BT5528, an EphA2-targeting Bicycle Toxin Conjugate (BTC): profound efficacy without bleeding and coagulation abnormalities in animal models

ABSTRACT# 164

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ABSTRACT

- BT5528 is a EphA2-targeting *Bicycle* Toxin Conjugate being developed to treat solid tumours
- BT5528 shows profound efficacy in a range of Epha2-expressing tumour models
- BT5528 maintains efficacy even in large (>1000mm3), heterogeneous PDX tumours
- BT5528 shows no bleeding or coagulation toxicity in rats or NHPs

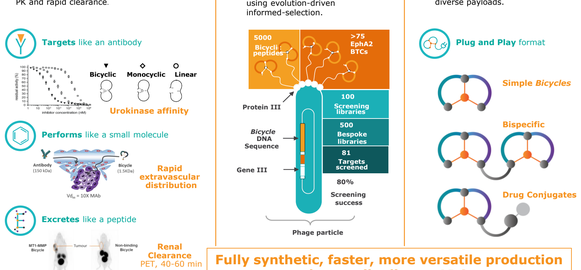
INTRODUCTION

Bicycles® are novel therapeutic agents: bicyclic peptides constrained via a chemical scaffold, which confer structural stability leading to high affinity and selectivity comparable with antibodies. The small size of *Bicycles* (1.5-3 kDa) allows rapid tissue penetration and extravasation. *Bicycles* are fully synthetic, allowing simple conjugation to form a Bicycle Toxin Conjugate, allowing targeted delivery of a cytotoxic payload.

Ephrin receptor A2 (EphA2) is a member of the Ephrin receptor family of cell-cell junction proteins and is highly overexpressed in several solid tumours and associated with poor prognosis. Despite the value of the target, clinical development of an antibody drug conjugate targeting EphA2 (MEDI-547) was stopped after severe adverse events, including bleeding and hepatotoxicity were seen (Annunziata et al, 2013).

Bicycle binders for EphA2 were identified using a proprietary phage display peptide technology consisting of highly diverse phage libraries of Bicycles, conjugated to cleavable linkers & toxins to form Bicycle Toxin Conjugates (BTCs). The small size of BTCs offers a significant advantage over other targeted cytotoxic approaches such as antibody-drug conjugates due to rapid extravasation, improved tumour penetration and renal elimination. We selected the candidate BTC BT5528 from a panel of >75 BTCs, based on in vivo efficacy, tolerability and drug-like properties.

WHY *BICYCLES*



RESULTS

Figure 1: *In vitro* profile of BT5528.

As shown in the table, BT5528 binds to EphA2 with high affinity, across a range of relevant species. There is no significant binding to 15 related Eph receptors.

High Content Screening in EphA2-expressing HT-1080 cells was used to measure binding of BTCs. Binding of BTC is detected using anti-MMAE antibody, (green), which colocalizes with membrane stain (purple). The cell nucleus is shown in blue. Clear membrane binding is seen with BT5528, but not non-binding BTC

Binding affinity (SPR)	BT5528 K_i (nM)
Human EphA2	0.9
Cyno EphA2	1.0
Rat EphA2	2.0
Mouse EphA2	2.7

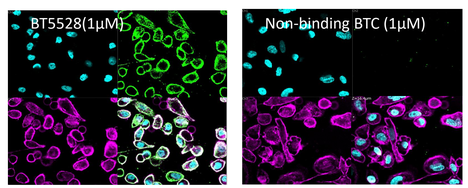
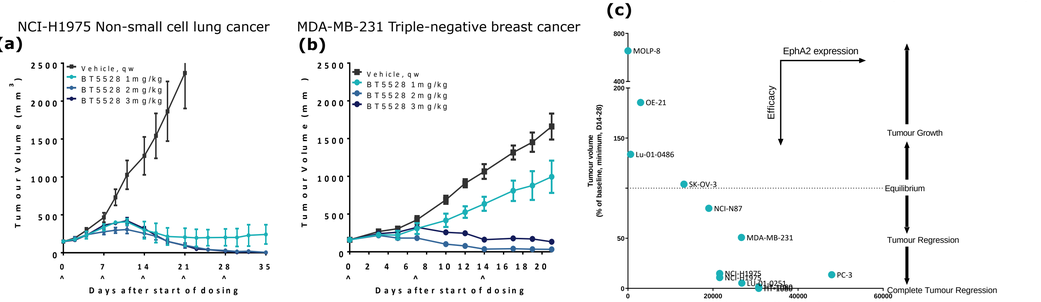
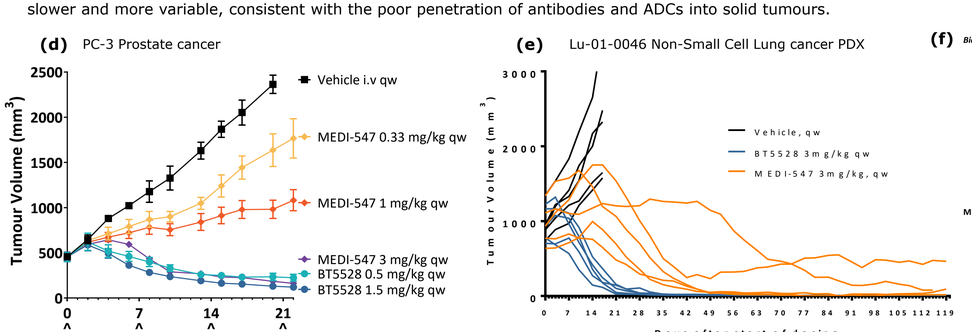
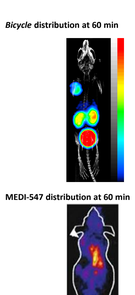


Figure 2: *In vivo* efficacy of BT5528 across multiple cell xenograft models.



As shown in (a)-(e), BT5528 shows profound efficacy across a range of xenograft models. Across a wide range of models, efficacy correlates with tumour expression of EphA2 (c). Regression of tumours can be seen with doses as low as 0.5mg/kg qw, compared to 3mg/kg qw for the EphA2 ADC MEDI-547 (d). In large, heterogeneous PDX models, (e), BT5528 shows rapid regression in all treated animals, reflecting the rapid and complete tumour penetration of BTCs (as shown in PET imaging, (f)). Efficacy seen with MEDI-547 is slower and more variable, consistent with the poor penetration of antibodies and ADCs into solid tumours.

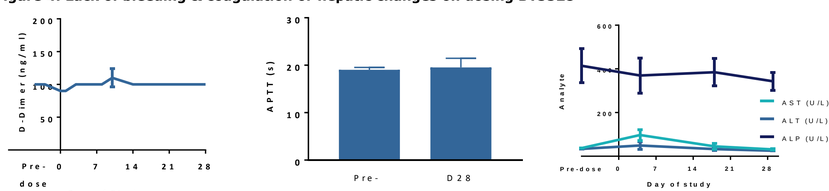




The EphA2-targeted ADC, MEDI-547, was progressed into clinical trials. As described in Annuziata et al (2013), the clinical trial was terminated early, due to treatment-related bleeding and coagulation events (hemorrhage-related, n=3; epistaxis, n=2) occurring in 5/6 patients receiving the starting dose. These events were preceded by findings in preclinical species, with the dose-limiting toxicology in monkey being disseminated intravascular coagulation (DIC). Increased activated partial thromboplastin time (APTT) and increased fibrin D-dimer were also reported, together with changes in liver function parameters (ALT, AST, ALP, serum albumin). The events observed in humans were considered to be consistent with the preclinical findings, in particular to the observation of DIC.

Figure 4 shows the lack of effect of BT5528 on toxicological parameters described for MEDI-547. In NHP exploratory toxicology studies, BT5528 at doses ~MTD does not produce changes in D-Dimer (a), APTT (b) or liver enzymes (c). Additionally, no signs of bleeding were seen in macro- or micro-scopic pathology analysis.

Figure 4: Lack of bleeding & coagulation or hepatic changes on dosing BT5528



In 28d exploratory toxicology studies, weekly dosing of BT5528 to NHPs at doses ~MTD showed no significant changes seen in D-Dimer, APTT or liver enzyme parameters.

CONCLUSION/SUMMARY

- BT5528 shows profound efficacy in a range of tumour models
- Efficacy of BT5528 correlates with tumour expression of EphA2
- BT5528 shows efficacy comparable to the EphA2 ADC MEDI-547, despite a shorter terminal half-life and intermittent dosing schedule
- BT5528 maintains efficacy even in large (>1000mm3), heterogeneous PDX tumours, reflecting the rapid and complete penetration of *Bicycle* Toxin Conjugates into solid tumours
- BT5528 shows no bleeding or coagulation toxicity in preclinical species
- IND-enabling studies for BT5528 are currently underway

REFERENCES

Annunziata, C.M. *et al.* (2013). Phase 1, open-label study of MEDI-547 in patients with relapsed or refractory solid tumours. Invest. New Drugs *31*, 77–84.

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