BT7455, a fully synthetic *Bicycle* tumor-targeted immune cell agonist®, leads to potent EphA2-dependent CD137 agonism and robust anti-tumor efficacy

Abstract #
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ABSTRACT

- To address the limitations of antibody-based agonists of immune costimulatory receptors, 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].
- We used a suite of in vitro and in vivo assays to characterize BT7455 pharmacology and mechanism of action. These included primary immune cell/tumor cell co-culture assays and efficacy and gene expression profiling studies in a syngeneic mouse model.
- BT7455 engages EphA2 and CD137 with high affinity, resulting in picomolar potency in co-culture assays consisting of EphA2-expressing tumor cells and CD137-expressing Jurkat NF-kB-luciferase reporter cells. Moreover, BT7455 led to EphA2-dependent production of interleukin-2 (IL-2) and interferon gamma (IFNγ) 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). BT7455 treatment also led to the increase in checkpoint gene expression, suggesting that combination with checkpoint inhibitor therapy may be effective.
- BT7455 exhibited linear pharmacokinetics in non-human primates and appears well-tolerated at exposures more than the predicted efficacious exposure in humans without significant elevation of cytokines or liver enzymes.

INTRODUCTION

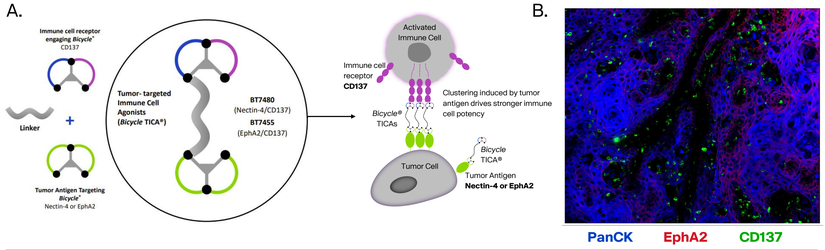

Figure 1: The concept of a *Bicycle* tumor-targeted immune cell agonist® (*Bicycle* TICA®) and evidence of co-expression of CD137 and EphA2 in Head and Neck squamous cell carcinoma (HNSCC). CD137/4-1BB, a member of the TNF receptor superfamily, is a signal 2 costimulatory receptor that drives T cell function and survival and is a validated immunotherapy target. (A) *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. (B) A MultiOmyx™ multiplexing immunofluorescence (IF) assay was developed and used to assess the presence of EphA2-positive tumor cells and CD137-positive immune cells in HNSCC. Image shows one representative tumor out of N=6.

RESULTS

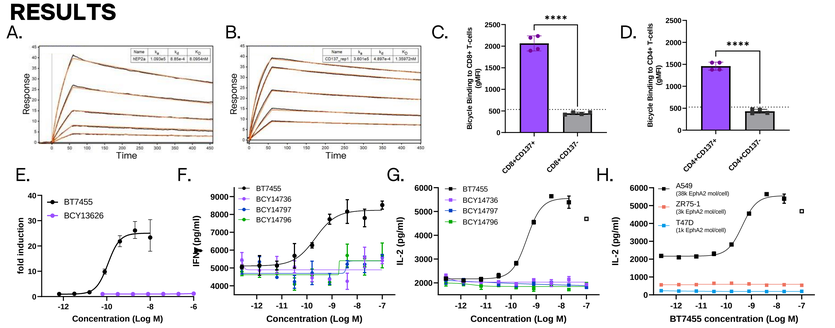

Figure 2: BT7455 bound simultaneously to CD137 and EphA2 proteins (shown by surface plasmon resonance; SPR) and bound specifically to CD137-positive T cells, which led to potent EphA2-dependent activity *in vitro*. (A-B) Biotinylated hCD137 or hEphA2 was immobilized on the SPR chip and each cycle was set up to capture BT7455 with the immobilized protein followed by injection of the second protein (2-3 fold dilution series) followed by regeneration of the surface. The sensorgrams are showing the capture of hEphA2 by the immobilized hCD137-BT7455 complex (A) or the capture of hCD137 by the immobilized hEphA2-BT7455 complex (B). (C,D) Human PBMCs were stimulated with anti-CD3 and treated with AF488-tagged BT7455, which bound to CD8+CD137+ T cells (C) and CD4+CD137+ T cells, but not CD137-negative cells as monitored by flow cytometry (n=4 +/-SD; 2 replicates each from 2 independent PBMC donors; ****p<0.0001). The dotted line represents the average background MFI (media only wells). (E) Jurkat-CD137 reporter cells were co-cultured with EphA2-expressing A549 tumor cells, treated with BT7455 or a non-binding analogue (BCY13626), and downstream CD137-mediated NF-κB activation was measured by luminescence (n=3, +/-SD). (F-G) PBMCs were stimulated with anti-CD3 and co-cultured with A549 cells, treated with BT7455 or non-binding analogues (BCY14736, BCY14797, and BCY14796), and IFNγ and IL-2 levels secreted into the media were measured by Luminex (n=3, +/-SEM). (H) As in (G), except PBMCs were co-cultured with ZR75-1 or T47D tumor cells that express low levels of EphA2. BT7455 activity was dependent on the presence of tumor cells, i.e. A549, that express high levels of EphA2. EphA2 receptor expression was estimated by flow cytometry using Quantibrite reference standards.

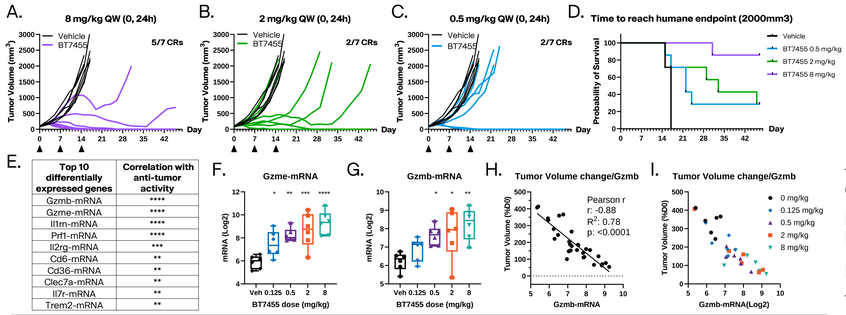

Figure 3: BT7455 led to complete responses in the MC38 syngeneic mouse model and transcriptional profiling identified several pharmacodynamic gene changes that correlated with anti-tumor activity. (A-C) BT7455 was delivered IV in dose response (8, 2, or 0.5 mg/kg; 0 & 24h on Days 0, 7, and 14) to MC38 tumor bearing hCD137-C57BL/6 mice. CR denotes the number of complete responder mice (n=7 per dose group). (D) Survival data corresponding to panels A-C. (E-G) MC38 tumors from mice treated with vehicle or BT7455 (0.125, 0.5, 2, or 8 mg/kg; 0 & 24h) were processed for transcriptional analysis by NanoString. (E) The top 10 differentially expressed genes (BT7455 treatment vs vehicle) also correlated with anti-tumor activity. (F-G) Granzyme e and Granzyme b expression are shown as representative examples of genes whose expression dose responsively increased in response to BT7455. (H-I) The increase in Granzyme b gene expression significantly correlated with decreased tumor volume in response to BT7455. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, 1-way ANOVA with Dunnett's post test.

RESULTS

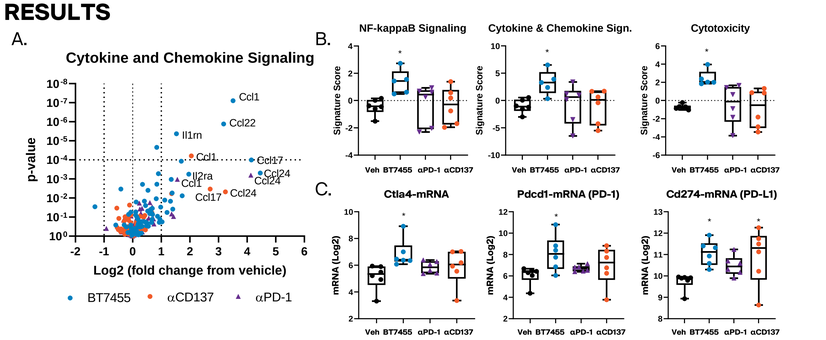

Figure 4: Geneset analysis revealed that BT7455 treatment effect on tumor tissue is differentiated from the effect of anti-PD-1 and anti-CD137 mAb treatments. MC38 tumors from huCD137-C57Bl/6 mice 48h after treatment with vehicle, BT7455 (8 mg/kg, 0 & 24h), anti-PD-1 (RMP1-14; 10 mg/kg), or anti-CD137 (Urelumab analogue; 2 mg/kg). Transcriptional analysis was performed on total RNA from tumor using NanoString. (A-B) More significant changes were observed in cytokine and chemokine signaling, NF-kB signaling, and cytotoxicity genesets after BT7455 treatment compared to anti-PD-1 or anti-CD137 treatment. (C) Immune checkpoint gene expression was significantly increased in response to BT7455. *p<0.05, 1-way ANOVA with Dunnett's post test.

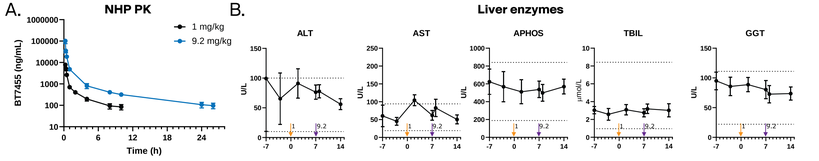

Figure 5: BT7455 exhibited dose linear exposure and was well tolerated in non-human primates (NHPs) up to 9.2 mg/kg. (A) Animals (n=3 +/- SD) were dosed 1 mg/kg IV on Day 0 and 9.2 mg/kg on Day 7. Exposures at 9.2 mg/kg are higher than those predicted to be required for a human efficacious dose. (B) A clinical chemistry panel indicated that liver enzymes were generally within the normal range (indicated by the dotted horizontal lines). Samples were collected on Day -7, Day 2,-3, 7 (pre-9.2 mg/kg dose), 8, and 14. Plasma cytokines (30-plex NHP ProcartaPlex Panel; Invitrogen) were also monitored at 1h and 24h post-dose and were not elevated in response to BT7455 (data not shown).

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

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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.

To learn more about CD137 *Bicycle* TICA® mechanism of action, see **Poster #1356** at this conference.

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