Bicycle® Radionuclide Conjugates for radioisotope delivery to solid tumors

Poster #

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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, de novo binders to important targets in oncology can be identified with ideal properties for radioisotope delivery[3,4,5]
- In this example, bicyclic peptides with high affinity to MT1-MMP were identified, optimized and incorporated in Bicycle® Radionuclide Conjugates (BRCs) for imaging and TRT.

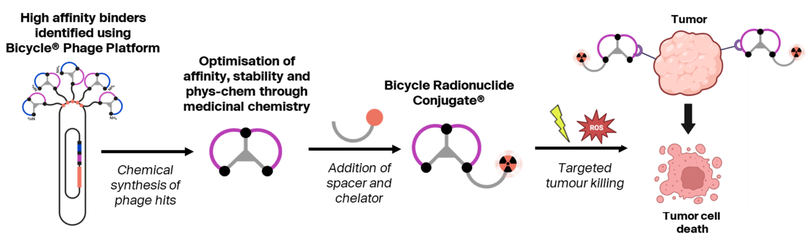

Figure 1: Overview of identification and design of Bicycle Radionuclide Conjugate® for TRT using Bicycle's proprietary phage platform.

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 is anticipated to spare 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 developed in the emerging field of theranostics.

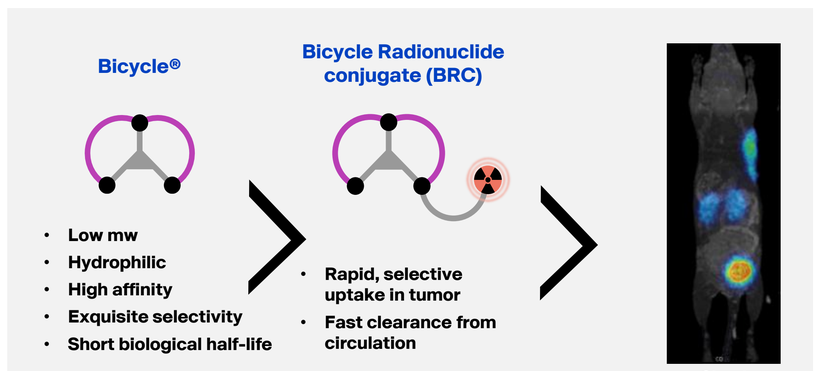

Figure 2: Left: Properties of Bicycle molecules that render them most suitable for radioactive payload delivery. Right: PET image of a MT1-MMP targeting ^{68}Ga-BRC in a HT1080 tumour bearing mouse model at 2h p.i.

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[1,2].
- Early positron emission tomography (PET) imaging in preclinical models highlighted the promise of MT1-MMP as a target for cancer diagnosis and potential therapy[5].
- 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

CO-CRYSTAL STRUCTURE OF MT1-MMP BINDING BICYCLE® PEPTIDE

- A co-crystal structure of MT1-MMP protein and bicyclic peptide was obtained
- This structural information was used to study molecular interactions and guide chemical optimisation

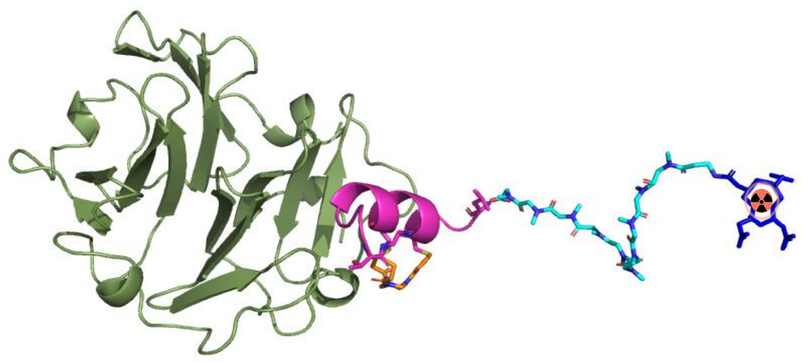

Figure 3: Bicycle Radionuclide Conjugate® binding to MT1-MMP protein in green (derived from Bicycle® co-crystal structure, linker and chelator added as illustration)

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

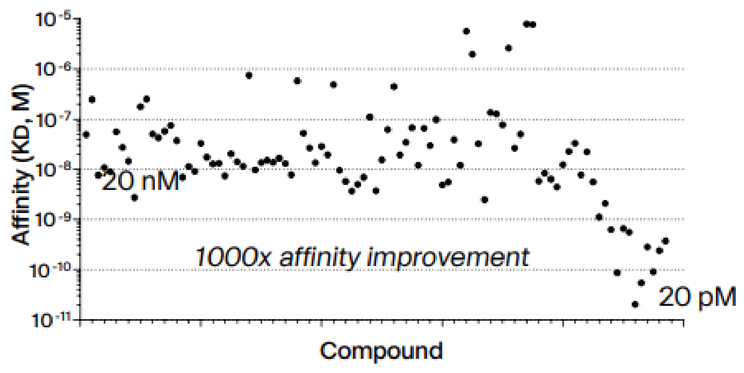

Figure 4: Graph showing affinity (K_D, M) of compounds for MT1-MMP over course of optimisation

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

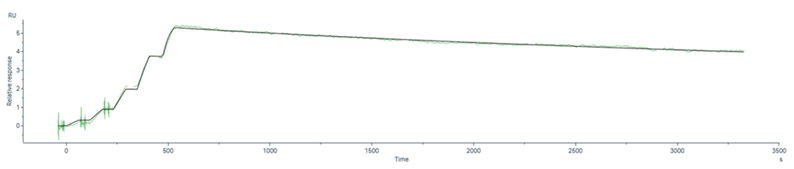

Figure 5: Bicycle® molecule binding to human MT1-MMP characterized using a 5-point 2-fold titration up to 10nM; fitted with a 1:1 binding model

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

- Internalisation of Bicycle® molecules into MT1-MMP positive cells was assessed using fluorescence imaging and gamma counting.
- 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 in both assays were observed

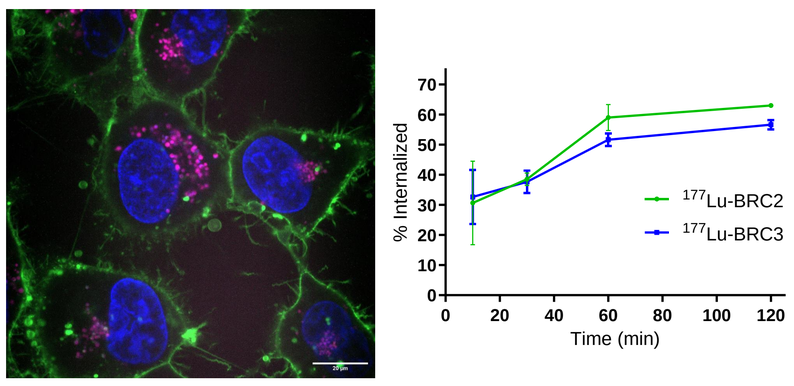

Figure 6: Left: MT1-MMP expressing cells incubated with Bicycle fluorophore conjugate (Alexa Fluor 647, red) at 100 nM concentration for 4 hours, washed, the nuclei counterstained with Hoechst (blue) and the cell membrane counterstained with CellMask (green). Images of live cells taken on an Olympus IX53 using a 100X objective. Right: % Internalisation of MT1-MMP targeting ^{177}Lu-BRCs in HT1080 cells over 120 minutes post incubation.

OPTIMISATION OF BRC® BIODISTRIBUTION PROFILE

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

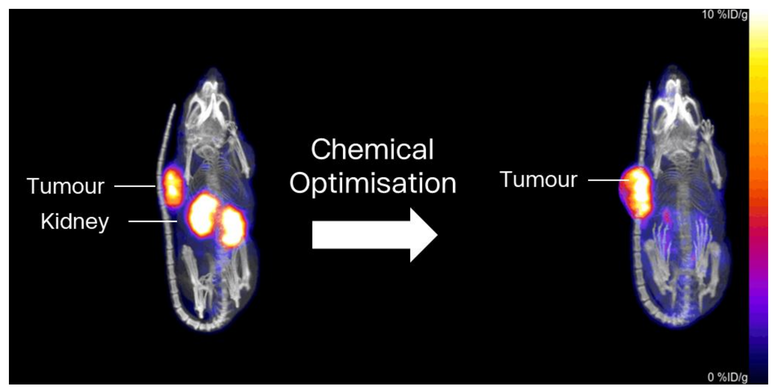

Figure 7: SPECT/CT images of early ^{111}In-labeled BRC showing high kidney uptake (left) and optimized ^{111}In-labeled BRC with much reduced kidney uptake (right) in HT1080 tumor-bearing athymic nude mice 24 hours p.i.

CONCLUSIONS

- Bicycle® molecules are suitable vectors for delivering radionuclides to tumours due to their favourable properties, including specific tumour uptake, rapid tumour penetration and rapid renal elimination.
- The biodistribution profile of the Bicycle Radionuclide Conjugates (BRCs) can be optimised to maintain high tumour uptake whilst significantly reducing kidney levels, as exemplified in this work using Indium-111 labelled BRCs targeting MT1-MMP.
- These data, coupled with previously disclosed preclinical data demonstrating the utility of BRCs labelled with Lead-212, highlight the potential for BRCs to be developed using a range of isotopes.
- BRCs emerge as promising agents for a theranostic approach.

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