ABSTRACT

Bicycle Toxic Conjugates (BTCs) targeting EphA2 for the treatment of solid tumours: Discovery and selection of BT5528

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INTRODUCTION

Ephrin receptor A2 (EphA2) is a member of the Ephrin receptor family of cell-cell junction proteins highly overexpressed in several solid tumours and associated with poor prognoses in patients. Bicycles are novel therapeutic agents: bicyclic peptides constrained via a chemical scaffold, which confer structural stability leading to high affinity and selectivity comparable to antibodies. The small size of bicycles (1.5-3 kDa) allows extensive tissue penetration, a short duration of systemic exposure and liver-opening rapid renal elimination. Bicycles are fully synthetic, allowing simple conjugation to a wide range of toxins and linkers.

METHODS

Bicycle binders were identified by proprietary phage display technology. Bicycles are synthesised by standard Fmoc solid phase synthesis and a proprietary cyclisation step. A range of linker- linkers were then conjugated to Bicycles (to either N- or C- termini) using standard conjugation chemistry, allowing rapid production of a matrix of BTCs. The affinity of Bicycles and BTCs for EphA2 was evaluated by competition fluorescence polarization, using a Bicycle conjugated to fluorescein as a tracer. A typical competition binding curve is shown in Figure 2.

Bio-distribution was evaluated using a Bicycle-DOTA conjugate, incorporating the radiosotope Ga-68. Mice bearing HT-1080 tumour xenografts were administered the radiotracer and scanned using microPET for 60 minutes. Efficacy was evaluated using a range of xenograft models in nude mice, with BTCs or vehicle dosed by intravenous bolus.

RESULTS

Figure 3: Optimization of Bicycle and BTCs. Bicycles were identified and conjugated to DOTA for imaging and to toxin-linker for efficacy studies. PET imaging shows clear visualisation of tumour and clearance through kidney to bladder, but with evidence of binding in liver (c). Efficacy was seen in HT-1080 xenograft study, but with dose-limiting weight loss (d). Liver toxicity was seen in the study (e).

CONCLUSION/SUMMARY

- Bicycles with high affinity for EphA2 were identified by phage display.
- Early BTCs using these Bicycles showed good efficacy in vivo xenograft models, but with poor physicochemical properties, and poor tolerability profiles consistent with liver toxicity.
- Screening a more diverse Bicycle library identified alternative Bicycle binders with a different mode of binding to EphA2 and a better physicochemical profile.
- Binders were further optimized and conjugated to produce a matrix of >75 BTCs which were evaluated for efficacy and tolerability.
- The Bicycle conjugate BT5528 showed the most optimal efficacy in vivo and tox experiments.

REFERENCES


Figures:

- Figure 2: Representative competition binding curve for measurement of binding to EphA2 by Bicycle and BTCs.
- Figure 3: Optimization of Bicycle and BTCs. Bicycles were identified and conjugated to DOTA for imaging and to toxin-linker for efficacy studies. PET imaging shows clear visualisation of tumour and clearance through kidney to bladder, but with evidence of binding in liver (c). Efficacy was seen in HT-1080 xenograft study, but with dose-limiting weight loss (d). Liver toxicity was seen in the study (e).