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Bicycle Radionuclide Conjugates (BRCs) as agents for tumour targeting

Abstract #

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ABSTRACT

USING THE BICYCLE PHAGE DISPLAY PLATFORM TO DESIGN NOVEL PEPTIDE BASED RADIOLIGANDS

- Targeted Radionuclide Therapy (TRT) is emerging as a promising therapeutic approach for cancer treatment. TRT is centered on delivering a cytotoxic radioactive payload to cancer cells via target receptors on the membrane.
- Membrane type 1 matrix metalloproteinase (MT1-MMP) is overexpressed in many solid tumours such as breast and non-small cell lung cancer making it a high value target for cancer therapy.[1,2]
- Using Bicycle's proprietary phage platform, bicyclic peptides with high affinity to MT1-MMP were identified, optimized and incorporated in Bicycle Radionuclide Conjugates (BRCs) for diagnostic imaging and TRT.

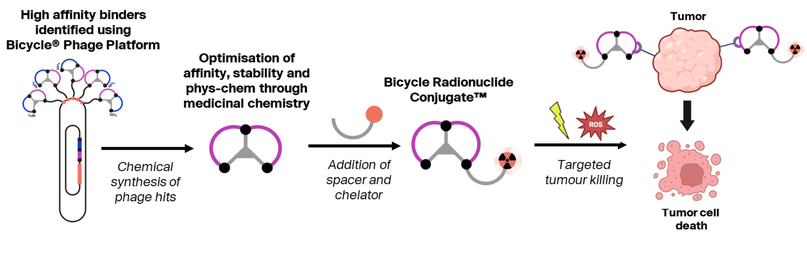

Figure 1: Overview of identification and design of Bicycle Radionuclide Conjugate for TRT using Bicycle's proprietary phage platform.

INTRODUCTION

- Bicycle® molecules are short linear peptides stabilized by a central chemical scaffold.
- The scaffold constrains the peptide in its bioactive form, resulting in high affinity whilst also imparting stability compared to their linear counterparts.
- The small size (1-3kDa) enables rapid penetration in tumours, allowing rapid delivery of payload.
- The relatively large binding footprint allows for exquisite selectively to close analogues of target protein.
- Bicycle® molecules have a short biological half-life, which allows fast clearance from circulation. This spares healthy tissue from prolonged radiation exposure, making Bicycle® molecules an ideal modality for targeted radionuclide delivery.
- Due to their fast clearance from circulation and rapid penetration in tumours at early timepoints, BRCs are well suited for both cancer diagnosis (through imaging) and therapy and can be applied in the new emerging field of thernaostics.

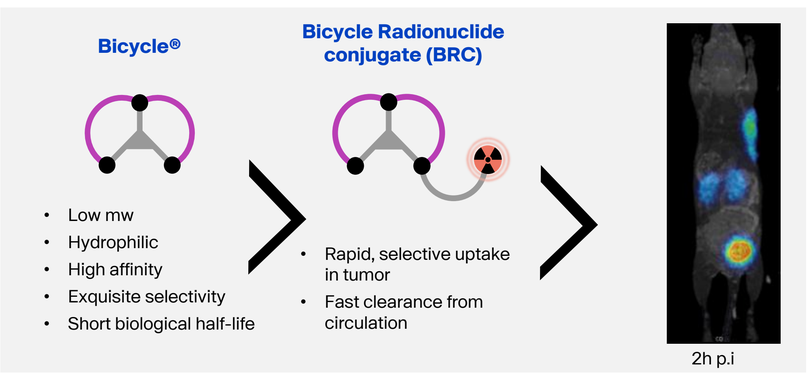

Figure 2: Left: Properties of Bicycle® molecules that render them most suitable for radioactive payload delivery. Right: PET image of a MT1-MMP targeting ^{68}Ga-BRC in a HT1080 tumour carrying mouse at 2h p.i.

MT1-MMP AS A TARGET FOR RADIOTHERANOSTIC APPROACH IN CANCER

- Membrane type 1 matrix metalloproteinase (MT1-MMP) plays a role in cancer metastasis and overexpression in solid tumours such as non-small cell lung cancer, esophageal and triple negative breast cancer.
- Early positron emission tomography (PET) imaging in preclinical models highlighted the promise for MT1-MMP as a target for cancer diagnosis and potential therapy.[3]
- In this study BRCs targeting MT1-MMP were optimised to selectively deliver high levels of radioactivity to tumours whilst minimising uptake in healthy tissue.

METHODS

STUDY APPROACH

- PET imaging to assess selectivity and biodistribution of MT1-MMP targeting BRCs in a mouse tumour xenograft model.
- Affinity improvement through structural activity relationship (SAR) exploration and co-crystal structure guided design.
- In-vitro profiling of BRCs in cellular uptake assays to measure internalisation.
- Iterative rounds of medicinal chemistry design to optimize the biodistribution profile to increase tumour uptake and tumour-to-tissue ratios.

RESULTS

PET IMAGING OF EARLY MT1-MMP TARGETING BRC SHOWS SELECTIVE UPTAKE INTO MT1-MMP EXPRESSING TUMOUR

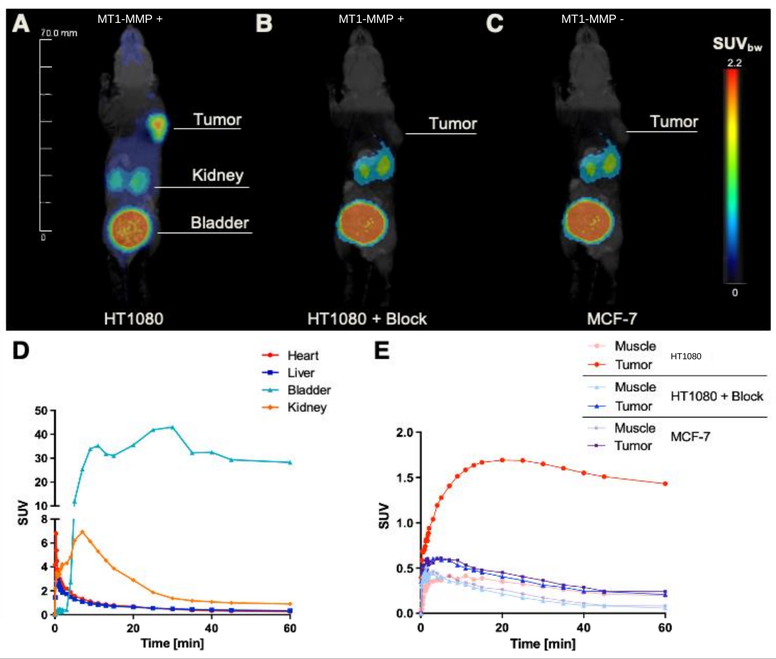

Figure 3:Whole-body maximum intensity projections of 150 pmol ^{68}Ga-labeled BCY1 in HT1080 (A, B) and MCF-7 (C) tumor-bearing BALB/c nu/nu mice (right flank) 60 min p.i. obtained from small animal PET/MR imaging. Blocking experiments (B) were performed with an excess of non-labeled peptide (30 nmol) 5 min prior to radiotracer administration. (D) Corresponding time activity curves (TAC) for organs of interest from 0 to 60 min p.i. of 150 pmol of [^{68}Ga]Ga-BCY1 in the HT1080 xenograft. (E) Detailed TACs of tumor and muscle tissue of all animals.

^{177}Lu LABELLED BRC IS RETAINED IN TUMOUR OUT TO LATER TIMEPOINTS

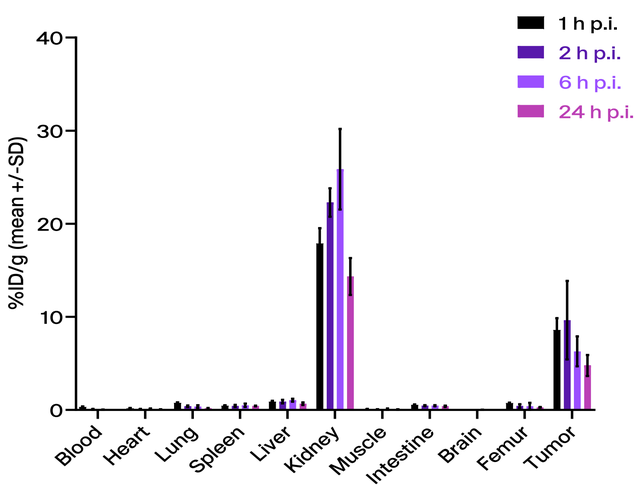

Figure 4:Organ distribution of 150 pmol ^{177}Lu-labeled BCY at 1, 2, 6 and 24 h p.i. in HT1080 mouse xenograft. Data are expressed as mean % ID/g tissue ± SD (n=3)

CO-CRYSTALLISATION WITH MT1-MMP PROTEIN

- A co-crystal structure of MT1-MMP protein and bicyclic peptide was obtained using co-crystallisation techniques and analysed using x-ray crystallography.
- This structural information was used to study molecular interactions and guide chemical optimisation.

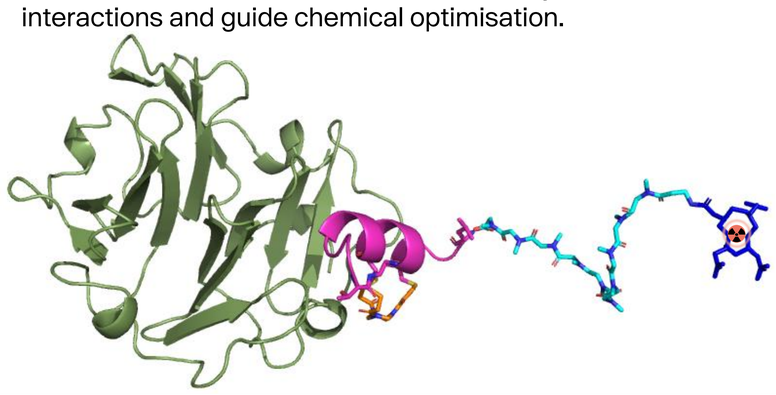

Figure 5: Illustration of Bicycle Radionuclide Conjugate binding to MT1-MMP protein in green (derived from Bicycle co-crystal structure)

IMPROVING BRC AFFINITY FOR MT1-MMP THROUGH SAR EXPLORATION

- Optimisation to improve binding kinetics with the aim of increasing tumour uptake and retention.
  - >100 compounds were designed and synthesised
  - Highly potent binders with affinity (K_D) of 20 pM and off-rate of 1.3E-4 s^{-1} were identified

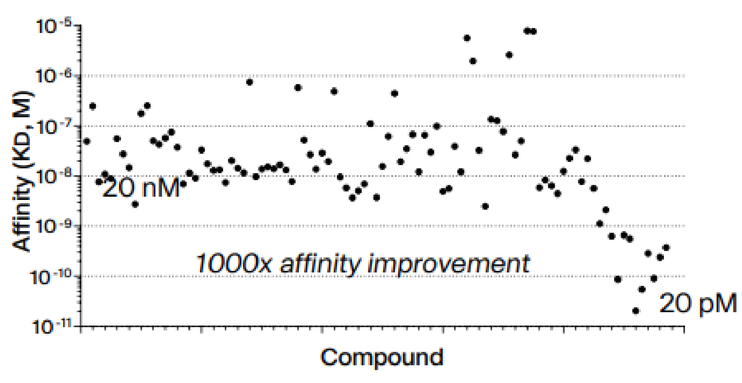

Figure 6: Graph showing affinity (K_D, M) of compounds for MT1-MMP over course of optimisation

BINDING KINETICS OF HIGH AFFINITY BINDERS WITH SLOW DISSOCIATION

- Bicycle® molecules can bind to target protein with high affinity (K_D) and slow dissociation (Kd) and have comparable binding kinetics to monoclonal antibodies.
- Slow dissociation rate (Kd) of BRCs in the range of 1.3E-4 s^{-1} could result in long residence time at the tumour receptor

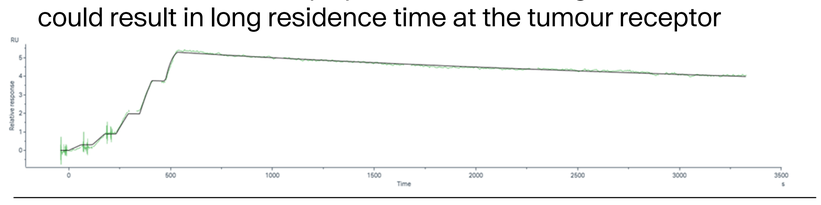

Figure 7: Bicycle® molcule binding to human MT1-MMP characterized using a 5-point 2-fold titration up to 10nM; fitted with a 1:1 binding model

MT1-MMP BRCS SHOW RAPID INTERNALISATION IN IN-VITRO CELLULAR ASSAYS

- Internalisation of Bicycle® molecules into MT1-MMP positive cells were assessed using both fluorescence imaging as well as gamma counting of radioactivity.
- MT1-MMP expressing cells after incubation with fluorophore conjugated bicycle molecules were imaged using confocal microscopy. Pink punctate signal indicates internalised bicycle fluorophore conjugate.
- ^{177}Lu-BRCs were incubated with HT1080 cells and internalised fractions were collected and radioactivity measured using a gamma counter.
- High levels of internalisation into both cell lines were observed.

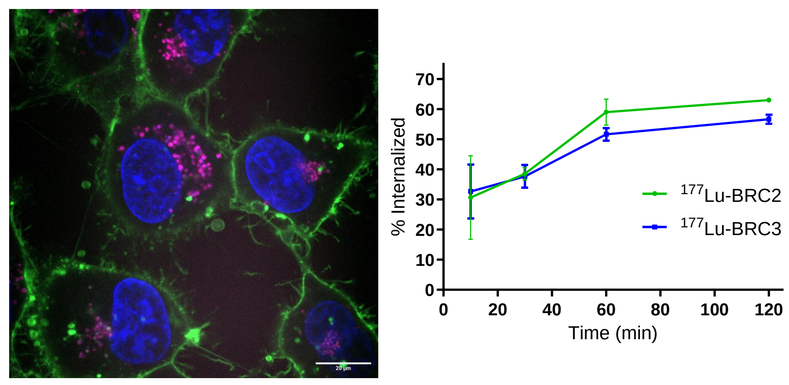

Figure 8: Left: MT1-MMP expressing cells incubated with Bicycle fluorophore conjugate (Alexa Fluor 647, red) at 100 nM concentration for 4 hours, washed, the nuclei counterstained with Hoechst (blue) and the cell membrane counterstained with CellMask (green). Images of live cells taken on an Olympus IX53 using a 100X objective. Right: % Internalisation of MT1-MMP targeting ^{177}Lu-BRCs in HT1080 cells over 120 minutes post incubation.

OPTIMISATION OF BIODISTRIBUTION PROFILE OF BRCS

- Chemical optimisation led to BRCs with increased tumour uptake and retention, along with reduced kidney uptake / retention.
- Medicinal chemistry can be used to optimise the in-vivo biodistribution profile of BRCs.

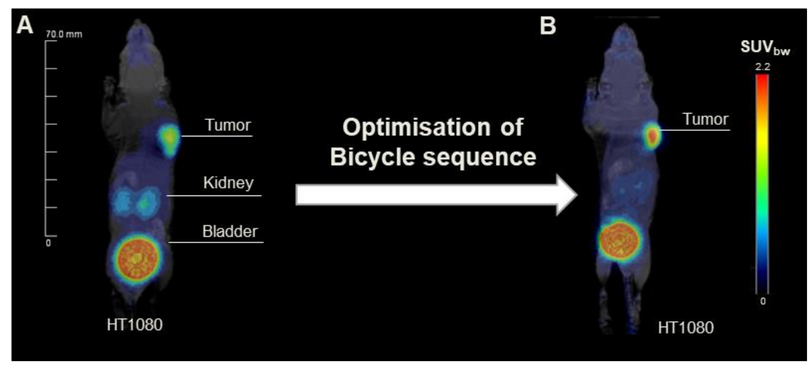

Figure 9:Whole-body maximum intensity projections of 150 pmol ^{68}Ga-labeled BRC1 (A) and optimized BRC4 (B) in HT1080 tumor-bearing BALB/c nu/nu mice (right flank) 60 min p.i. obtained from small animal PET/MR imaging.

CONCLUSIONS

HIGH TUMOUR UPTAKE, LOW HEALTHY TISSUE UPTAKE

- Bicycle® molecules are suitable vectors for delivering radionuclides to tumours due to their favourable pharmacokinetic properties (specific tumour uptake, rapid penetration and rapid renal clearance).
- Due to their rapid clearance from blood circulation and minimal non-specific uptake into healthy tissue (apart from kidneys), they can be used effectively as diagnostic PET agents.
- The biodistribution profile of the Bicycle Radionuclide Conjugates (BRCs) can be optimised to maintain high tumour uptake whilst significantly reducing kidney levels.
- BRCs emerge as promising agents for a theranostic approach.

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German Cancer Consortium
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