EphA2-dependent CD137 agonism and anti-tumor efficacy by BT7455, a *Bicycle* tumor-targeted immune cell agonist®

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ABSTRACT

- We have developed a new class of modular synthetic drugs, termed *Bicycle*® tumor-targeted immune cell agonists (*Bicycle*® TICAs), which are multifunctional molecules composed of constrained bicyclic peptides (*Bicycles*) [1].
- The first molecule of this class, BT7480, a Nectin-4-dependent CD137 (4-1BB) agonist, entered clinical trials in 2021 in patients with solid tumors associated with Nectin-4 expression (NCT05163041).
- Compelling preclinical data characterization of BT7480 [2] led us to develop a second *Bicycle* TICA™ molecule, BT7455, which is designed to deliver highly potent CD137 agonism to Ephrin receptor A2 (EphA2)-positive cancers.
- EphA2 is a receptor tyrosine kinase overexpressed in several human cancers and its high expression correlates with poor clinical prognosis in certain cancer types [3, 4].
- BT7455 pharmacology was assessed *in vitro* using surface plasmon resonance, receptor occupancy assays, and PBMC/tumor cell co-culture bioactivity assays. BT7455 *in vivo* activity was evaluated in efficacy studies in syngeneic EphA2-positive mouse tumor models and pharmacodynamic studies using transcriptional profiling of the tumor immune microenvironment.
- BT7455 engages EphA2 and CD137 with high affinity resulting in potent EphA2-dependent production of interleukin-2 (IL-2) and interferon gamma (IFN-γ) in primary human PBMC/tumor cell co-culture assays.
- Treatment of MC38 tumors in immunocompetent mice with BT7455 with an intermittent dosing regimen led to robust anti-tumor activity, including complete responses.
- Gene expression profiling of BT7455-treated tumors revealed modulation of the tumor immune microenvironment, including a rapid increase in cytokine expression (both myeloid and T cell origin) and an increase in cytotoxic cell scores. The kinetics and extent of the immune microenvironment modulation differentiated BT7455 from both a checkpoint inhibitor (anti-mouse PD-1) as well as an anti-CD137 agonist antibody (Urelumab analogue). Further studies in the mouse model paired with single cell RNA sequencing were used to investigate the immune cell types responsible for the early burst of cytokine and chemokine gene expression following CD137 *Bicycle* TICA™ treatment.

INTRODUCTION

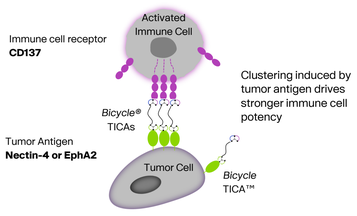
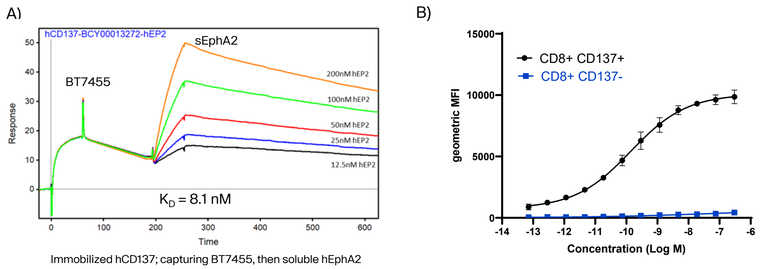


Figure 1. The concept of a *Bicycle* tumor-targeted immune cell agonist® (*Bicycle* TICA™) CD137/4-1BB, a member of the TNF receptor superfamily, is a signal 2 costimulatory receptor that drives T cell function and survival and is a validated immunotherapy target. *Bicycle* binders to CD137 and to the tumor antigen EphA2 were identified via phage display and were linked together to form EphA2/CD137 *Bicycle*® TICAs. An extensive medicinal chemistry campaign yielded the development candidate BT7455. CD137 requires trimerization and clustering for its activation and we hypothesize that by binding to EphA2 on tumor cells, BT7455 would be able to cluster and activate CD137 on immune cells in the tumor microenvironment

RESULTS: SPR AND CELL BINDING



Immobilized hCD137; capturing BT7455, then soluble hEphA2

Figure 2. BT7455 bound simultaneously to CD137 and EphA2 by SPR and bound specifically to CD137 expressing immune cells A) Surface plasmon resonance: 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

RESULTS: RECEPTOR OCCUPANCY ASSAYS

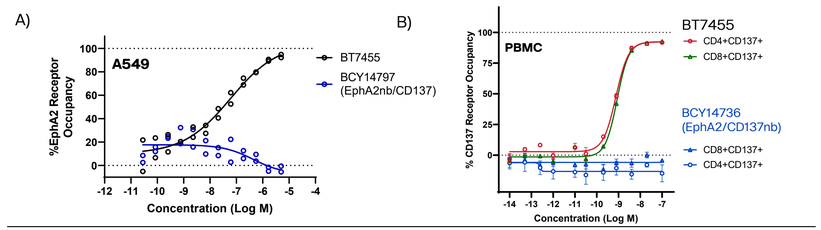


Figure 3. Cell based receptor occupancy assays for CD137 and EphA2 show competitive binding by BT7455 but not non-binder controls. Receptor occupancy was calculated from flow cytometry data using the formula: %RO={1-[(MFI-Emin)/(Emax-Emin)]}x100. **A)** EphA2 RO assay in A549 cells shown with BT7455 (black) and control non-EphA2 binding analogue of BT7455 (BCY14797; blue). Unoccupied receptors were detected with anti-EphA2 (clone 1C1). Mean % EphA2 RO EC50 value for BT7455 in A549 and PC3 tumor cell lines was 52 nM (n=5). **B)** CD137 RO assay shown with BT7455 and control non-CD137 binding analogue of BT7455 (BCY14736) in OKT3-stimulated PBMCs. Unoccupied receptors were detected with an AF647-conjugated CD137 binding *Bicycle* dimer probe (BCY16776; 10 nM). Mean % CD137 RO EC50 value for BT7455 across CD4+ and CD8+ T cells from 2 donors was 0.72 nM. nb=non-binding

RESULTS: CO-CULTURE ASSAYS

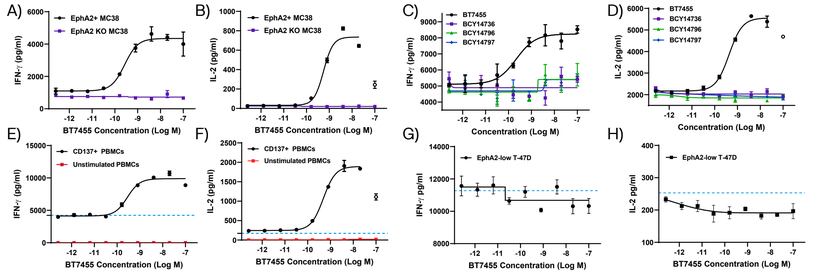


Figure 5. BT7455 elicits potent EphA2-binding dependent CD137 agonism in vitro. A&B) BT7455 shows response in a PBMC/tumor cell co-culture assay with MC38 cells (black) but not MC38 cells with EphA2 expression knocked-out (EphA2 KO, purple). **C&D)** BT7455 elicits activity in a co-culture assay with PBMCs and A549 tumor cells (black circles), while nonbinding (nb) analogues of BT7455 are inactive; BCY14736 (EphA2/CD137nb, purple), BCY14796 (EphA2nb/CD137nb, green), or BCY14797 (EphA2nb/CD137, blue). **E&F)** BT7455 shows response in a PBMC/PC3 tumor cell co-culture assay PBMCs that were stimulated with anti-CD3 (black) to induce CD137 expression but not unstimulated PBMCs (red). **G&H)** BT7455 is inactive in the assay when PBMCs are co-cultured with the EphA2-low expressing tumor cell line T-47D. Dotted blue lines in E-H represent anti-CD3 stimulated cell culture medium controls with no BT7455 added.

RESULTS: IN VIVO RESPONSE

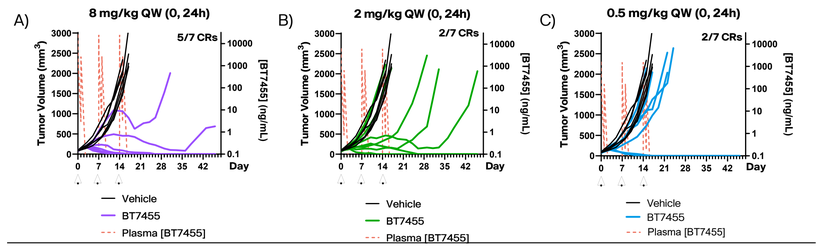
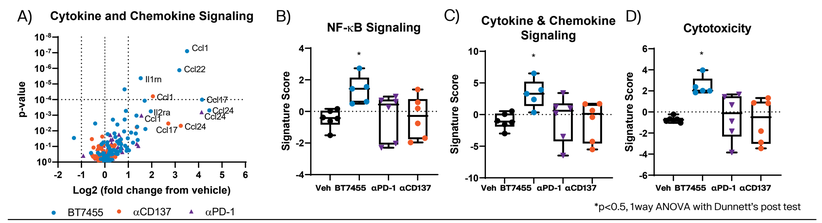


Figure 6. Intermittent BT7455 exposure led to complete responses in the MC38 syngeneic mouse model. MC38 tumor bearing hCD137-C57BL/6 mice were dosed with vehicle or BT7455 (8, 2 or 0.5 mg/kg) i.v. on days 0, 7, 14 at 0 and 24h. Simulated BT7455 plasma concentration during the study is overlaid on the tumor growth curves (orange dotted lines). Numbers of complete responders (CRs) are listed in **(A-C)**.

RESULTS: GENE EXPRESSION ANALYSIS



*p<0.5, 1way ANOVA with Dunnett's post test

Figure 7. 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, where as the effect of anti-CD137 and anti-PD1 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.

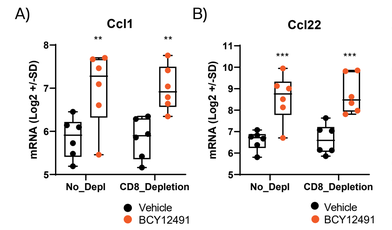


Figure 8. Increase in T cell chemotactic cytokines was not dependent on CD8+ T cells. MC38 tumor bearing huCD137-C57Bl/6 mice were depleted of CD8+ cells prior to treatment initiation with BCY12491 (EphA2/CD137 *Bicycle* TICA™, 15 mg/kg, 0 & 24h). Transcriptional analysis of the tumor tissue was performed 48h after treatment initiation by NanoString PanCancer IO360 assay. Increase in Ccl1 mRNA **(A)** and Ccl22 mRNA **(B)** levels in response to BCY12491 – treatment was not affected by depletion of CD8+ cells. **p<0.01, ***p<0.001: 2way ANOVA with Sidak's post-test.

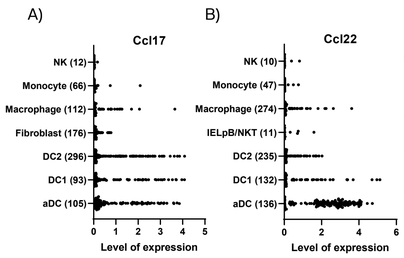


Figure 9. Myeloid cells including monocytes, macrophages and dendritic cells can be the main sources of cytokines modulated by CD137 agonizing *Bicycle*® TICAs. MC38 tumors in huCD137-C57Bl/6 mice were harvested for scRNAseq and a total of about 45K cells were sequenced. Markers for cell type identification for the preliminary analysis are derived from the cell atlas by Park et al.[5] Cell populations for top 1000 gene expressing cells from vehicle treated tumors are shown for two of the transcripts of interest. Preliminary analysis reveals that DC1 (XCR1+CLEC9A+), DC2 (SIRPA+CLEC10A+) and aDC (LAMP3+CCR7+) cell populations are significant sources of Ccl17 **(A)** and Ccl22 **(B)** transcription.

CONCLUSIONS

- BT7455 is a highly potent EphA2 expression dependent CD137 agonist.
- BT7455 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.
- BT7455 is currently being evaluated in IND-enabling studies.

REFERENCES

1. Upadhyaya P, Lahdenranta J, Hurov K, et al., Anticancer immunity induced by a synthetic tumor-targeted CD137 agonist. *JITC* 2021; 9:e001762.
2. Hurov K, Lahdenranta J, et al. BT7480, a novel fully synthetic *Bicycle* tumor-targeted immune cell agonist™ (*Bicycle* TICA™) induces tumor localized CD137 agonism. *JITC* 2021; 0:e002883.
3. Campbell C, et al., A survey of EphA2 expression by immunohistochemistry (IHC) in tumor tissue microarrays (TMAs) to support BT5528 indication selection. *Cancer Res* 2020; 80 (16_Supplement): 5300.
4. Xiao, et al., Targeting EphA2 in cancer. *Journal of Hematology & Oncology* 2020; 13:114.
5. Park, et al., A cell atlas of human thymic development defines T cell repertoire function. *Science* 2020; 367:3224.

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