BT7480, a synthetic *Bicycle* tumor-targeted immune cell agonist® (*Bicycle* TICA®), induces reprogramming of the tumor immune microenvironment through tumor localized CD137 agonism

Johanna Lahdenranta[1], Kristen Hurov[1], Punit Upadhyaya[1], Heather Cohen[1], Elizabeth Repash[1], Jun Ma[1], Eric Haines[1], Carly Campbell[1], Mike Kelly[2], Phil Jeffrey[2], Kevin McDonnell[1], Philip Brandish[1], and Nicholas Keen[1]

[1]Bicycle Therapeutics, Lexington, MA, [2]Bicycle Therapeutics, Cambridge, UK

Bicycle

Abstract Number A067

Abstract

Bicycles are fully synthetic constrained peptides with antibody-like affinities and specificities, readily penetrate tumor tissue, have relatively short half-lives, and can be chemically linked together to make multifunctional molecules. BT7480 is a first-in-class, Nectin-4/CD137 *Bicycle* tumor-targeted immune cell agonist® (*Bicycle* TICA®) designed to activate CD137 through co-ligation of CD137 on immune cells and Nectin-4 on tumor cells. Nectin-4 is reported as highly expressed in wide range of solid tumors including bladder, pancreas, breast, ovary, esophagus, head and neck, stomach, and lung cancers (1, 2). Nectin-4 and CD137 are co-expressed in many of these tumor types (3, 4) and thus may benefit from Nectin-4 targeted CD137 agonism.

- We used a suite of in vitro and in vivo assays to characterize BT7480 pharmacology and mechanism of action. These include primary human peripheral blood mononuclear cell (PBMC)/tumor cell co-culture assays and efficacy and transcriptional profiling studies in a syngeneic mouse tumor model.
- BT7480 elicited potent Nectin-4-dependent CD137 agonist activity in vitro as measured by increase in interferon gamma and interleukin-2 production from stimulated PBMCs in Nectin-4 dependent manner. Treatment of immunocompetent mice bearing Nectin-4-expressing tumors with BT7480 led to a wide reprogramming of the tumor immune microenvironment including an early increase in several T-cell chemotactic cytokines that preceded T cell infiltration and upregulation of cytotoxicity-related genes. We demonstrated that BT7480 anti-tumor activity with complete tumor regressions was not dependent on continuous circulating drug levels, but that plasma drug exposure for approximately two days per weekly cycle was sufficient for optimal anti-tumor activity. In rat and non-human primate safety studies BT7480 appears well tolerated at doses that are far greater than those we believe to be clinically relevant.

Introduction

BT7480 uses Nectin-4 on tumor cells as a scaffold for CD137 clustering and activation

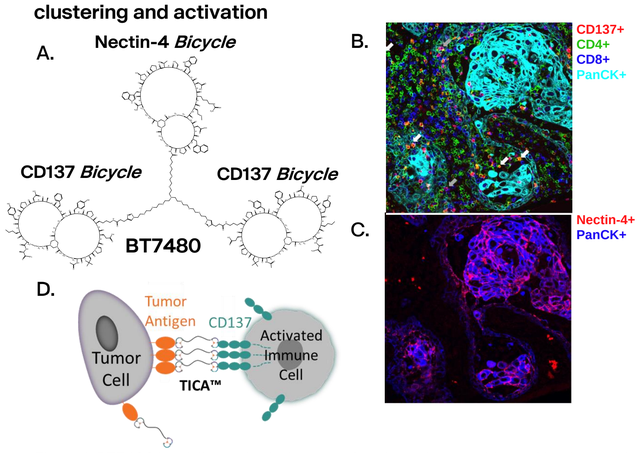
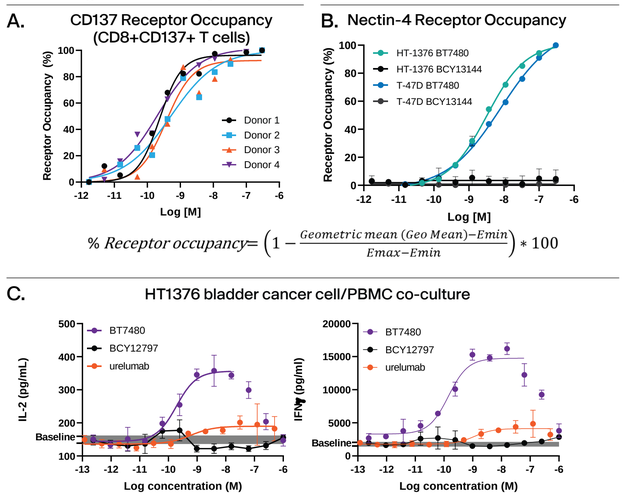


Figure 1: BT7480 is a fully synthetic, hetero- trimeric conjugate with one Nectin-4 and two CD137 binding *Bicycles*. CD137 is expressed by immune cells (B) and Nectin-4 is expressed on cancer cells (C) in a variety of solid tumortypes[1,-4], shown here in Head and Neck squamous cell carcinoma. (D) We hypothesized that by using Nectin-4 on cancer cells, BT7480 would be able to cluster and activate CD137 on immune cells in the tumor microenvironment .

Results

BT7480 binds potently and specifically to both tumor and immune cell targets and elicits potent Nectin-4 –dependent CD137 agonist activity in vitro



$$\% \, Receptor \, occupancy = \left(1 - \frac{Geometric\,mean\,(Geo\,Mean) - Emin}{Emax - Emin}\right) * 100$$

Figure 2: (A) Human PBMCs were pre-stimulated with anti-CD3 and treated for 1 hour with BT7480. Unbound and total CD137 receptors were detected by flow cytometry using an AlexaFluor 488-labeled CD137 Bicycle dimer (BCY15416) and a non-competitive CD137 antibody, respectively. (B) HT-1376 and T-47D cells were treated with BT7480 or a non-Nectin-4 binding analog of BT7480 (BCY13144) for 1 hour. BT7480 Nectin-4 receptor occupancy was determined by flow cytometry using a competitive Nectin-4 antibody. CD137 and Nectin-4 receptor occupancy was calculated as shown. (C) Human PBMCs were stimulated with anti-CD3 and co-cultured with HT-1376 cells and treated with BT7480, BCY12797 (non-binding BCY), or anti-CD137 antibody agonist and IFNγ and IL-2 in the cell supernatants were measured after 48 hours. Gray bars indicates untreated control levels. Data were fit using log (agonist) versus response (three parameter) or log (agonist) versus response—variable slope (four parameter).

BT7480 pharmacokinetics in rodents and non-human primates

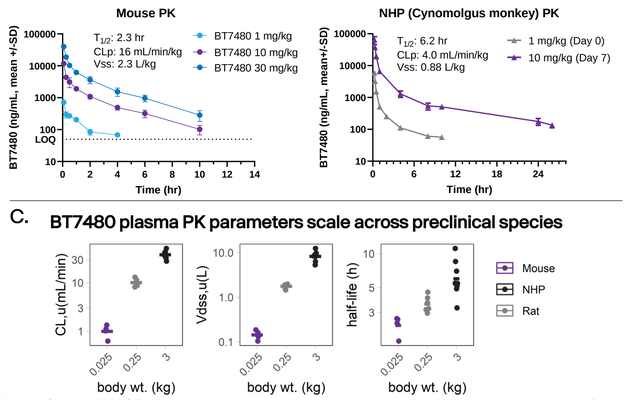


Figure 3: (A) BT7480 plasma concentrations and PK parameters in plasma after a 1, 10 and 30 mg/kg iv bolus dose in CD-1 mice. (B) BT7480 plasma concentrations and mean PK parameters after an iv infusion of 1 mg/kg (on day 0) or 10 mg/kg (on day 7) over 15 min. (C) Unbound clearance (CL), volume of distribution (Vdss), and terminal half-life of BT7480 plotted against mean body weight (wt) of mouse (n= 4), rat (n= 6), and NHPs (n= 8).

Results

BT7480 leads to tumor regressions and complete responses in vivo with an intermittent drug exposure in the periphery

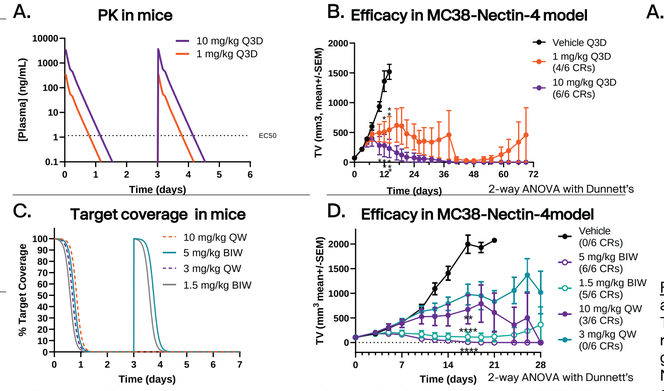


Figure 4: (A) Simulated plasma concentration profiles for 1 and 10 mg/kg BT7480 administered Q3D. [BT7480] was maintained at or above the average EC50 required to induce IFNγ and IL-2 secretion in PBMC co-culture assay for approximately 1 day of the Q3D dosing cycle. (B) 5 doses of BT7480 given Q3D showed significant anti-tumor activity in MC38-Nectin-4 tumor bearing huCD137-C57Bl/6 mice. (C) We next simulated target coverage–time profiles for BT7480 dosed BIW with 1.5 mg/kg and 5 mg/kg or QW with 3 and 10 mg/kg. (D) The BIW dosing regimens (1.5 or 10 mg/kg) that lead to ~48h weekly target coverage showed optimal anti –tumor activity.

PK and in vivo modeling predicts potential target coverage and efficacy in humans with QW dosing

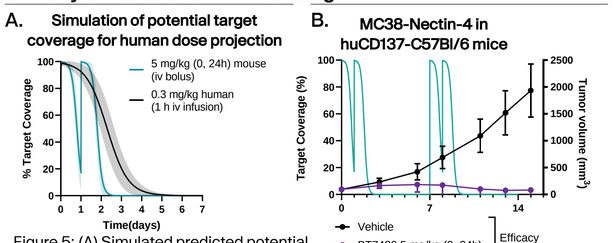


Figure 5: (A) Simulated predicted potential target coverage in human with a weekly BT7480 dosing of 0.3 mg/kg matches that with 0h and 24h dosing at 5 mg/kg in mice. (B) Simulated target coverage–time profile of BT7480 dosed at 5 mg/kg at 0 h and 24h in mice overlayed with the MC38-Nectin-4 tumor growth in huCD137 C57Bl/6 mice treated with vehicle or QW dosing of BT7480 at 0h and 24h.

BT7480 leads to CD8+ cell infiltration into tumor tissue

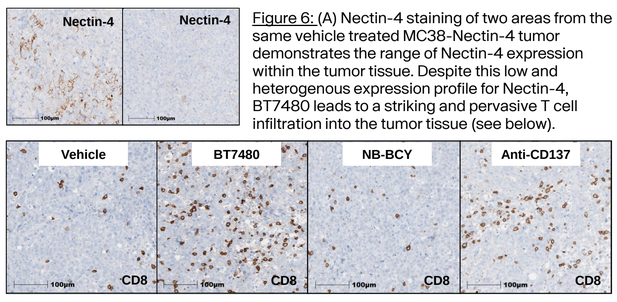


Figure 6: (A) Nectin-4 staining of two areas from the same vehicle treated MC38-Nectin-4 tumor demonstrates the range of Nectin-4 expression within the tumor tissue. Despite this low and heterogenous expression profile for Nectin-4, BT7480 leads to a striking and pervasive T cell infiltration into the tumor tissue (see below).

Figure 5: (B) Tumor harvested at 144-hours after treatment of MC38-Nectin-4 bearing C57Bl/6 mice with vehicle, 5 mg/kg BT7480 or NB-BCY (BCY12797) at 0 and 24 hours or 2 mg/kg anti-CD137 antibody agonist Q3D and stained for mouse CD8 are shown.

Results

BT7480 causes rapid activation of tumor immune signaling followed by cytotoxic cell infiltration in a mouse tumor model

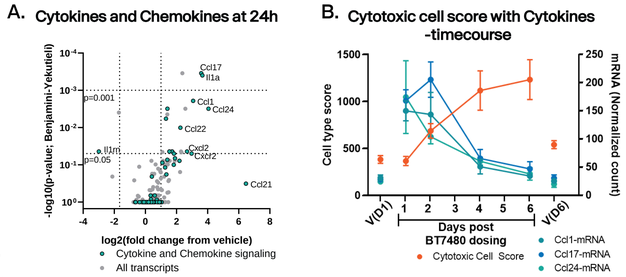
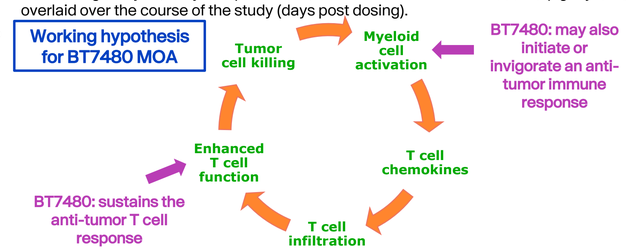


Figure 7: MC38-Nectin-4 tumor bearing mice were treated with 5 mg/kg BT7480 at 0 and 24h. Tumors were harvested for gene expression analysis by NanoString. (A) Transcriptional changes induced by BT7480 at 24-hour time point. Gray circles represent all measured transcripts and aqua circles identify transcripts belonging to the gene set for 'Cytokine and chemokine signaling'. (F) Cytotoxic cell scores (defined by NanoString analysis, left y-axis) and Ccl1-, Ccl17- and Ccl24- mRNA counts (right y-axis) overlaid over the course of the study (days post dosing).



BT7480 dosing in rat and NHP is well tolerated with no adverse findings

Species*	Dose (mg/kg)	Clinical observations	Hematology findings	Clin. Chem. findings
Rat	30	None	None	None
	100	None	None	None
	300	None	None	None
NHP	30	None	None	None
	100	None	None	None
	300	None	None	None

BT7480 doses (single dose) up to 300 mg/kg (15 min intravenous infusion) were well tolerated in a dose range finding study in rat and NHP

Conclusions

- We have demonstrated that BT7480 is potent, specific, effective, and well tolerated in preclinical species and are therefore uniquely positioned to test the hypothesis in humans that intermittent CD137 agonism may benefit cancer patients. BT7480 entered first-in-human clinical trial at the end of 2021 (NCT05163041).

References: [1] Challita-Eid *et al.* Cancer Res 2016; 76(10): 3003-13, [2]Campbell *et al.* AACR; Cancer Res 2021 ; 81(13_Suppl): Abstract #1197, [3] Hurov, Lahdenranta *et al.* JITC 2021; 9(11):e002883, [4] Cohen *et al.* SITC; JITC 2021; 9 (Issue Suppl 2): Abstract#2

Bicycle Therapeutics, Inc.
4 Hartwell Place
Lexington, MA
02421-3122

Bicycle Therapeutics plc
Portway Building
Granta Park
Cambridge CB21 6GP

bicycletherapeutics.com