Novel Multimers of Bicyclic Peptides Cluster and Activate CD137 (4-1BB): A Costimulatory T-Cell Checkpoint Receptor

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ABSTRACT

• Agonism of CD137 to activate T cell mediate anti-tumour effects is a promising immunotherapeutic approach validated by numerous in vivo models.
• CD137 agonism requires clustering of receptors into trimeric complexes; would be difficult achieve with small molecule drugs.
• CD137 agonistic antibody has difficulty in clinical testing but have shown hepatotoxicity and limited efficacy.
• Bicycles are bicyclic peptides constrained via a trifunctional chemical scaffold and bind targets with high affinity and selectivity.
• Bicycle binders to CD137 were identified by phage display before chemical optimisation to generate plasma stable monomeric antagonists of CD137.
• Systematic chemical elaboration generated of a matrix of dimeric, trimeric and tetrameric synthetic CD137 agonists with a broad range of activity in a cell-based functional assay.
• Bicycle multimers exhibit high avidity to CD137 on the cell surface resulting in receptor agonism even with short exposure.
• The Bicycle platform is efficient, flexible and adaptable and lends itself to multiple applications in oncology and beyond.

INTRODUCTION

CD137 (4-1BB) is a co-stimulatory T-cell receptor expressed on activated CD4+ and CD8+ cells. Ligation and clustering of the receptor by CD137L (4-1BBL), expressed on antigen presenting cells (APCs) promotes T cell survival and proliferation in vivo (Fig.1). In an immune oncology setting, agonistic antibodies of CD137 have demonstrated strong anti-tumour activity in mouse models, including inducing a memory response (Malero et al. 1997). Human trials have been less successful due to hepatotoxicity (urelumab, BMS, Segal et al. 2017) or lack of single agent activity (utomilumab, Pfizer, Sigal et al. 2018). Development of an agonist of CD137 that can overcome weak agonism and excessive activity in the liver could lead to a potent anti-tumour agent.

METHODS

Phage selections: Bicycle phage libraries were targeted against immobilized soluble recombinant human CD137. After 4 rounds of selection and amplification, individual phage clones were sequenced and a phage-based Bicycle display was identified.

Bicycle synthesis: Peptides made using standard solid phase Fmoc peptide chemistry followed by cyclisation on TATa scaffold. Lysines or D-lysines were substituted in all positions of the Bicycle for the attachment of discrete lengths of PEG linker and multimerisation on central hinge.

Fluorescence polarisation: Bicycles were made with a short linker and coupled to a fluorophore probe. Binding to CD137 indicated by an increase in mI in assay.

Surface plasmon resonance: Bicycles flowed over immobilised CD137 (Promega), and association and dissociation curves were measured using Biacore instrument.

CD137 agonism reporter gene assay: CD137 NF-kB luciferase reporter cells were used following manufacturer's instructions (Promega).

Syngeneic humanised mouse CDX model: Mice expressing human CD137 implanted with MC38 cell line xenografts. Tumours grown to ~100 mm³ before dosing with CD137 agonists.

RESULTS

A diverse library of >1012 Bicycles were screened on phase against human CD137 protein, hits from phage screening were synthesised. A peptide sequence was identified with Kd of 1.4 pM as determined by fluorescence polarization (FP) and chosen for optimisation on phase by use of custom phage libraries. An improved Bicycle binder with Kd of 67 nM (FP) was found and a single amino acid substitution gave a plasma stable lead Bicycle with Kd of 32 nM (measured by surface plasmon resonance, SPR). Competition experiments showed that the Bicycle binding site had overlap with both the natural ligand and utoxilumab but not urelumab (data not shown).

Fig. 2: Homotandem Bicycles show increased affinity to CD137 versus monomeric Bicycles. SPR measurements with mono- and dimeric Bicycles shows that the dimer form exhibits similar association rates but much reduced rate of dissociation leading to an increase in affinity of over 20 fold. Teiding with trimeric and tetrameric Bicycles showed even slower k on values and higher affinities (data not shown, poor fit to binding models as result of multiple interactions).

Fig. 3: Activity of trimer and tetramer Bicycles in a CD137 agonism reporter gene assay. Synthetic Bicycle trimers and tetramers are significantly smaller than biological activators of CD137 (soluble CD137L, utoxilumab and urelumab) and demonstrate a range of activities. Monomeric and dimeric Bicycles show no agonism (not shown); tetrameric Bicycles generally give higher fold induction at lower concentrations versus trimeric Bicycles.

Fig. 4: In vivo PK in mice (left-hand graph): concentrations of Bicycle multimers in plasma after i.v. dosing at 5mg/kg. Bicycles show half-life of 20-40 hrs after single i.v. dose.

CD137 Bicycle multimers maintain activity after washout (right-hand graph): Bicycle multimers were incubated with reporter cells (same) for 30, 60, or 120 min followed by washout of the compound or the compound was left on the cells for the full duration of the assay (no washout). Activity was assayed as an increase in reporter assay. In all cases the Bicycle multimers were retained on the cells and showed higher induction versus the CD137; control. For BCY19 and BCY794, 30 min exposure was sufficient to produce almost the same level of agonism as the 6 h no washout sample.

Multimeric Bicycles can overcome issues hindering development of an anti-tumour agent based on CD137 agonism. By tuning cellular activity to be around that of the natural ligand, they can avoid the lack of activity observed with very weak CD137 agonist antibodies. In addition, due to their tunable half life and lack of an Fc domain that could interact with Fc receptors, it is anticipated that Bicycle would not block the on-target hepatotoxicity seen with superagonists like urelumab.

CONCLUSIONS

• Phage display of Bicycle peptides identified binders specific for human CD137 protein that shows tolerance to conjugation.
• Chemical optimisation of the affinity matured phase hit lead to generation of a plasma stable antagonist that demonstrated good in vivo PK with well defined permitted sites of chemical elaboration.
• Assembling multimers of Bicycles by click chemistry generate larger multimers that were potent agonists of CD137 in an in vitro cell-based assay, comparable to or better than the natural ligand and clinical antibodies.
• When dose i.v. multimer Bicycle exhibit in vivo half lives typical of an unmodified Bicycles of 20-30 mins.
• Activity advantage of multimeric Bicycle means that even when exposed to CD137 on cells for a short period of time, they are able to be retained on the receptor and maintain a lasting effect.
• Bicycle multimers represent novel small molecule agonists of CD137 that could form the basis of safe and effective anti-tumour agents.

REFERENCES