888 AN INTEGRATIVE APPROACH TO OPTIMIZE A SYNTHETIC EPHA2/CD137 AGONIST: BALANCING POTENCY, PHYSIOCHEMICAL PROPERTIES, AND PHARMACOKINETICS TO ACHIEVE ROBUST ANTI-TUMOR ACTIVITY

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Background CD137 (4-1BB) is a resurging target in immunotherapy after the first generation of monoclonal antibodies were limited by hepatotoxicity[1] or lack of efficacy.[2] A new generation of CD137 agonists are now in clinical development but they exclusively utilize large molecules derived from recombinant technology and are associated with long circulating terminal half-lives.[3–6] Unlike checkpoint inhibition where complete saturation of the receptors drives the reversal of immunosuppression, intermittent target engagement that reflects the physiological context of T cell co-stimulation may be more appropriate for a CD137 agonist.[7] Bicyclic peptides or *Bicycles* are a class of small (MW~2kDa), highly constrained peptides characterized by formation of two loops cyclized around a symmetric scaffold. To develop a differentiated tumor antigen dependent CD137 agonist for treating EphA2 expressing solid tumors, we integrated structure activity relationship (SAR) data from biochemical binding studies and in-vitro and in-vivo models to understand the relationship between exposure, target engagement and preclinical efficacy.

Methods Over 150 different EphA2/CD137 tumor-targeted immune cell agonists (*Bicycle* TICAs) were synthesized by linking *Bicycle*® binders to EphA2 to those binding CD137.[8] The molecules were assessed in vitro using a CD137 reporter assay and by measuring cytokine production from primary human PBMC in tumor cell co-cultures. The pharmacokinetics were evaluated in rodents using Phoenix WinNonlin. The in vivo activity was determined in syngeneic mouse tumor models by measuring tumor growth kinetics and using tumor immune cell and transcriptional profiling by IHC and NanoString.

Results Evaluation of the *Bicycle* TICAs in co-culture assays with EphA2-expressing tumor cell lines and Jurkat reporter cells overexpressing CD137 or human PBMCs demonstrated that constructs bearing two CD137 binding *Bicycles* to one EphA2 binding *Bicycle* (1:2 format) were more potent than the 1:1 format.[8] Several *Bicycle* TICAs with amino acid substitutions to the EphA2 binding *Bicycle* maintained sub-nanomolar potency in-vitro and exhibited a plasma terminal half-life (t1/2) in rodents ranging from 0.4 and 4.0 h. Modifications that conferred aqueous solubility of greater than 10 mg/mL were considered suitable for further development. Treatment of MC38 tumors in immunocompetent mice with this series of molecules demonstrated that low MW *Bicycle* TICAs with sub-nanomolar potency and a t½ of ~1 h in mouse maintained target coverages necessary to produce robust modulation of the tumor immune microenvironment and tumor regression.

Conclusions A differentiated EphA2-dependent CD137 agonist was developed that exploits intermittent rather than continuous exposure for robust anti-tumor activity.

REFERENCES

1. Segal NH, Logan TF, Hodi FS, *et al*. Results from an integrated safety analysis of urelumab, an agonist anti-CD137 monoclonal antibody. *Clin Cancer Res* 2017;**23**(8):1929–1936.
2. Segal NH, Aiwu RH, Toshihiko D, *et al*. Phase I study of single-agent utomilumab (PF-05082566), a 4-1BB/CD137 agonist, in patients with advanced cancer. *Clin Cancer Res* 2018;**24**(8):1816–1823.
3. Chester C, Sanmamed MF, Wang J, Melero I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. *Blood* 2018;**131**(1):49–57.
4. Hinner MJ, Aiba RSB, Jaquin TJ, *et al*. Tumor-localized costimulatory T-cell engagement by the 4-1BB/HER2 bispecific antibody-anticalin fusion PRS-343. *Clin Cancer Res* 2019;**25**(19):5878–5889.
5. Claus C, Ferrara, C, Xu W, *et al*. Tumor-targeted 4-1BB agonists for combination with T cell bispecific antibodies as off-the-shelf therapy. *Sci Transl Med* 2019;**11**(496):eaav5989.
6. Eskiocak U, Guzman W, Wolf B, *et al*. Differentiated agonistic antibody targeting CD137 eradicates large tumors without hepatotoxicity. *JCI Insight* 2020;**5**(5):e133647.
7. Mayes PA, Hance KW, Hoos A. The promise and challenges of immune agonist antibody development in cancer. *Nat Rev Drug Discov* 2018;**17**:509–27.
8. Upadhyaya P, Lahdenranta J, Hurov K, *et al*. Anticancer immunity induced by a synthetic tumor-targeted CD137 agonist. *J Immunother Cancer* 2021;**9**:e001762.

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