MT1-MMP Immunohistochemistry (IHC) analysis of tumor microarrays (TMAs) using a novel scoring system guides patient selection for BT1718 expansion cohorts

ABSTRACT# A047

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ABSTRACT

- BT1718 is a targeted Bicycle toxin-conjugate designed to deliver the anti-tubulin agent, DM1 to tumors expressing membrane type 1-matrix metalloprotease (MT1-MMP; MMP14; MT1)
- In vivo preclinical studies demonstrate that anti-tumor activity of BT1718 is dependent on the level of tumor MT1-MMP expression
- In patient tumors MT1-MMP expression has been reported in tumor and stromal cells, both of which may contribute to the potential for anti-tumor effects following BT1718 dosing
- BT1718 is currently being investigated in a Phase 1/2 clinical trial, which includes both dose escalation (ongoing) and dose expansion cohorts
- Expansion cohorts selected for BT1718 Phase 1/2 trial: lung squamous and all-comers basket

INTRODUCTION

MT1-MMP is a membrane-type matrix metalloproteinase (MMP) involved in extracellular matrix degradation

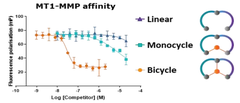
- Established role in cell invasion and metastasis
- Various tumor types have reportedly high MT1-MMP expression including breast, ovarian, lung, and bladder cancer
- MT1-MMP expression is associated with poor outcomes in various tumor types
- MT1-MMP is expressed at low levels in normal tissue
- MMPs are difficult pharmacological targets due to their high sequence similarity and compensatory roles

WHY BICYCLES?

New Class of Therapies

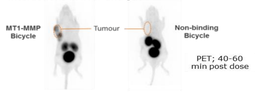
Novel modality delivers high affinity, favourable PK and rapid clearance.

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

- A clinical grade MT1-MMP IHC assay was developed on the Ventana platform using a Millipore MT1-MMP primary antibody (MAB3328) at 1:6000 and detected using Optiview chemistry
- TMAs (n=8) were selected covering multiple cancer indications (Figure 1A) with reportedly high MT1-MMP expression. After staining, MT1-MMP expression levels were estimated by consensus review of two pathologists using an H-score scale (staining intensity*percent positivity). H-scores (0-300) were derived separately for tumor membrane (TM), cytoplasm (TC), and stroma (TS) for each case
- H-score analysis and modeling was performed in R/R Studio (packages: ggplot2, tidyverse, reshape2, naniar)

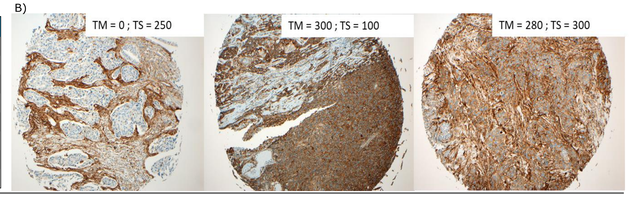
RESULTS *DISTRIBUTION ANALYSIS*

Figure 1: Tumor membrane, tumor cytoplasm and stromal staining of MT1-MMP was quantified for >600 tumor cores

A)

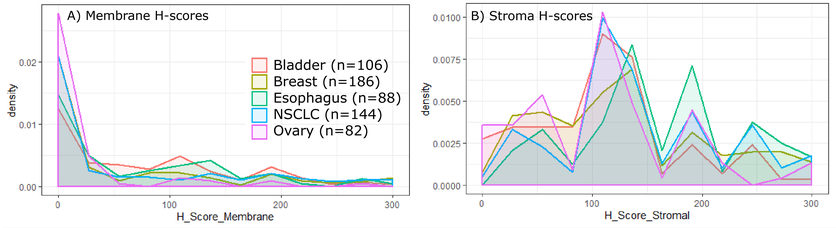
TMA	Indication	N
BC00019	Pan-squam	61
BC11115c	Ovary	81
BL2082a	Bladder	105
BR1901	Breast	92
BR1902	Breast	93
ES1501	Esophageal	25
ES2081	Esophageal	58
LC1921b	NSCLC	139

B)



A) TMAs included in analysis. N=# of cores with TM/TC/TS score. TMAs mostly from primary tumors. B) Representative images of lung cancer cores and corresponding tumor membrane (TM) and tumor stromal (TS) H-scores.

Figure 2: Distribution pattern of MT1-MMP H-scores in tumor membrane and stroma

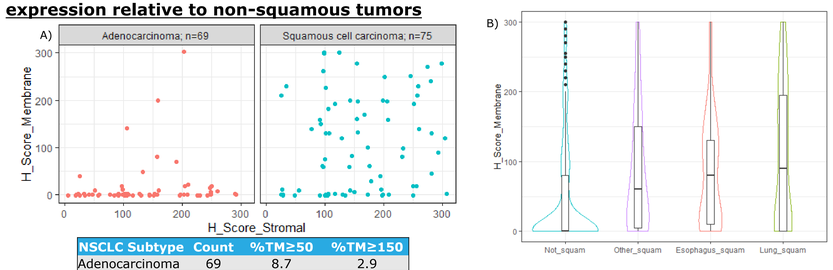


Indication	N	Tumor membrane (%)						Tumor stromal bins (%)					
		0-49	50-99	10-149	150-199	200- 249	250+	0-49	50-99	10-149	150-199	200- 250	250+
Bladder	106	44.3	17.0	18.9	10.4	6.6	2.8	17.0	18.9	33.0	14.2	8.5	8.5
Breast	186	65.6	8.6	8.1	4.3	6.5	7.0	13.4	21.5	23.7	13.4	13.4	14.5
Esophagus	88	53.4	11.4	18.2	11.4	1.1	4.5	5.7	12.5	20.5	18.2	21.6	21.6
NSCLC	144	64.6	7.6	7.6	5.6	7.6	6.9	10.4	8.3	34.0	15.3	14.6	17.4
Ovary	82	89.0	1.2	6.1	1.2	1.2	1.2	19.5	17.1	35.4	7.3	15.9	4.9

Density plots of MT1-MMP tumor membrane (A) and stroma (B) H-scores across 5 indications. Across all indications the most frequent TM H-score bin was 0-49, whereas TS H-scores were more evenly distributed.

RESULTS *SQUAMOUS HISTOLOGY ANALYSIS*

Figure 3: Squamous tumors are enriched for higher MT1-MMP tumor membrane expression relative to non-squamous tumors

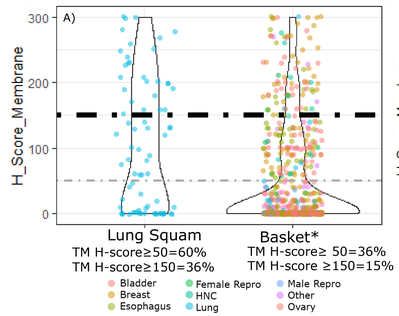


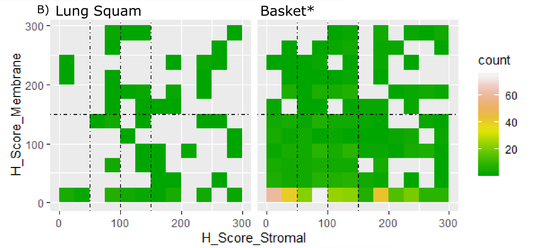
NSCLC Subtype	Count	%TM≥50	%TM≥150
Adenocarcinoma	69	8.7	2.9
Squamous	75	60	36

A) MT1-MMP TM and TS H-scores plotted in NSCLC cancer cores grouped by subtype (left: adenocarcinoma, right: squamous cell carcinoma). B) TM H-scores plotted for 4 groups: lung squamous, esophageal squamous, other squamous (not lung/esophageal), and non-squamous. Boxplots ordered by median TM H-score.

RESULTS *EXPANSION COHORT IDENTIFICATION*

Figure 4: Squamous lung and all-comers basket selected as expansion cohorts

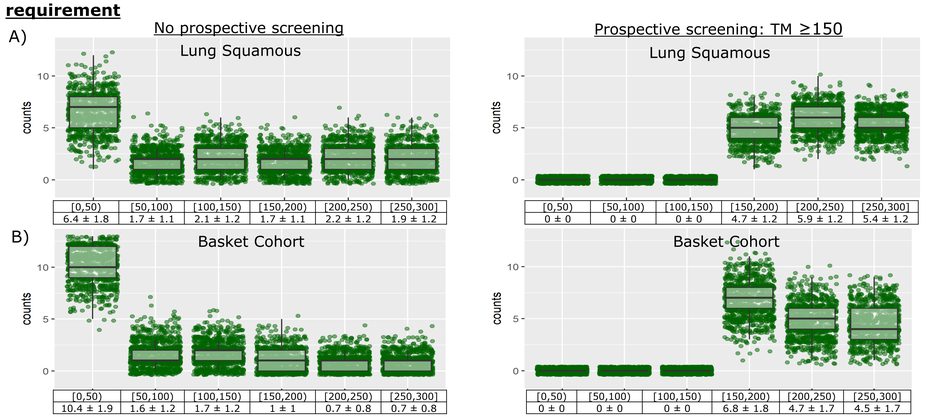




A) MT1-MMP TM H-scores in lung squamous and basket (*all-comers excluding lung squamous) cohorts. Repro= reproductive. B) Distribution (2D) binning of TM and TS H-scores in hypothetical lung squamous and basket cohorts. Horizontal line: TM H-score=150.

RESULTS *RATIONALE FOR PROSPECTIVE SCREENING AT TM H-SCORE≥150*

Figure 5: Modelling of patient enrollment into 2 expansion cohorts testing with TM H-score≥150 requirement



Modeling of patient enrollment into two expansion cohorts (N = 16): lung squamous (A) and basket solid tumor cohort excluding lung squamous (B) with and without prospective screening. TMA data was sampled 1000X randomly to predict the TM H-score distribution expected in cohorts of N=16. In the prospective screening setting cores with a TM H-score<150 were excluded.

CONCLUSION/SUMMARY

- An indication and patient selection strategy was developed for BT1718 using a novel IHC scoring method that determines MT1-MMP expression levels on tumor sub cellular compartments.
- Expansion cohorts with prospective selection were selected for the BT1718 Phase 1/2 trial to increase the likelihood of enrollment of patients with tumors that have moderate to high MT1-MMP expression.
- Enrollment to expansion cohorts will commence following establishment of the once weekly BT1718 RP2D; dose escalation is ongoing.