BT8009, a *Bicycle*® Toxin Conjugate targeting Nectin-4, shows target selectivity, and efficacy in preclinical large and small tumor models.

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- BT8009 is a *Bicycle* Toxin Conjugate (*BTC*) in which a Nectin-4 binding *Bicycle*® (bicyclic peptide) is conjugated via an inert sarcosine spacer chain, and a cleavable linker, to the antimitotic toxin monomethyl auristatin E (MMAE).
- BT8009 has low nanomolar (3nM) affinity for Nectin-4 and high selectivity (>1000 fold) over other Nectins and Nectin-like family members.
- BT8009 is active in multiple Nectin-4 positive CDX and PDX models, leading to stable diseases and tumor regressions with durable responses.
- Efficacy data has now been extended to demonstrate efficacy of BT8009 in large (~1000mm^3) xenograft tumors, with rapid and near complete responses observed.
- BT8009 shows excellent efficacy in xenograft models expressing Nectin-4 target and the pharmacokinetic profile enables a rapid attainment of high tumor exposure levels with reduced systemic exposure.

INTRODUCTION

Nectin-4 is a cell adhesion molecule important in adherens junction formation, with a restricted distribution in normal tissues, but over expressed in multiple tumor types (e.g. bladder, breast, gastric, lung, esophageal and pancreatic cancers (1-3)). As such it is a suitable target for directed delivery of a cytotoxic agent. A Nectin-4 ADC, Enfortumab vedotin, is in phase 3 trials for metastatic urothelial cancer.

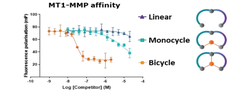
The Bicycle phage display platform was used to identify a Nectin-4 binding parent *Bicycle* which was optimized for affinity stability and hydrophilicity. Conjugation of this *Bicycle* peptide, through a cleavable linker, to MMAE results in the *BTC,* BT8009. BT8009 targets Nectin-4 and releases MMAE on cleavage by the enzymes upregulated in the tumor micro-environment, in order to kill adjacent tumor cells.

Why Bicycles?

New Class of Therapies

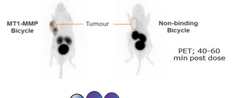
Novel modality delivers high affinity, favourable PK and rapid clearance.

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



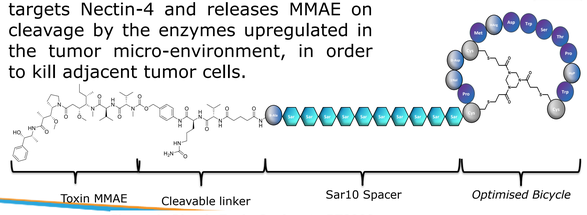


Figure 1: *Bicycle* Toxin Conjugate BT8009

METHODS

Bicycle binders are identified using phage display technology. The *Bicycle* was 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, valine-citrulline p-aminobenzyloxycarbonyl-MMAE (vc-PABC-MMAE), was conjugated to the *Bicycle* to generate BT8009.

Surface Plasmon Resonance (SPR) was used to confirm affinities for Nectin-4 and 2º targets. High content imaging and immunocytochemistry demonstrated BT8009 binding to cell surface of Nectin-4 expressing cells (MDA-MB-468).

Expression of Nectin-4 in tumor cells, or patient-derived tumor samples was evaluated by FACS. Where protein was not measured RNA expression was provided by the model supplier.

Efficacy was evaluated using a range of xenograft models in nude mice. Xenografts were established as subcutaneous cell- or patient derived tumors. Dosing was normally initiated from an average tumor size of ~200mm^3. For some experiments tumors were allowed to grow to ~1000mm^3 before drug treatment commenced. Test agents (*BTC*s or vehicle) were administered by i.v. dosing as a bolus, unless stated otherwise.

RESULTS

Parent Bicycle

Ac C P F G C M K N W S W P I W C

Amino Acids Important for target engagement Identified

KD (nM)	CHIlogP	$T_{1/2}$*
9.82	2.08	1.3h

Stabilised Bicycle

Ac C P F d C M hArg N W S W P I W C

Modifications improve stability and hydrophilicity

KD (nM)	CHIlogP	$T_{1/2}$*
19.52	1.72	>24h

Solubility = 4.9 ug/ml
LogD = -0.7

Optimised Bicycle

Ac C P 1Nal d C M hArg D W S T P Hyp W C

Further changes improve affinity and hydrophilicity

KD (nM)	CHIlogP	$T_{1/2}$*
0.9	1.37	>57.8h

Solubility = 420 ug/ml
LogD < -2.2

* T1/2 human plasma *in vitro*

Figure 2. Identification and optimization of Nectin-4 binding Bicycle.

	hNectin sub type KD (nM)				hNecl KD (nM)				
	4	1	2	3	1	2	3	4	5
BT8009	3.02	No binding	No binding	No binding	No binding	No binding	No binding	Weak @ 5 uM	No binding

Table 1: BT8009 shows excellent specificity for hNectin-4 over the other Nectin/Nectin-like family members in SPR. Biotinylated Nectin-4 *Bicycle* (1 μM) was assessed for binding to 5,528 human cell membrane proteins expressed in fixed HEK cells and only bound Nectin-4.

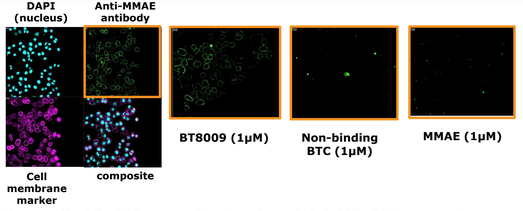


Figure 3: BT8009 binds Nectin-4 on MDA-MB-468 cells. Cells preincubated with BT8009, MMAE, non-binding *BTC* were washed and retained MMAE detected with anti-MMAE antibody. BT8009 remains bound to cells.

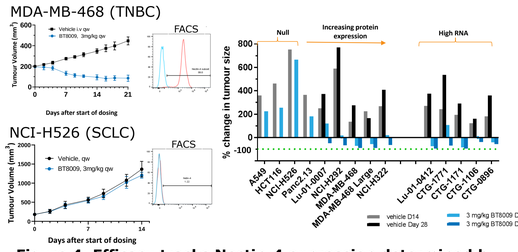


Figure 4: Efficacy tracks Nectin-4 expression determined by FACS. CDX models were selected for "null" and increasing expression of Nectin-4 by FACS. High expression models show excellent efficacy.

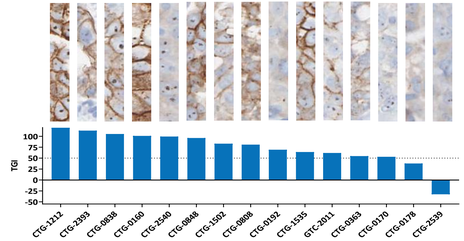


Figure 5: Efficacy tracks Nectin-4 expression as determined by IHC. BT8009 was tested in 15 NSCLC PDX models at 3 mg/kg qw. Tumor growth inhibition correlates well with Nectin-4 expression, determined by IHC. An IHC assay for human tissue is being developed to support patient selection.

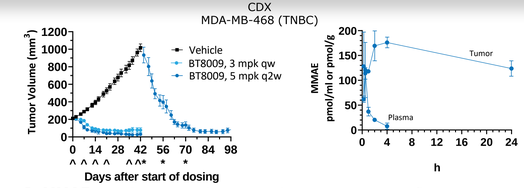


Figure 6: MMAE is retained in tumor but rapidly cleared from plasma, thereby reducing systemic exposure.

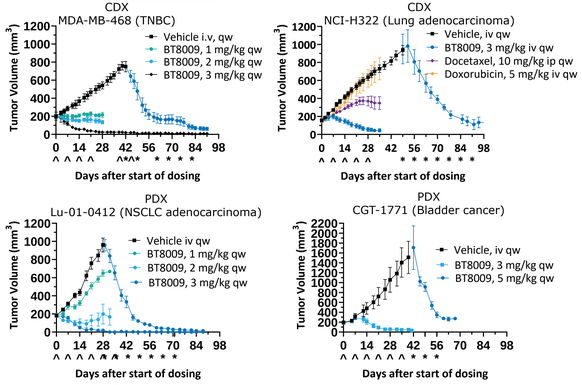


Figure 7: BT8009 delivers outstanding efficacy against large CDX and PDX tumors, with rapid tumor regression.

CONCLUSION/SUMMARY

Using phage display technology a Nectin-4 binding *Bicycle* was identified. The Parent *Bicycle* was optimized for affinity, stability and hydrophilicity. BT8009 was synthesized by conjugation through an inert spacer and a cleavable linker to the toxin MMAE. The binding peptide and the *BTC* are highly selective for the target protein. The pharmacokinetic profile of BT8009 enables a rapid attainment of high tumor levels of MMAE, with corresponding reduced systemic exposure. BT8009 shows excellent efficacy in large tumor CDX and PDX models expressing Nectin-4 target.

IND enabling studies for BT8009 are ongoing.

REFERENCES

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