AN INTEGRATIVE APPROACH TO OPTIMIZE A SYNTHETIC EphA2/CD137 AGONIST: BALANCING POTENCY, PHYSICAL CHEMICAL PROPERTIES, AND PHARMACOKINETICS TO ACHIEVE ROBUST ANTI-TUMOR ACTIVITY

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Background CD137 (4-1BB) is a resurging target in immuno-therapy after the first generation of monoclonal antibodies were limited by hepatotoxicity1 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 efficacity.

Methods Over 150 different EphA2/CD137 tumor-targeted immune cell agonists (Bicycle TICAs) were synthesized by linking Bicycle8 binders to EphA2 to those binding CD137.9 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 activity was determined in syngeneic mouse tumor models by evaluating 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 activity was determined in syngeneic mouse tumor models by evaluating 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 microenvironment and tumor regression.

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 t1/2 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


