Tumor-targeted activation of CD137 using Bicycle® molecules: New insights into mechanism of action and discovery of BT7455, a clinical candidate for the treatment of EphA2-expressing cancers

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ABSTRACT

Clinical trials for tumor patients have validated CD137 agonism as an activator of the immune system to eliminate tumor rejection. We have demonstrated that small, chemically synthetic bicyclic peptide can drive tumor-localized agonism of CD137 and anti-tumor immunity in mouse models. Here, we report the next steps of our work—delineating the preclinical mechanism of action of CD137 fused peptides and extending our program to serve patients whose tumors express EphA2.

BT7455 is a receptor tyrosine kinase agonist in several human cancers and its high expression correlates with poor clinical prognoses in certain cancer types, including bladder, ovarian, head & neck, and breast (3, 4).

The Bicycle MultiOmyx™ imaging panel [5] was developed to evaluate the expression of CD137 and EphA2 in squamous cell carcinoma samples. Human PBMC/Tumor cell co-culture assays were used to assess BT7455 in vitro bioactivity. Synergistic mouse tumor models were used to evaluate BT7455 Bicycle anti-tumor activity in vivo. Pharmacodynamic activity was evaluated by transcriptional profiling using Nanostring assays.

INTRODUCTION

Figure 1. The concept of a Bicycle tumor-targeted immune cell agonist® (Bicycle TICA®) and evidence for co-localization of CD137 and EphA2 in head and neck squamous cell carcinoma (HNSCC).

• CD137/4-1BB, and BCY14796 [6], which exhibit very high receptor affinity and clustering for their agonist, are a promising lead for development into a Bicycle TICA® molecule that would elicit and activate CD137 on immune cells in the tumor microenvironment.

• Bicycle® binders to CD137 and to the tumor antigen EphA2 were identified via phage display and were linked together to form a BT7455/Bicycle TICA®. An extensive medicinal chemistry campaign yielded the development candidate BT7455 and a closely related tool compound BCY14908. Figure 2. The Bicycle MultiOmyx™ imaging panel was developed and used to quantify simultaneously the presence of EphA2-positive and CD137-positive cells in HNSCC (representative image from 1 patient (n = 17 patients total)).

Figure 2. BT7455 bound simultaneously to CD137 and EphA2 proteins and bound specifically to CD137 expressing immune cells. A) Surface plasmon resonance (SPR): Biotinylated human CD137 was immobilized on the SPR chip and each cycle was set up to capture BT7455 with the immobilized protein followed by injection of the second protein, EphA2-2-3 fold dilution series followed by regeneration of the surface. B) Flow cytometry: Human PBMCs were stimulated with anti-CD3 and treated with AF647-tagged BT7455 and were bound to CD8+CD137+ T cells, but not CD8–negative T cells. The average EC50 for binding to CD8+CD137+ T cells is 0.14 nM (n=4, 2 replicates each from 2 independent PBMC donors); MFI mean fluorescence intensity.

Figure 3. BT7455 elicits potent EphA2-binding dependent CD137 agonism in vitro, A&B) BT7455 elicits activity in a co-culture system with J509 and 1000 tumor cells (black circled). B) Non-binding (rib) analogues of BT7455 are inactive: BCY14738 (EphA2/CD137/rib), purple; BCY1766 (EphA2/CD137/rib), green; or BCY1767 (EphA2/CD137/okay). C&D) BT7455 shows responses in a P815/P2C tumor cell co-culture assay when PMBCs were stimulated with anti-CD3 (black) to induce CD137 expression but not when PMBCs were unstimulated (red). Dotted line represents anti-CD3 stimulated cell culture medium controls with no BT7455. Figure 4. BT7755 leads to tumor regressions and complete responses in vivo without continuous drug exposure in the periphery. A) MCB38 tumor bearing CD137-SCID/BL6 mice were dosed i.v. with BT7455 (at 8, 2, or 0.5 mg/kg on days 0, 7, 14 and 0 at 0 and 24. Simulated BT7455 plasma concentration during the study is overlaid on the tumor growth curves (trange dotted lines). Numbers of complete responders (CRs) are shown in the figure. B) BT7455 concentration and PK parameters in plasma after a 5 mg/kg bolus dose for 24 h. Data is shown as mean (solid line) with 95% confidence interval (hashed lines) of total BT7455 concentration.

Figure 5. BT7455 treatment effect is differentiated from the effects of anti-PD-1 or anti-CD137 mAb treatment in the same xenograft model. Total RNA was prepared from MC38 tumors in huCD137/huPD-1 C57Bl/6 mice 48h after treatment with vehicle (V), BT7455 (8 mg/kg i.p., 0 & 24h), pembrolizumab (3 mg/kg on day 0 and in combination (n=10 per cohort). Signature profiles of individual animals by treatment group and number of complete responders (CRs). The combination of BCY12491 and pembrolizumab led to more CRs when compared to either treatment alone (p<0.02 or 3 x SDs).

CONCLUSIONS

BT7455 is EphA2-dependent CD137 agonist that has optimal target binding, pharmacodynamic, and pharmacokinetic properties that enable intermittent dosing for curative effect through modulation of the tumor immune microenvironment in syngeneic mouse models. EphA2/CD137 Bicycle TICA® synergizes with anti-PD-1 therapy leading to complete responses in a preclinical mouse model.

BT7455 is currently being evaluated in IND-enabling studies.