BT5528, a Bicycle Toxin Conjugate targeting EphA2: mechanism of action and clinical translation

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ABSTRACT# C066

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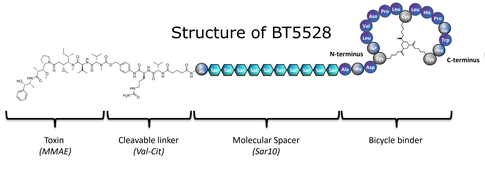
BT5528 is a Bicycle Toxin Conjugate (BTC) targeting the tumor expressed antigen EphA2
Increased EphA2 expression has been reported in multiple tumor types (including NSCLC, ovarian cancer, TNBC, gastric/upper GI, pancreatic and urothelial cancers) and so represents a attractive tumor binding target. The identification, characterization and initial *in vivo* profiling of BT5528 has previously been described. This poster describes a more detailed analysis of the mechanism of action of BT5528.

INTRODUCTION

Ephrin receptor A2 (EphA2) is a member of the Ephrin receptor family of cell-cell junction proteins highly overexpressed in several solid tumors and associated with poor prognosis in patients.
This has been a "high-value target" for pharma companies, with multiple programs in discovery and clinical stages but previous approaches have failed to offer an appropriate balance of efficacy and safety
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. Bicycles can be simply conjugated to form a range of Bicycle conjugates, including Bicycle Toxin Conjugates such as BT5528

Structure of BT5528

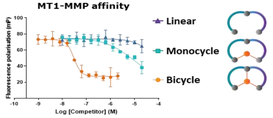


WHY BICYCLES?

New Class of Therapies

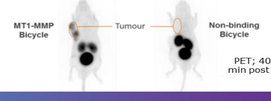
Novel modality delivers high affinity, favourable PK and rapid clearance.

Large binding footprint allowing targeting of protein-protein interactions



Low molecular weight (1.5–2 kDa), delivering attractive PK and profound tissue penetration

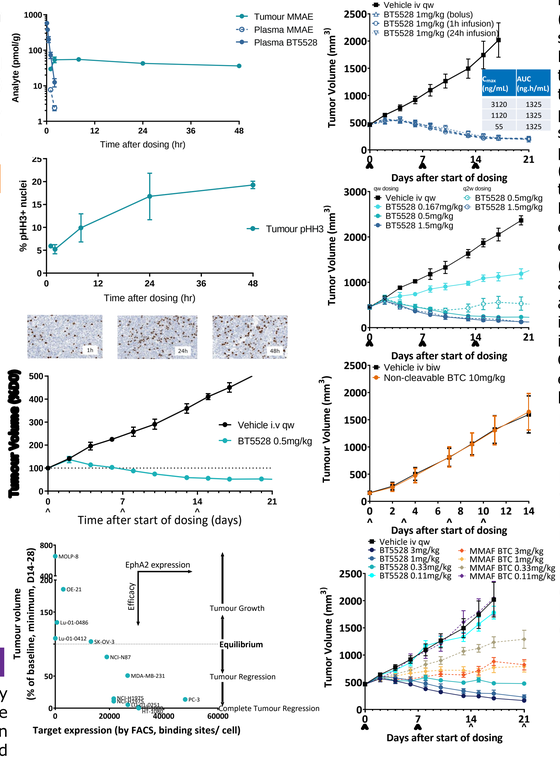
Renal elimination minimising cell interactions in liver and gut



METHODS

Bicycle binders were identified by proprietary phage display technology. *Bicycles* were synthesised by standard Fmoc solid phase synthesis and a proprietary cyclization step. Amino acid substitutions were made to optimise affinity, stability and hydrophilicity. The toxin-linker vc-PABC-MMAE was then conjugated to the *Bicycle* to generate BT5528. Efficacy and pharmacokinetics/ pharmacodynamics and distribution were evaluated using a range of xenograft models in nude mice. Xenografts were established subcutaneously cell- or patient derived tumors. Dosing was normally initiated from an average tumor size of ~200mm^3, though dosing was initiated from average sizes of up to ~1000mm^3 for specific experiments. Test agents (BTCs or vehicle) were normally administered by i.v. dosing as a bolus (over a few seconds), though administration using a syringe pump or osmotic minipump was used for for specific experiments. For the metastatic xenograft model, PC-3-Luc cells were administered via intracardiac injection and monitored using bioluminescence imaging. Expression of EphA2 in tumor cells or patient-derived tumor samples was evaluated by measurement of EphA2 antibody binding (PE conjugated) using flow cytometry.
PET imaging was performed using Bicycle binder-DOTA conjugate, incorporating ^{68}Ga.

RESULTS



C_{max} (ng/mL)	AUC (ng.h/mL)
3120	1325
1120	1325
55	1325

Mechanism of action work with BT5528

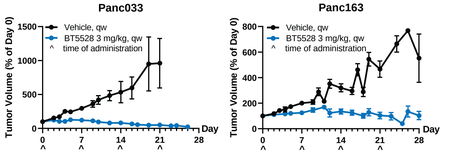
(a) Intravenous dosing of BT5528 provides short systemic exposure, and limited systemic exposure of MMAE payload. BT5528 efficiently delivers MMAE to target, with high concentrations measured in tumor out to at least 48h post-dose. (b) tumor cells show pharmacodynamic response to BT5528 dosing, with a steady increase of cell numbers staining positive for pHH3+. tumor volume decreases from 2d post-dose (c), indicating tumor cell death after dosing BT5528.
tumor killing with BT5528 requires expression of EphA2, with a clear relationship between EphA2 expression on cell surface (measured by FACS) and efficacy (d).
(e) Efficacy of BT5528 is independent of the rate of administration, with equivalent efficacy seen after administering the same dose as a bolus or as 1 or 24h infusions. (f) Efficacy can also bee seen with dosing as infrequently as every 2 weeks.
Compared to BT5528, reduced efficacy is seen with equivalent constructs with either lacking a cleavable linker (g) or carrying the non-permeant toxin MMAF (h)

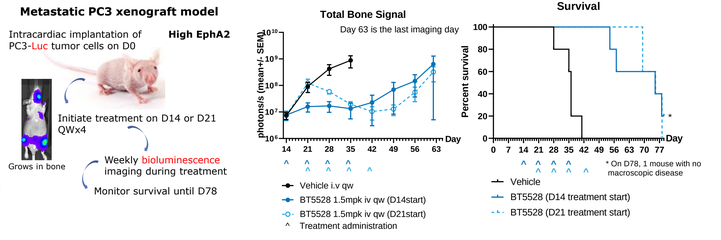
BT5528 toxicology

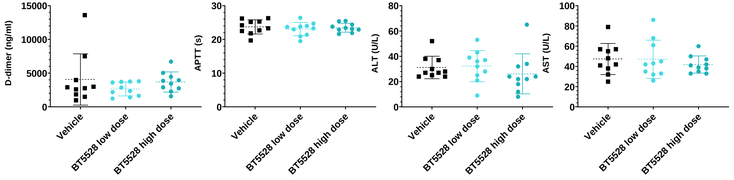
A previous Antibody Drug Conjugate targeting EphA2 (MEDI-547) showed good preclinical efficacy[1], but bleeding/ coagulation events and effects on liver were observed at the starting dose in a Ph1 clinical trial[2]. The clinical events were precedented by nonclinical findings observed in toxicity studies in NHP and rat. In preclinical toxicology with BT5528, no coagulopathy, DIC-like syndrome or bleeding events were seen, no changes seen in measures of bleeding/ coagulation, and no increases seen in tests of liver enzymes in plasma (l).

BT5528 in complex models

BT5528 shows profound efficacy in NSCLC PDX models, even when dosing is initiated at ~1000m^3 tumor volume (i). Profound efficacy is also seen in PDX models of pancreatic ductal carcinoma (j) and in models of metastatic disease (k).







CONCLUSION/SUMMARY

BT5528 is a Bicycle Toxin Conjugate targeting the tumor cell marker EphA2.
Mechanism of action studies show that BT5528 has a limited systemic exposure but efficiently delivers toxin payload to tumor, resulting in extended pharmacodynamic effects and tumor regression. Efficacy can be seen across a range of dose rates and intervals, and requires target expression, linker cleavage and includes a significant bystander component.
BT5528 maintains efficacy in a range of "hard to hit" models, including complex PDX models with very large tumor volumes, pancreatic tumors and metastatic models.
Clear differentiation is seen from previous ADC approach to targeting EphA2 in terms of efficacy, but also importantly in terms of toxicology, where no evidence was seen of bleeding/coagulation or liver toxicity in preclinical studies.
BT5528 is currently progressing towards FIH clinical trials.

REFERENCES

Jackson *et al.,* Cancer Research 68 (22): 9367–74 (2008)
Annunziata *et al.,* Investigational New Drugs 31 (1): 77–84 (2013)