A novel fully synthetic dual targeted EphA2/4-1BB Bicycle® peptide induces tumor localized 4-1BB agonism

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ABSTRACT

**4-1BB (CD137) is a member of TNF superfamily involved in the stimulation of several immune cells. Agonism of this receptor in a preclinical immunotherapy model with rationally designed bispecific Bicycle® peptides showed efficiency in clinical models with limited success in clinical trials due to hepatotoxicity.** Bicycle® represent a new therapeutic modality – fully synthetic, constrained bicyclic peptides. We recently showed that Bicycle® CD137 agonists with rapid clearance, minimal liver exposure induce CD137 mediated anti-tumor activity while avoiding liver toxicity. Moreover, our platform allows for rapidly developing a portfolio of fully synthetic tumor-targeted immune cell agonists (TICAs).

**EphA2/CD137 TICA demonstrates tumor-specific signaling in in vitro and in vivo models.** Immunopharmacological characterization of EphA2 in a tumor antigen which is overexpressed in human cancers and correlates with poor prognosis.

**Here, we present preclinical data demonstrating the potent immunomodulatory activity of EphA2/CD137 TICAs which engage EphA2 and CD137 simultaneously with high affinity resulting in potent potentiating effect. EphA2/CD137 TICAs potentiate tumor target dependent cytokine secretion in immune co-culture experiments and promote caspase activity in T cell mediated killing assays.**

**In vivo testing of EphA2/CD137 TICAs in humanized tumor bearing HT29 xenografts show an increased percentage of CD8+ cells in tumor but not in the circulation, suggesting a local tumor specific stimulation of T cells without systemic CD137 agonism. Interestingly, EphA2/CD137 TICA showed a robust anti-tumor activity in a syngeneic MC38 mouse model.**

**Together, these studies define the unique ability of EphA2/CD137 dual targeting Bicycle® to precisely and potently stimulate target-specific immune cells in tumors without systemic immune stimulation in very promissing and provides us rationale for developing first-in-class Bicycle® to target EphA2+ cancers.”

RESULTS

**Figure 1A:** EphA2/CD137 TICAs demonstrate cytokine secretion and caspase activity in a target-dependent manner.

**Figure 1B:** EphA2/CD137 TICAs promote cytokine secretion and caspase activity in a target-dependent manner.

**Figure 2:** EphA2/CD137 TICAs promote tumor killing.

**Figure 3:** EphA2/CD137 TICAs promote cytokine secretion and caspase activity in a target-dependent manner. (A) PBMCs from healthy donors were co-cultured with tumor cells (SI) in presence of anti-CD3, anti-CD28 and anti-CD137 antibodies. Supernatant was harvested after 24h, analyzed by ELISA and intracellular cytokine staining for IFNγ, TNFα, IL-17, IL-23, IL-2, IL-10 and IL-6. (B) EphA2/CD137 TICAs induced A549 tumor cell killing by CD3-stimulated PBMCs. Cell killing was measured by quantitating Caspase 3/7 activity in cancer cells by Incucyte (error bars = SD).

**Figure 4:** EphA2/CD137 TICAs promote synergistic effect in co-culture assays in a target-dependent manner.

**Figure 5:** EphA2/CD137 TICAs demonstrate tumor localization and potent anti-tumor activity.

**Figure 6:** EphA2/CD137 TICAs demonstrate tumor localization and potent anti-tumor activity.

CONCLUSIONS

We have successfully synthesized EphA2/CD137 TICAs that engage EphA2 and CD137 simultaneously with high affinity resulting in promiscuous potency.

EphA2/CD137 Bicycle® are highly potent in both reporter cell and primary T-cell assays in a tumor-targeted manner.

EphA2/CD137 Bicycle® show robust anti-tumor activity in HT29/PBMC engraftment model and syngeneic MC38 mouse model.

PK/PD modeling predicts a potential for weekly dosing of these molecules in the clinic.

The Bicycle® platform enables a discovery strategy to synthesize monoclonal antibodies with human IgG Fc-properties across wide range of tumor targets as well as other immune cell receptors.


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