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

Abstract # 5301

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ABSTRACT

- Clinical studies in cancer patients have validated CD137 agonism as an activator of the immune system to enable tumor rejection. We have demonstrated that small, chemically synthetic bicyclic peptides can drive tumor-localized agonism of CD137 and anti-tumor immunity in mouse models [1, 2]. Here, we report the next stage of our work – delving into the preclinical mechanism of action of these novel agents and extending our program to serve patients whose tumors express EphA2.
- EphA2 is a receptor tyrosine kinase overexpressed in several human cancers and its high expression correlates with poor clinical prognosis in certain cancer types, including bladder, ovarian, head & neck, and lung cancer [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. Syngeneic mouse tumor models were used to evaluate CD137 Bicycle TICA® anti-tumor activity in vivo. Pharmacodynamic activity was evaluated by transcriptional profiling using Nanostring assays.

INTRODUCTION

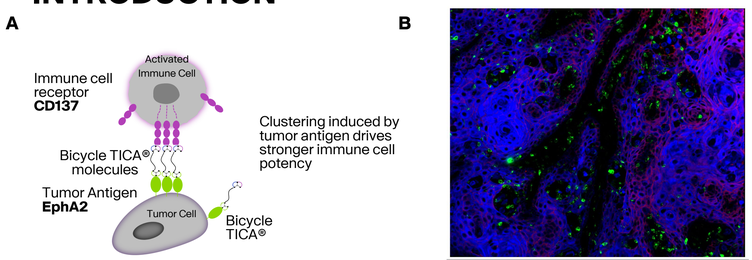


Figure 1. The concept of a Bicycle tumor-targeted immune cell agonist® (Bicycle TICA®) and evidence of co-localization of CD137 and EphA2 in head and neck squamous cell carcinoma (HNSCC). **A)** CD137/4-1BB, a member of the TNF receptor superfamily, is a signal 2 costimulatory receptor that drives T cell function and survival and is a validated immunotherapy target. CD137 requires trimerization and clustering for its activation, and we hypothesize that by binding to EphA2 on tumor cells, a CD137 agonistic Bicycle TICA® molecule would be able to cluster 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 an EphA2/CD137 Bicycle TICA®. An extensive medicinal chemistry campaign yielded the development candidate BT7455 and a closely related tool compound BCY12491. **B)** 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)).

BIOCHEMICAL AND CELL BINDING PROPERTIES

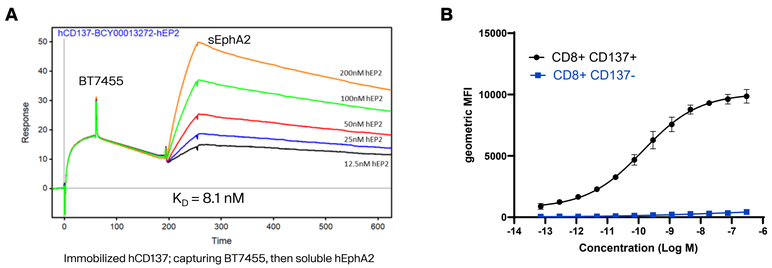


Figure 2. BT7455 bound simultaneously to CD137 and EphA2 proteins and bound specifically to CD137 expressing immune cells **A)** Surface plasmon resonance (SPR): Biotinylated human CD137 was immobilized on the SPR chip and each cycle was set up to capture BT7455 with the immobilized protein followed 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 (BCY26048), which bound to CD8+CD137+ T cells, but not CD137-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

BT7455 ACTIVITY DEPENDS ON EPHA2 AND CD137

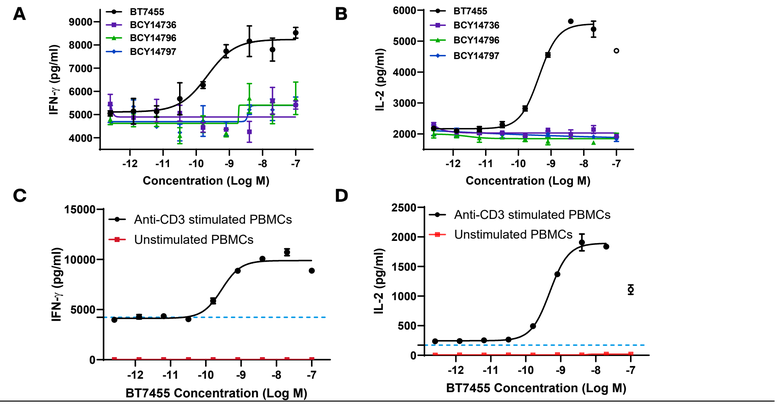
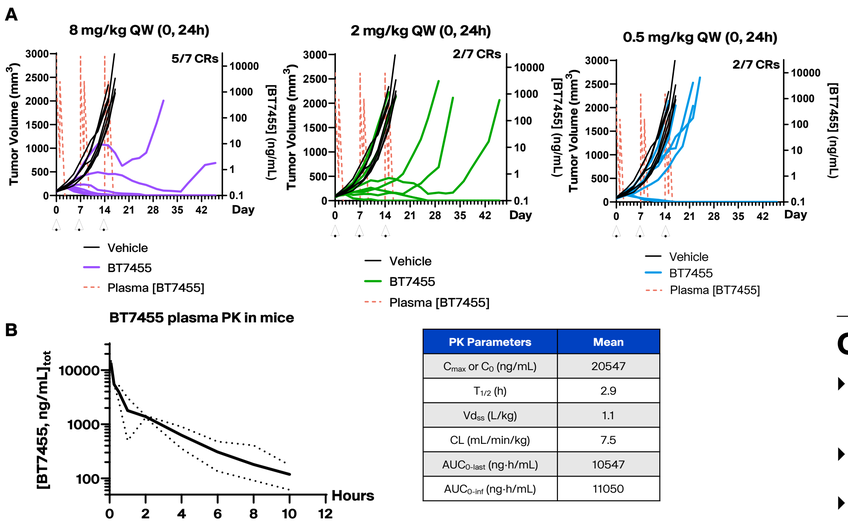


Figure 3. BT7455 elicits potent EphA2-binding dependent CD137 agonism in vitro. A&B) BT7455 elicits activity in a co-culture assay with PBMCs and A549 tumor cells (black circles), while non-binding (nb) analogues of BT7455 are inactive; BCY14736 (EphA2/CD137nb, purple), BCY14796 (EphA2nb/CD137nb, green), or BCY14797 (EphA2nb/CD137, blue). **C&D)** BT7455 shows response in a PBMC/PC3 tumor cell co-culture assay when PBMCs were stimulated with anti-CD3 (black) to induce CD137 expression but not when PBMCs were unstimulated (red). Dotted blue lines represent anti-CD3 stimulated cell culture medium controls with no BT7455 added.

BT7455 IS EFFICACIOUS IN MOUSE TUMOR MODELS



PK Parameters	Mean
C_{max} or C_0 (ng/mL)	20547
$T_{1/2}$ (h)	2.9
Vd_{ss} (L/kg)	1.1
CL (mL/min/kg)	7.5
$AUC_{0\text{-last}}$ (ng·h/mL)	10547
$AUC_{0\text{-inf}}$ (ng·h/mL)	11050

Figure 4. BT755 leads to tumor regressions and complete responses in vivo without continuous drug exposure in the periphery. A) MC38 tumor bearing hCD137-C57BL/6 mice were dosed i.v. with vehicle or BT7455 (at 8, 2 or 0.5 mg/kg) on days 0, 7 and 14 at 0 and 24h. Simulated BT7455 plasma concentration during the study is overlaid on the tumor growth curves (orange 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 iv bolus dose in naïve CD-1 mice. Data is shown as mean (solid lines) with 95% conference interval (dashed lines) of total BT7455 concentration.

IN VIVO IMMUNE SIGNALING IN RESPONSE TO BT7455

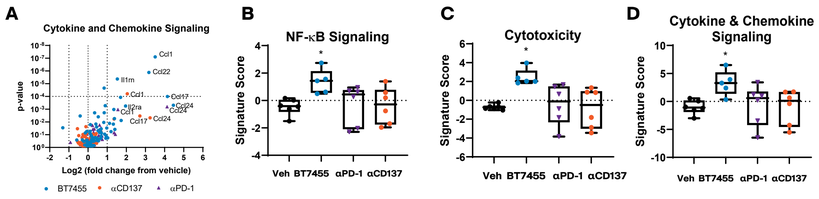


Figure 5. BT7455 treatment effect is differentiated from the effects of anti-PD-1 or anti-CD137 mAb treatments by gene set analysis. Total RNA was prepared from MC38 tumors in huCD137-C57Bl/6 mice 48h after treatment with vehicle (Veh), BT7455 (8 mg/kg, 0 & 24h), αPD-1 (RMP1-14; 10 mg/kg), or αCD137 (Urelumab analogue; 2 mg/kg). Transcriptional analysis was performed using NanoString. **A)** BT7455 induced a burst of cytokine and chemokine expression, whereas the effect of anti-CD137 and anti-PD-1 was more modest. Significant changes were observed in NF-κB signaling **(B)**, cytokine and chemokine signaling **(C)**, and cytotoxicity **(D)** gene sets after BT7455 treatment but not after anti-PD-1 or anti-CD137 treatment. *p<0.05, 1way ANOVA with Dunnett's post test

SYNERGY OF EPHA2/CD137 BICYCLE TICA® WITH ANTI-PD-1 THERAPY IN VIVO

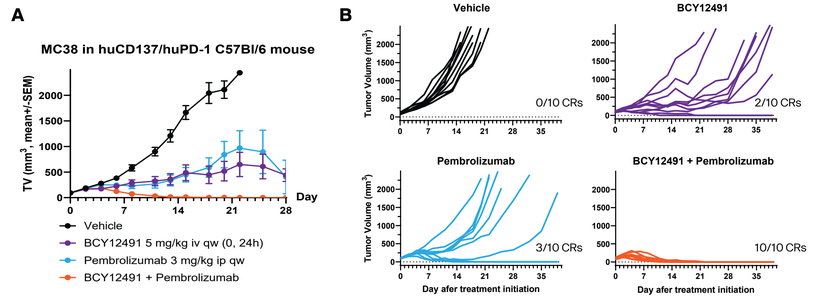


Figure 6. BCY12491 (a BT7455 tool molecule) synergizes with the anti-PD-1 therapy pembrolizumab leading to complete responses in the MC38 syngeneic mouse model. A) Tumor volume of MC38 tumor bearing huCD137/huPD-1-C57BL/6 mice dosed i.v. with either vehicle, BCY12491 at 5 mg/kg once per week (qw) at 0 and 24h, pembrolizumab at 3 mg/kg i.p. qw or in combination (n=10 per cohort). **B)** Spider plots 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 (10/10 versus 2 or 3/10 CRs).

CONCLUSIONS

- BT7455 is and EphA2-dependent CD137 agonist that has optimal target binding, pharmacologic, 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 treatment leading to complete responses in a preclinical mouse model
- BT7455 is currently being evaluated in IND-enabling studies

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