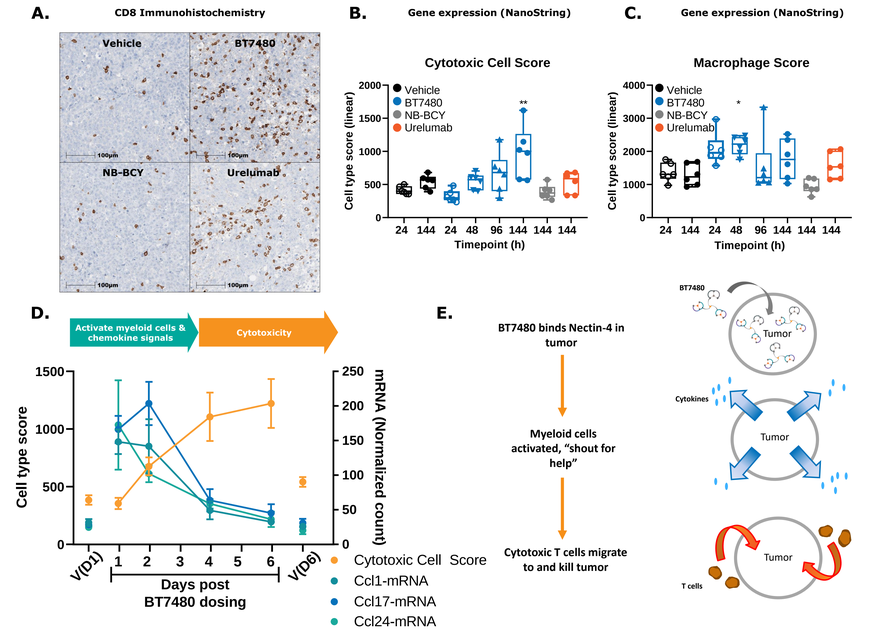

Figure 4: BT7480 treatment led to significant immunomodulation in tumor tissue, including early activation of myeloid cells. MC38-Nectin-4 tumor bearing mice (huCD137-C57Bl/6) were treated with Vehicle, 5 mg/kg BT7480 or a non-CD137 binding control *Bicycle* (NB-BCY; BCY12797) iv at 0h and 24h or 2 mg/kg anti-CD137 mAb (Urelumab) BIW. Tumors were harvested at 24, 48, 96 or 144 h as indicated, and processed for both immunohistochemistry (IHC) and transcriptional analysis performed by NanoString. (A) Representative images of tissue sections harvested at 144 h from tumors and stained for mouse CD8. (B) Transcriptional profiling showed an increase in the cytotoxic cell score and (C) macrophage score in tumor tissue after BT7480 treatment over time. *p<0.05, **p<0.01, 1wayANOVA with Dunnett's. (D) Cytotoxic cell scores (left y-axis) and Ccl1-, Ccl17- and Ccl24- mRNA counts (right y-axis) overlaid over the course of the study (days post dosing). (F) Working hypothesis for the BT7480 mechanism of action based on immune cell IHC and transcriptional profiling data.

CONCLUSIONS/SUMMARY

- BT7480 is a Nectin-4-targeted CD137 agonist and is the lead compound in a new class of synthetic tumor antigen-dependent immune agonists
- BT7480 drives complete tumor regressions and durable tumor immunity in mouse models, and this was achieved with intermittent dosing
- Initiation of a first-in-human study of BT7480 is planned for 2021

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); [5] Upadhyaya et al, *JITC* 9: e001762 (2021)

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