BT1718, a novel *Bicycle Drug Conjugate*® shows potent anti-tumor activity in diverse cell-derived and patient-derived tumor xenograft models

ABSTRACT#

bicycle therapeutics

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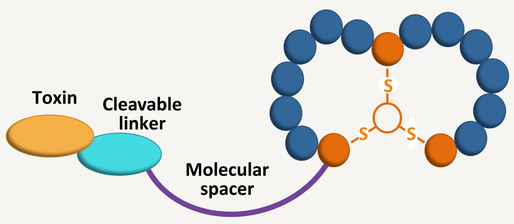
ABSTRACT

- Bicycle Therapeutics has developed a proprietary phage display technology which enables efficient identification of high affinity / high selectivity bicyclic peptides (*Bicycles*®)
- *Bicycles* can be efficiently linked to therapeutic payloads to produce *Bicycle Drug Conjugates* (*BDC*®) which enable antigen-mediated delivery of cytotoxins to tumours
- BT1718 is a BDC comprising a *Bicycle* binder of membrane type 1-matrix metalloprotease (MT1-MMP; MMP14) covalently linked through a hindered disulfide cleavable linker to the potent anti-tubulin agent DM1
- BT1718 demonstrated MT1-MMP-dependent cell killing of non-small cell lung cancer (NCSLC) cells *in vitro* and *in vivo* across a panel of human lung tumour xenograft mouse models, including patient-derived xenograft models (PDX)

INTRODUCTION

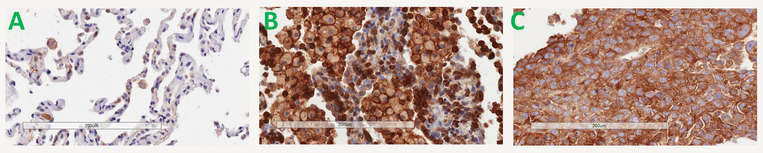
BT1718 is a *Bicycle Drug Conjugate (BDC)* comprising a constrained bicyclic peptide that binds with high affinity and specificity (Table 1) to membrane type 1-matrix metalloprotease (MT1-MMP; MMP14) covalently linked through a hindered disulfide linker to the potent anti-tubulin agent DM1 (Figure 1).



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 (n=20)	2.6 (n=3)
Human/cyno MT1 ectodomain	100%	1.2 (n=5)	Not tested
Human/cyno MT1 catalytic domain	N/A	> 100 (n=3)	Not tested
Mouse/rat MT1 PEX	99.5%	1.2 (n=2)	2.7 (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

Figure 1 & Table 1 : Design of the BT1718 *Bicycle Drug Conjugate* construct and table showing selective pharmacology of the *Bicycle* component where the binding affinity of BT1718 to MT1-MMP from human, cynomolgus, rat & mouse, and to related human MMPs was evaluated. BT1718 binds to MT1-MMP via the hemopexin domain, with high affinity and exquisite selectivity. When used *in vitro in* cytotoxicity assays, BT1718 kills MT1-MMP expressing EBC-1 cells with an IC_{50}~1nM

MT1-MMP is naturally involved in tissue remodeling, however overexpression of the cell-surface protease in certain tumour types (e.g. triple negative breast cancer, non-small cell lung cancer and soft tissue sarcoma) is well established (Figure 2) and over-expression is linked to increased tumor aggressiveness and invasiveness, as well as poor patient prognosis for many cancer indications. Expression of MT1-MMP is elevated in tissue from NSCLC patients vs controls (Table2).



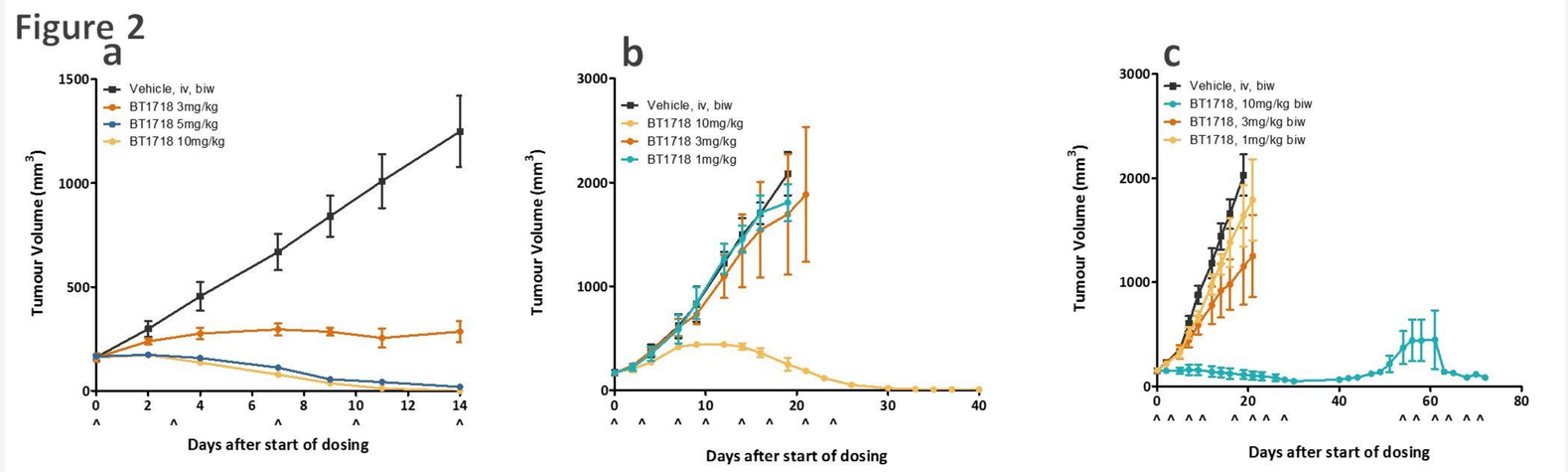
	Samples positive	Samples positive %	Samples negative	Samples negative %
NSCLC (94)	71	75.5	23	24.5
Control (80)	20	25	60	75

Zhou *et al., Oncology Letters.* 7:1395-1400 (2014)

Figure 2 & Table 2 : Expression of MT1-MMP in tissue sections, visualized by immunohistochemistry using anti-MT1-MMP antibody. Low expression of MT1-MMP is seen in normal lung tissue (A), with higher expression in NSCLC tumour (B), and in PDX tumour (C).

METHODS & RESULTS

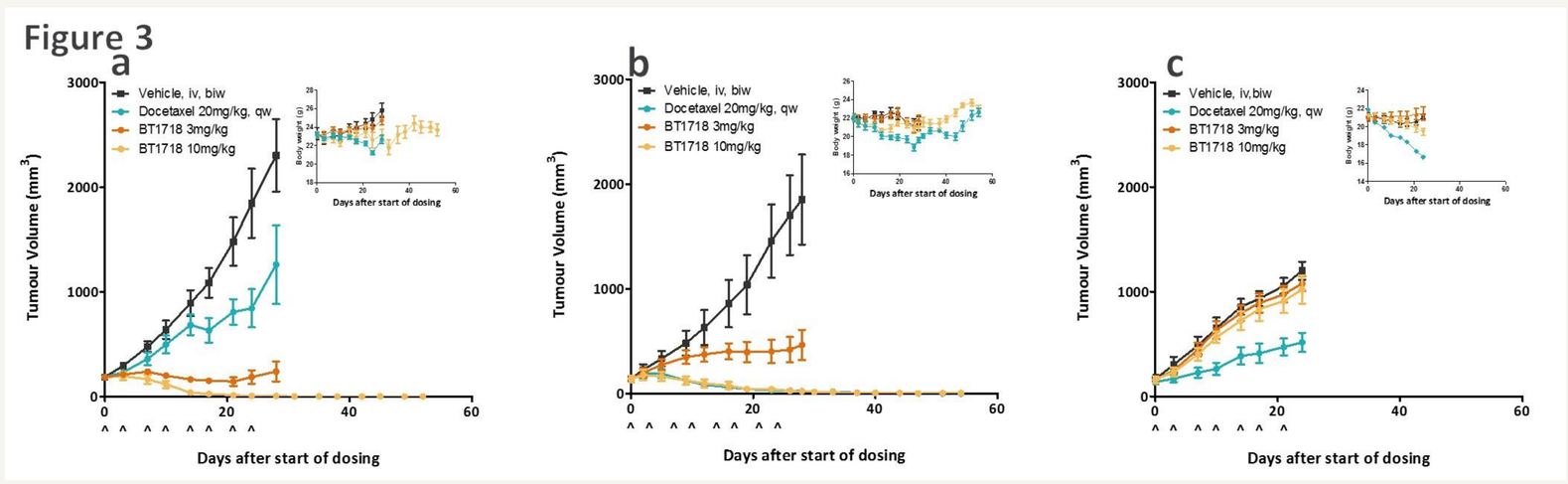
Figure 2



High MT1-expressing EBC-1 NSCLC xenograft	High MT1-expressing NCI-H1975 NSCLC xenograft	High MT1-expressing NCI-H292 NSCLC xenograft
Insensitive to EGFR inhibitors; sensitive to Met inhibitors	T790M EGFR-expressing, erolitinib resistant	EGFR overexpressing
Squamous cell carcinoma (Met amplified)	Adenocarcinoma	Mucoepidermoid pulmonary carcinoma

Figure 2 : Efficacy of BT1718 in cell-derived NSCLC xenograft models. BT1718 was dosed at 1, 3, 10 mg/kg, twice weekly, i.v. (a) In EBC-1 xenografts, complete clearance of tumour was seen within 14 days at 10mg/kg & 5mg/kg, whilst a dose of 3mg/kg elicited tumour stasis. (b) In NCI-H1975 xenografts, complete clearance of tumour was seen by 28 days at 10mg/kg with lower doses being comparable to the vehicle control group. (c) In NCI-H292 xenografts, tumours were reduced to minimal volume after 28 days with bi-weekly dosing at 10mg/kg, with lower doses being partially efficacious. After the 8th dose, on day 28, dosing was ceased. In this model a rare relapse and re-growth of the tumour was observed and animals were subsequently re-dosed with BT1718 at 10mg/kg (when tumours had reached ~150mm³). The tumours were responsive to additional doses of BT1718 and the re-growth was successfully treated and tumour cleared.

Figure 3



High MT1-expressing Patient-derived Xenograft	High MT1-expressing Patient-derived Xenograft	Low MT1-expressing Patient-derived Xenograft
Docetaxel resistant	Docetaxel sensitive	Docetaxel resistant
74 year old male, grade 3 primary tumour	68 year old male, grade 3 primary tumour	59 year old male, grade 3 primary tumour

Figure 3 : Efficacy of BT1718 in patient-derived xenograft models. BT1718 was dosed at 3 or 10mg/kg twice weekly, i.v. Docetaxel was dosed at 20mg/kg once weekly, i.v. (a) In high MT1-MMP expressing PDXs, good efficacy was seen with 3 & 10mg/kg BT1718, with complete clearance of tumour within 20 days at the 10mg/kg dose and tumour stasis at the 3mg/kg dose. Docetaxel, as standard of care comparator, shows a small reduction in the growth of the tumour. (b) In high MT1-MMP expressing PDXs, efficacy was seen at both 3 & 10mg/kg BT1718, with complete clearance of tumour within 20 days at 10mg/kg. Docetaxel, as standard of care comparator, shows comparable tumour clearance to BT1718 at 10mg/kg. However, at this dose significant weight loss (>10%) was seen in the Docetaxel group (see inset graph) and not in the BT1718 cohorts. (c) In low MT1-MMP expressing PDXs, efficacy was not observed with BT1718 at 3 or 10mg/kg, Docetaxel slowed the rate of tumour growth but again, exerted a significant effect on body weight (>10% loss in 20 days).

Figure 4 a

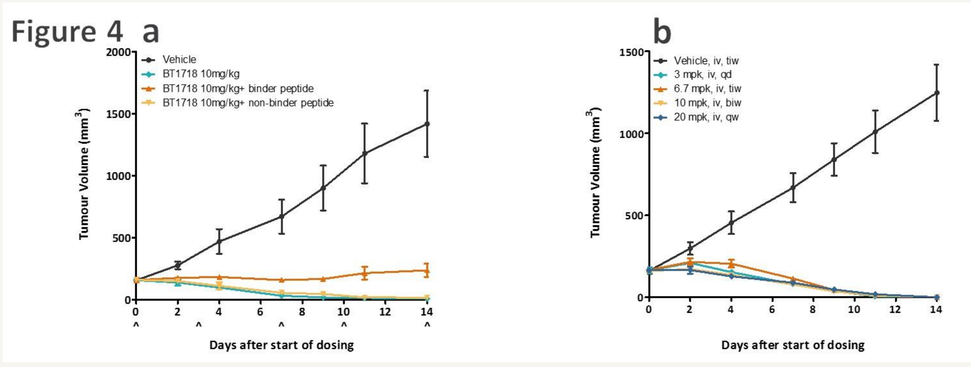


Figure 4 : The target-dependence of BT1718 activity was determined using EBC-1 xenografts across a range of dose schedules. (a) Target dependence was evaluated by co-dosing an unconjugated *Bicycle* MT1-binder. BT1718 efficacy was inhibited by co-injection of 100x molar excess of an MT1-binding *Bicycle*, but efficacy was unaffected by injection of 100x fold molar excess of a non MT1-binding *Bicycle*. (b) The same total weekly dose of BT1718 (20mg/kg) is equally efficacious when dosed across a range of dose splitting paradigms: either in once, twice, three or seven injections weekly.

Figure 5

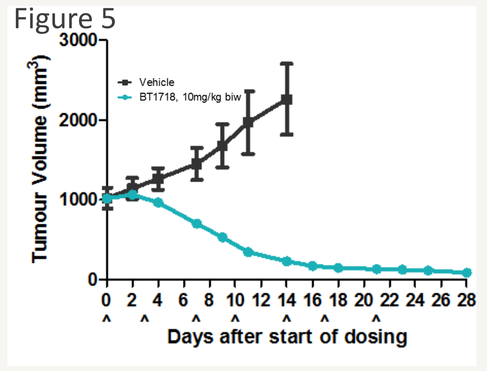


Figure 5 : The ability of BT1718 to distribute to and clear extremely large tumours was tested using EBC-1 xenografts, which were allowed to grow to a volume of 1000mm³ before dosing using an i.v. dose of 10mg/kg twice weekly. Large tumours are effectively cleared by BT1718 with similar elimination kinetics ~20 days seen when treating smaller tumours.

CONCLUSION/SUMMARY

- BT1718 has high affinity & selectivity for MT1-MMP, which is highly upregulated in NSCLC and other tumours
- BT1718 rapidly clears tumours in a range of lung cancer models in an MT1-MMP dependent manner
- Comparable efficacy in observed in cell-derived and patient-derived xenografts independent of sensitivity of the model to current standard of care therapies
- BT1718 is a first-in-class cytotoxic *Bicycle Drug Conjugate* with great potential for treatment of lung cancer and is progressing forward to clinical trials in collaboration with Cancer Research UK

REFERENCES

Zhou *et al., Oncology Letters.* 7:1395-1400 (2014)

–**Targets** like an antibody –**Performs** like a small molecule –**Excretes** like a peptide

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