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BT7455, A FULLY SYNTHETIC *BICYCLE* TUMOR-TARGETED IMMUNE CELL AGONIST[®], LEADS TO POTENT EPHA2-DEPENDENT CD137 AGONISM AND ROBUST ANTI-TUMOR EFFICACY

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Background 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 comprised 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. Compelling preclinical data characterizing BT7480² 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}

Methods BT7455 bioactivity was assessed *in vitro* using a CD137 reporter assay and by measuring proinflammatory cytokine production in human PBMC/tumor cell co-cultures. BT7455 *in vivo* pharmacological activity was evaluated in efficacy studies in syngeneic EphA2-positive mouse tumor models and pharmacodynamic studies using transcriptional profiling of the tumor immune microenvironment by NanoString.

Results 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-κB-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 exhibits 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.

Conclusions BT7455 is a highly potent EphA2 expression-dependent CD137 agonist with 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 safety 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.

Ethics Approval All the procedures related to animal handling, care and treatment in the studies were performed according to the guidelines approved by the Institutional Animal Care and Use Committee of WuXi AppTec (Beijing, China), following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care.

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