Bicyclic Peptides for Positron Emission Tomography (PET) Imaging of MT1-MMP Expressing Tumors

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ABSTRACT# 4920

bicycle therapeutics

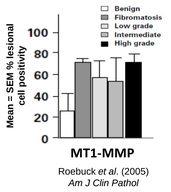
ABSTRACT

- *Bicycles®* are phage display derived bicyclic peptides constrained via a chemical scaffold
- This constraint stabilises the *Bicycle,* energetically favour positive binding conformations, and drives high affinity binding to the designated target whilst retaining excellent selectivity to the rest of the proteome
- *Bicycles* with K_D of around ~1.5 nM were identified, selectively binding tumor associated MT1-MMP (matrix metalloproproteinase 14)
- Radiolabelled DOTA *Bicycle* conjugates (^{68}Gallium or ^{177}Lu) exhibited selective tumor localization *in vivo* and rapid clearance of non-tumor bound peptide via the kidney/bladder, with liver metabolism bypassed
- Chemical stabilisation of *Bicycles* to serum proteases enhanced tumor accumulation

INTRODUCTION

MT1-MMP (MMP14)

- Cell-surface expressed metalloproteinase
- Plays a central role in cancer cell invasion and motility[1]
- Overexpressed in many cancers including triple negative breast, non-small cell lung, and soft tissue sarcoma
- Expression correlates with disease progression and poor patient outcome, and is associated with tumor metastasis[2-4]
- Not highly expressed in healthy tissue



Roebuck *et al.* (2005) *Am J Clin Pathol*

***Bicycles®* - A new therapeutic and diagnostic modality**

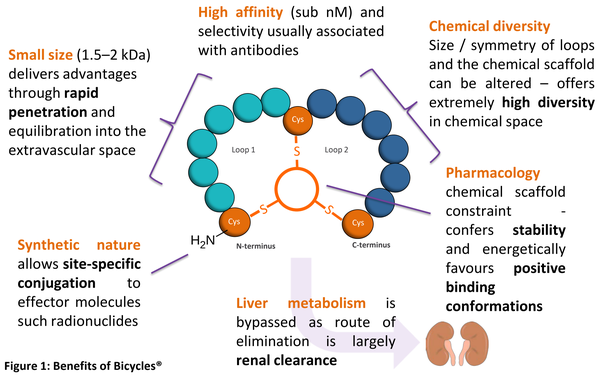


Figure 1: Benefits of Bicycles®

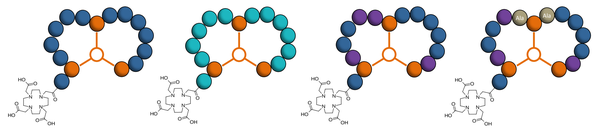
[1] Hofmann *et al.* (2000) *Journal of pathology 191*, 245-56
[2] Wu *et al.* (2014) *Tumour Biol* 35, 12489-95
[3] Shaverdashvili *et al.* (2014) *Pigment cell & melanoma research* 27, 287-96
[4] Shaverdashvili *et al.* (2015) *Oncotarget* 6, 33512-22
[5] Heinis *et al* (2009), *Nat Chem Biol* 5(7), 502-507

METHODS

- 6x6 *Bicycle* phage libraries were generated as described previously[5] and used to screen the hemopexin domain of MT1-MMP. Positive binders were subsequently identified using pyrosequencing & characterised via ALPHAscreen® binding assays
- Peptide synthesis was based on Fmoc chemistry, using a Symphony peptide synthesiser manufactured by Gyros Protein Technologies
- DOTA was coupled to the peptide chain during solid phase peptide synthesis using the protected precursor DOTA(tBu)$_3$
- Affinity and selectivity of Bicycles was measured using fluorescence polarisation (FP)
- ^{68}Ga (positron β+ emitter) and ^{177}Lu (γ and β emitter) labelling was performed by quantitative chelation at higher temperature (95 C, 10 min).
- For µPET imaging, mice were anaesthetized, placed in a small animal PET scanner (Inveon PET, Siemens) and injected with ^{68}Ga-labeled Bicycle peptides. Images were reconstructed iteratively and were converted to standardized uptake value (SUV) images. Quantitation was done using a ROI (region of interest) technique and expressed as SUVmean
- For organ distribution the ^{177}Lu-radiolabeled peptide was injected via the tail vein of Balb nu/nu xenograft mice (50 kBq per mouse) transplanted with the respective cell line. At indicated time points p.i the animals were sacrificed and organs and tumor harvested. The radioactivity was measured with a γ-counter along with a sample of injection solution to calculate the percentage injected dose per gram of tissue (%ID/g)

RESULTS

DOTA Conjugated *Bicycle* Peptides



Bcy1	Bcy2	Bcy3	Bcy4
Initial Hit	Non-binder	Optimised Hit	Non-binder
	All D-amino acids	*Stabilised with Non natural amino acids*	*Double Alanine Analogue of Bcy3*

Figure 2: Pictorial representation of DOTA conjugated Bicycle® peptides

Peptide	K_D (nM) (Hemopexin domain)[a]	$t_{1/2}$ (hrs) (human plasma)[b]	$t_{1/2}$ (hrs) (mouse plasma)[b]
Bcy1	0.51 ± 0.03	30.3 ± 4.7	3.9 ± 0.3
Bcy2	>5000	NT	NT
Bcy3	0.52 ± 0.24	> 36	> 36
Bcy4	>5000	NT	NT

[a]Determined by fluorescence polarisation competition experiments
[b]Determined using quantitative LC-MS. Incubation time up to 24 hrs in plasma, containing 4 µM BDC. Based on acetylated, non DOTA conjugated peptide precursor.

Table 1: Affinity and *in vitro* half life of Bicycle® peptides

Binding and Internalization of DOTA Conjugated Bicycle® *in vitro*

Cell surface binding and internalisation of Bcy1 were determined on MT1-MMP+ HT1080 cells and non-expressing cell lines (DU-145, MBA-231, MCF-7). Bcy1 peptide was loaded with ^{177}Lu using standard complexation techniques.

- Bcy1 binds to the cell surface of MT1$^+$ HT1080 cells, but not to a range of MT1$^-$ cell lines (Fig 3)
- A small percentage of the total signal is recovered from cell lysates The higher signal at 37°C versus 4°C suggests energy dependent active uptake mechanism.

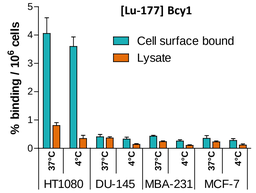


Figure 3: Radioactivity from Bcy1 released from cell surface and cell lysates in MT1-MMP+ and - cell lines

***In vivo* bio-distribution and selectivity of Bcy1 vs Bcy2**

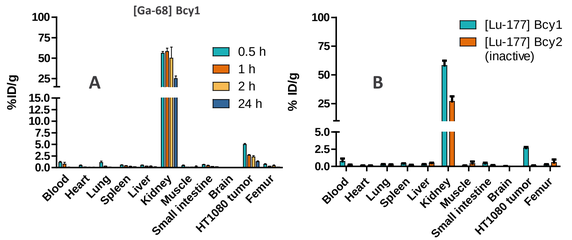


Figure 4: *(A)* Organ distribution profile of Bcy1 over 24 hours after dosing 150 pmol/mouse. The peptide is present for up to 24 hours in the tumor, with levels slowly decreasing from 0.5 hrs post injection. *(B)* direct comparison of Bcy1 (binder) and Bcy2 (non-binder) at 1 h p.i. at a dose of 150 pmol. Bcy2 does not accumulate in the tumor, confirming that the tumor signal for Byc1 is driven by MT1-MMP target binding.

Chemical Stabilization of Bcy1 produces optimized Bcy3, showing higher tumor uptake

- Stability of the core bicyclic sequence Bcy1 *in vitro* in mouse plasma was determined to be approximately 4 hours, whilst *in vivo* mouse PK studies indicated a half-life of approximately 14 min
- The mouse clearance rate exceeds the glomerular filtration rate for hydrophilic molecules, suggesting *in vivo* proteolytic instability
- Therefore, chemical optimisation was used to stabilise Bcy1 against proteases resulting in the production of Bcy3, which is retained the binding affinity of Bcy1 to the target but exhibited increased *in vitro* plasma stability (Table 1)

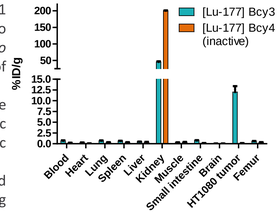


Figure 5: Stabilised Bicycle Bcy3 which shows a >3 fold greater tumor signal than non-stabilized bicycle Bcy1. Accumulation of Bcy3 in the tumor remains selective, compared to an inactive Ala/Ala mutant

PET imaging (40-60 mins)

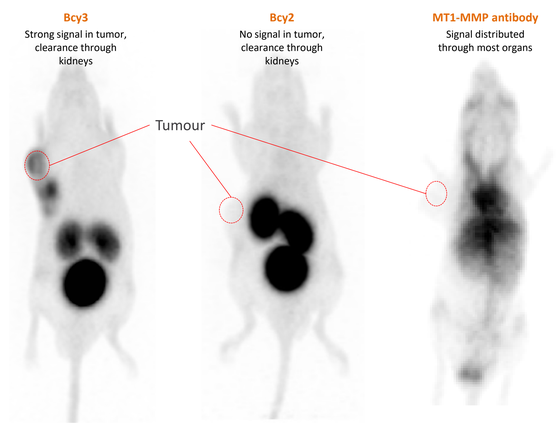


Figure 6: Rapid and target specific localization of Ga68-labelled Bcy1 to an MT1-MMP expressing tumour was observed. Non-binding Bcy2 does not localize to the tumour. Free labelled bicycle is only observed in the kidney and bladder consistent with renal elimination. The antibody shows no tumour penetration, and significant non-MT1-MMP1 expressing tissue accumulation (mostly liver)

- Rapid accumulation of Bcy3 was seen in the HT1080 tumor at 1hr (Figure 5)
- Tumour loading was higher with stabilised Bcy3 (>3x) than with un-stabilised Bcy1 (Fig 4B vs 5)
- Tumour accumulation was pharmacologically driven by affinity to the designated target (MT1-MMP) as shown by the absence of tumour loading with a non-binding control (Fig 5, 6).
- The distribution of a ^{68}Ga labelled anti MT1-MMP antibody showed marked differences to that of a *Bicycle* (Fig 6); accumulating in most organs
- *Bicycle* agents have potential utility in clinical imaging, delivering excellent signal to background contrasts and fast renal clearance

CONCLUSION/SUMMARY

- Radiolabelled *Bicycles* Bcy1 and Bcy3 demonstrated rapid tumor-specific target-mediated uptake and rapid renal clearance of un-bound *Bicycle* in HT1080 MT1-MMP+ mouse xenograft models in µPET studies
- Proteolytic stabilisation of the *Bicycle* enhanced tumor uptake
- *Bicycles* have great potential as diagnostic clinical imaging agents in the stratification and therapeutic management of patients

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

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