# Development of BT1718, a novel Bicycle Drug Conjugate for the treatment of lung cancer

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ABSTRACT# 1167/2

## ABSTRACT


- Bicycle® Therapeutics has developed a proprietary phage display platform technology allowing the selection of high affinity bicyclic peptide binding molecules (Bicycles ®)
- BT1718 is a Bicycle Drug Conjugate (BDC®) comprising a constrained bicyclic peptide (Bicycle®) that binds with high affinity and specificity to membrane type 1-matrix metalloprotease (MT1-MMP; MMP14) covalently linked through a hindered disulfide linker to the potent anti-tubulin agent DM1.
- BT1718 demonstrated MT1-MMP target-specific binding and MT1-MMP-dependent cell killing of lung tumor cells in vitro as well as efficacy across a panel of lung tumor xenograft mouse models.


## INTRODUCTION

BT1718 is a Bicycle drug conjugate (BDC) comprising a constrained bicyclic peptide that binds with high affinity and specificity to membrane type 1-matrix metalloprotease (MT1-MMP; MMP14) covalently linked through a hindered disulfide linker to the potent anti-tubulin agent DM1. MT1-MMP is naturally involved in tissue remodelling, however overexpression of the cell-surface protease has been tied to 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 (Table1)

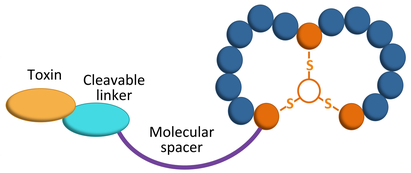



**Table 1: MT1-MMP (MMP-14) Protein Expression in samples**

| | 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)

**Table 2: Affinity and selectivity of the Bicycle® binder**

| 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) |
| 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 |

## METHODS & RESULTS

Figure 1. 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 section (b), and in PDX tumour section (c).

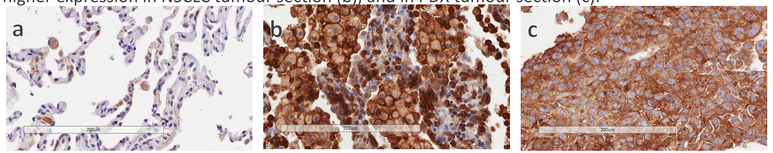



Table 2. The binding affinity of BT1718 to MT1-MMP from human, cyno, rat & mouse, and to related human MMPs was evaluated. BT1718 binds to MT1-MMP via the hemopexin domain, with high affinity and exquisite selectivity. In *in vitro* cytotoxicity assays, BT1718 kills MT1-MMP expressing EBC-1 cells with an $IC_{50}$~1nM.

Figure 2. Efficacy of BT1718 in NSCLC xenograft models. BT1718 was dosed at 1, 3, 10 mg/kg, twice weekly, iv. (a) EBC-1 xenograft, complete clearance of tumour was seen within 14d, with partial efficacy seen at 3mg/kg biw. (b) NCI-H1975 xenograft, complete clearance of tumour was seen by 28d. (c) NCI-H292, tumours were reduced to minimal volume after 28d, and dosing ceased. On relapse animals were re-dosed (when tumours reached ~150mm3). Significant clearance of tumour was seen in mice following re-dosing.

Figure 3. Efficacy of BT1718 in Patient-Derived Xenograft models. BT1718 was dosed at 3 or 10mg/kg twice weekly, iv. Docetaxel was dosed at 20mg/kg once weekly, iv. (a) High MT1-MMP expressing PDX, efficacy seen with 3 & 10mg/kg BT1718, with complete clearance of tumour within 24d at 10mg/kg. Docetaxel shows negligible effect on tumour. (b) High MT1-MMP expressing PDX, efficacy seen with 3 & 10mg/kg, with complete clearance of tumour within 28d at 10mg/kg. Docetaxel shows complete clearance of tumour, though with significant weight loss (>10%). (c) Low MT1-MMP expressing PDX, efficacy not seen with BT1718 at 3 or 10mg/kg, Docetaxel shows negligible effect on tumour, with significant weight loss (>10%).

Figure 4. Using EBC-1 xenograft model, we investigated the target-dependence of BT1718 activity and evaluated a range of dose schedules. (a) Target dependence was evaluated by co-dosing unconjugated peptide binder. BT1718 efficacy was inhibited by co-injection of 100x excess of MT1-binder, but not non-binder, peptide. (b) The same total weekly dose of BT1718 (20mg/kg) is equally efficacious when dosed in one, twice, three or seven injections.

Figure 4

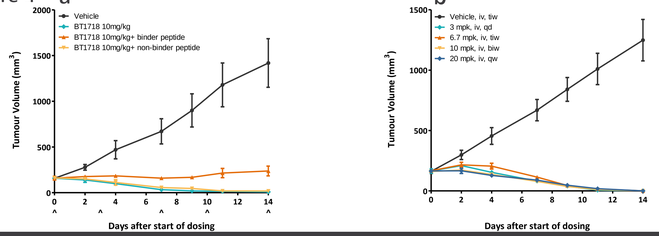
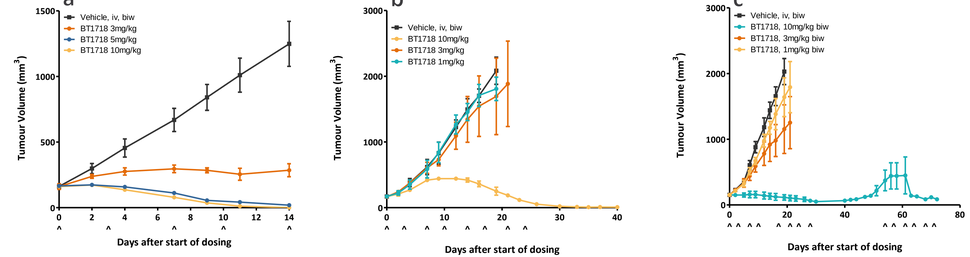
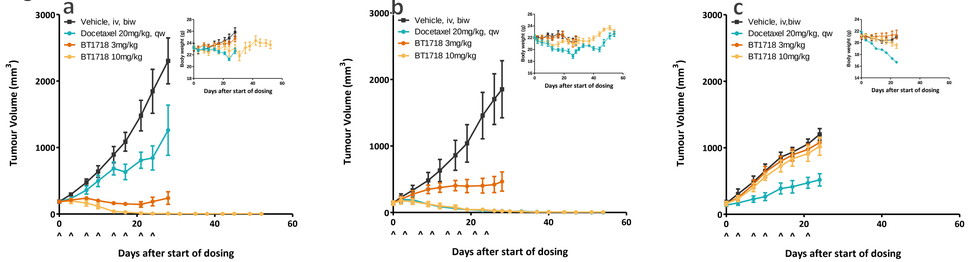



Figure 2




| 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 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 |

## CONCLUSION/SUMMARY

BT1718 is a first-in-class cytotoxic drug conjugate with great potential for treatment of lung cancer

BT1718 shows:

- High affinity & selectivity for MT1-MMP. MT1-MMP is highly expressed in human lung tumours & xenografts, not in normal lung
- Rapid clearance of tumours in a range of MT1-MMP expressing lung cancer models
  - Comparable efficacy in cell line and patient-derived xenografts
  - Efficacy in lines sensitive or resistant to Standard of Care treatment
  - Efficacy dependent on binding to tumour-expressed MT1-MMP

BT1718 is progressing forward to clinical trials in collaboration with Cancer Research UK

Bicycle Therapeutics will be presenting further data on posters 5144 / 16 & 3719/4



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