BT1718, a novel bicyclic peptide-maytansinoid conjugate targeting MT1-MMP for the treatment of solid tumours: Design of bicyclic peptide and linker selection

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ABSTRACT# 5144

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ABSTRACT

- Bicycle Therapeutics has developed a proprietary phage display platform technology allowing the selection of high affinity bicyclic peptide binding molecules (*Bicycles* ®)
- Membrane type 1-matrix metalloprotease (MT1-MMP/MMP14/MT1) is a promising target for a specific targeted toxin delivery approach in oncology
- *Bicycle* peptide N241 binds MT1 with a K_D of approximately 2nM, maintains species cross- reactivity and doesn't bind related MMP proteins
- *Bicycle Drug Conjugates*® (*BDCs*) with a variety of linkers and cytotoxic payloads were prepared which retain binding affinity to MT1
- The anti-tumour activity of select *BDCs* was demonstrated *in vitro* and *in vivo*

INTRODUCTION

MT1 is a cell-surface expressed metalloprotease which degrades the extracellular matrix both directly, through collagenolysis, and indirectly via activation of MMP2, promoting cell migration. MT1 is overexpressed in many tumour types including TNBC and NSCLC (fig. 1), is implicated in the invasion and metastasis of many cancers (Zarrabi *et al.*, 2011, Journal of Biological Chemistry, 286(38), 33167–33177) and is associated with poor patient prognosis. These properties make MT1 an attractive target for targeted tumour payload delivery.

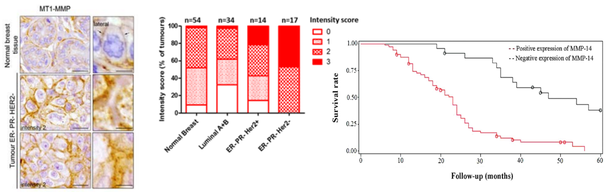


Figure 1: MT1 over-expression in triple negative breast cancer (TNBC) (Rosse et el., PNAS 111: E1872-1879) and MT1 correlation with prognosis in non-small cell lung carcinoma (NSCLC) (Zhou *et al.* 2014, Oncology Letters. 7, 1395-1400)

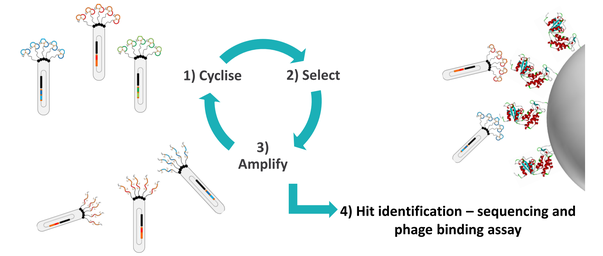


Figure 2: High diversity, high speed and high throughput Bicycle Therapeutics' phage display and cyclisation platform identifies high affinity *Bicycle* binders. The platform has now been validated on over 80 targets

***Bicycle* properties:**

- **High affinity and selectivity:** Bicycles are constrained via a central chemical scaffold conferring stability and positive binding confirmations
- **High diversity**: Size and symmetry of the peptide loops and the characteristics of the scaffold can be altered to deliver extremely high diversity in chemical space
- **High penetration:** Small size (1.5-2 kDa) delivers advantages in tissue penetration and extra-vascularisation
- **Low toxicity:** Fast, renal clearance avoids liver and GI toxicity often associated with other drug modalities

METHODS

- Selections were carried out against MT1 hemopexin domain (PEX) using a *Bicycle* phage library with 6 amino acids in each loop. Output clones were characterised using pyrosequencing & ALPHAscreen® binding assays
- Peptides were synthesised by Fmoc Solid Phase Synthesis, cleaved and cyclised with a tri-functional small molecule scaffold
- The lead peptide 17-69-07 underwent chemical modification in which non-natural amino acid mutations were made to increase stability in plasma, creating peptide N241
- Peptide N241 was conjugated to maytansinoid toxins via a panel of disulphide linkers varying in steric hindrance or a non-reducible maleimide thioether bond (table 2)
- Affinity and selectivity of N241 and BDCs were measured using fluorescence polarisation (FP) and Biacore™ standard methods
- Stability of the linkers was assessed by exposure to 10mM dithiothreitol (DTT) or *in vitro* human plasma and remaining parent molecule was measured using quantitative UV-LCMS/MS
- *In vitro* cytotoxicity was determined by ATP measurement and *in vivo* efficacy measured in xenograft models in mice implanted with tumours expressing MT1-MMP

RESULTS

INTIAL HIT
17-69
K_D approx. 500nM

AFFINITY MATURATION LEAD
17-69-07
K_D approx. 2nM
Plasma stability = 6 hrs
= newly optimised natural aa

CHEMICALLY OPTIMISED LEAD
17-69-07-N241 (N241)
K_D approx. 2nM
Plasma stability >>20hrs
= stabilising non-natural aa

BT1718
Bicycle Drug Conjugate
K_D approx. 3nM

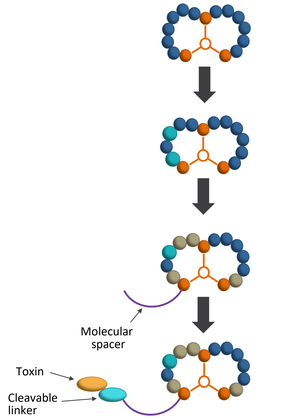


Figure 3: Development of BT1718

Protein	Sequence homology to human MT1 PEX	Affinity (K_D in nM ± SD) (FP direct)	Affinity (K_D in nM ± SD) (Biacore™)
Human/cyno MT1 PEX	100%	1.6 ± 1.1 (n=20)	2.6 ± 0.4 (n=3)
Human/cyno MT1 ectodomain	100%	1.2 ± 0.6 (n=5)	Not tested
Human/cyno MT1 catalytic domain	N/A	>> 100 (n=3)	Not tested
Mouse/rat MT1 PEX	99.5%	1.2 ± 0.1 (n=2)	2.7 ± 0.6 (n=2)
Dog MT1 PEX	99.5%	0.9 ± 0.4 (n=3)	2.4± 0.1 (n=2)
Human MT2-MMP ectodomain	66%	>> 500 (n=4)	>10000 (n=1)
Human MT3-MMP ectodomain	64%	>> 500 (n=5)	>10000 (n=1)
Human MT5-MMP ectodomain	58%	>> 2000 (n=2)	Not tested

Table 1: The *Bicycle* binder maintains affinity to human, rodent, dog and primate MT1-MMP and demonstrates impressive selectivity over related MMP proteins.

Stability:
Increasing stability of the disulphide bond was achieved by introducing methyl groups on carbon atoms adjacent to the disulphide linkage. BT17BDC21 includes a non-reducible maleimide thioether linkage which is proteolytically labile (table 2). More sterically hindered disulphide linkages were more resistant to reductive cleavage by both DTT and plasma, following the same rank order as demonstrated in antibody drug conjugates with the same linkers (Kellogg *et al.* 2011, Bioconjugate Chemistry, 22, 717-727). Affinity for target was maintained with all *BDCs* tested.

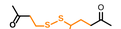
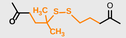
BDC	Linker	Toxin	Relative DTT stability	Plasma stability (h)	Binding affinity (nM)	*In vitro* cytotoxicity (nM)
BT17BDC17		DM-1	1	4	0.7	0.4
BT1718		DM-1	5	13	0.9	1.0
BT17BDC19		DM-4	30	24	0.6	0.2
BT17BDC20		DM-3	93	32	1.1	0.5
BT17BDC21	SMCC-Lys	DM-1	N/A	Not tested	1.1	8.9

Table 2: Structure, relative stability and affinity of *BDCs*. Relative DTT stability = rate of thiol-disulphide exchange normalised to that of BT17BDC17. Plasma stability = $t_{1/2}$ in *in vitro* human plasma (hours). Affinity = K_i (nM) measured by fluorescence polarisation competition. *In vitro* cytotoxicity = IC50 (nM) measured in ATP endpoint assay after 72 hours incubation with HT-1080 cells

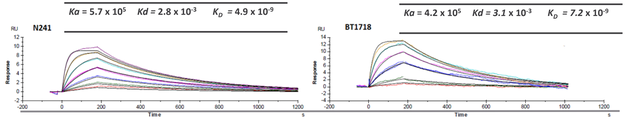


Figure 4: Surface plasmon resonance (Biacore) experiments on MT1-MMP hemopexin domain demonstrate that BT1718 maintains comparable binding affinity and kinetics compared to the unconjugated peptide N241

***In vitro* cytotoxicity:** All *BDCs* tested demonstrated a dose-dependent killing of HT-1080 cells. The least efficacious BDC was BT17BDC21 which would be expected to contain the most stable linker.

***In vivo* efficacy:** Treatment with *BDCs* containing the most labile linkers (BT17BDC17 or BT1718) showed rapid and complete tumour clearance (EBC-1 cells), while *BDCs* containing more stabilised linkers showed comparatively reduced efficacy (fig. 5) suggesting that target internalisation is not the sole mechanism of action for *BDC* efficacy and that extracellular cleavage and release of toxin within the local tumour environment likely also contribute. Only the most labile *BDC* (BT17BDC17) caused any significant toxicity (17% ± 9.7 body weight loss); all others were well tolerated (<10% body weight loss at 10mg/kg tiw). Optimal therapeutic index was achieved with BT1718. Testing of BT1718 in different dosing regimes in an additional model (HT-1080 cells) also demonstrated excellent tumour regression, with 10mg/kg biw leading to complete tumour clearance in all 3 animals within 23 days and no re-growth out to 70 days.

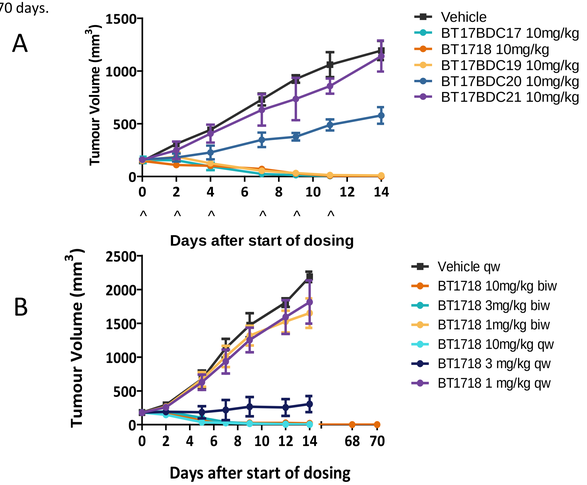


Figure 5: A: *In vivo* efficacy of *BDCs* with various linkers dosed at 10mg/kg iv tiw in a cell-derived xenograft model in mice implanted with MT1-positive EBC-1 cells. B: *In vivo* efficacy of BT1718 dosed at 1-10mg/kg iv qw or biw in a cell-derived xenograft model in mice implanted with MT1-positive HT-1080 cells. Dosing began when tumours were approx. 180mm^3. Data represents mean of n=3 animals ± SEM.

CONCLUSIONS

- Bicycle N241 demonstrates high affinity for the tumour target MT1 and excellent selectivity against other MMPs
- Conjugation of N241 to DM1 via a range of disulphide linkers produced a panel of *Bicycle Drug Conjugates* which maintain high affinity for MT1 and demonstrate *in vitro* and *in vivo* efficacy, exemplifying proof of concept for an exciting new targeted delivery modality
- Optimal therapeutic index was achieved using a mono-hindered disulphide bond (BT1718)
- BT1718 has demonstrated efficacy in a wide range of MT1 positive cell and patient derived xenografts and is currently progressing well through pre-clinical development
- BT1718 showcases the great potential of Bicycle Therapeutics' platform for the development of novel and transformational drugs for the treatment of a wide range of cancers

Bicycle Therapeutics will be presenting further data on posters 1167/2 & 3719/4

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