Development and clinical translation of a phage display-derived MT1-MMP-specific bicyclic peptide for radiotheranostic applications

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Background and Objective

Membrane type 1 matrix metalloproteinase (MT1-MMP) is a pivotal enzyme involved in extracellular matrix remodeling, contributing to tumor invasion, metastasis, and poor prognosis in various cancers, including non-small cell lung, urothelial, pancreatic, gastric, and breast cancers[1,2]. This study outlines the preclinical development and first in-human application of a phage display-derived MT1-MMP-specific bicyclic peptide, [^{68}Ga]Ga-BCY25286 (Figure 1), as a radiotheranostic agent for PET/CT imaging.

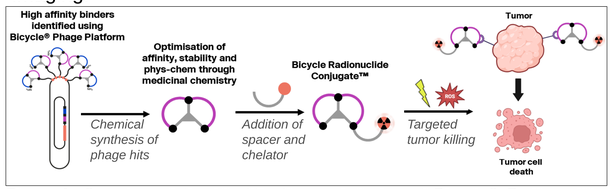

Figure 1: Overview of the identification and design of Bicycle® Radionuclide Conjugates (BRCs).

Materials & Methods

BCY25286 was radiolabeled with Ga-68 or Lu-177 and characterized for stability, binding affinity, and internalization, with preclinical evaluation including biodistribution and µPET/MR imaging in MT1-MMP$^+$ HT1080 and MT1-MMP$^-$ MCF-7 xenograft tumor-bearing nude mice xenograft models. In addition, imaging was performed in two patients: a 65-year-old with pulmonary adenocarcinoma underwent sequential PET/CT imaging with 270 MBq [^{18}F]FDG and 256 MBq [^{68}Ga]Ga-BCY25286, and an 84-year-old female with both invasive ductal breast cancer (G2) and high-grade urothelial cancer (G3) received [^{68}Ga]Ga-BCY25286 PET/CT imaging 60 minutes post-injection of 207 MBq, with a prior contrast-enhanced CT for comparison (ethical approval #25-1019-S1).

Results

Radiolabeling achieved >99% radiochemical purity for both radionuclides. [^{68}Ga]Ga-BCY25286 demonstrated highly MT1-MMP-specific binding (7.2 ± 1.6 nM), proteolytic stability up to 72 hours, and rapid background clearance, thereby enhancing imaging contrast within 60 minutes (Figure 2).

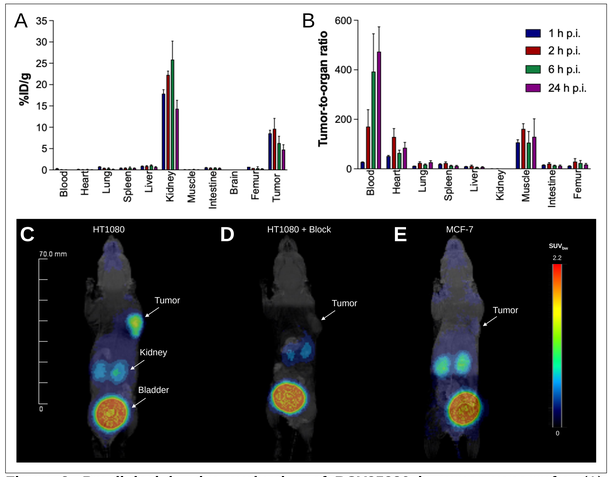

Figure 2: Preclinical *in vivo* evaluation of BCY25286 in tumor xenografts. (A) Organ distribution of 150 pmol ^{177}Lu-labeled BCY25286 at 1, 2, 6 and 24 h p.i., and (B) corresponding tumor-to-organ ratios in HT1080-tumor bearing BALB/c nu/nu mice. Data are expressed as mean % ID/g tissue ± SD (n=3). Whole-body maximum intensity projections of 150 pmol ^{68}Ga-labeled BCY25286 in HT1080- (C, D) and MCF-7- (E) tumor-bearing BALB/c nu/nu mice (right flank) 60 min p.i. obtained from small animal PET/MR imaging. Blocking experiments (D) were performed with an excess of non-labeled peptide (30 nmol) 5 min prior to radiotracer administration.

In mice, the tracer demonstrated high tumor uptake (10.6 ± 1.1 %ID/g at 1 h p.i.) with persistence up to 24 hours (Figure 2). In the first clinical case of a patient with advanced adenocarcinoma of the lung, [^{68}Ga]Ga-BCY25286 PET/CT imaging revealed high uptake in both primary tumour, biopsy-confirmed lymph node metastases at mediastinal levels 2R and 4R and distant metastases, corroborating the findings of [^{18}F]FDG-PET (Figure 3). While MT1-MMP-PET demonstrated lower SUV_{max} values in the primary tumor as compared to [^{18}F]FDG-PET (SUV_{max} 6.0 g/ml vs. 10.3 g/ml), SUV_{max} values in lymph node metastases were comparable (SUV_{max} 4.7 g/ml vs. 4.4 g/ml) and SUV_{max} values in bone metastases were higher in MT1-MMP-PET than in [^{18}F]FDG-PET (SUV_{max} 7.9 g/ml vs. 6.0 g/ml), with significant kidney retention due to renal excretion.

In the second patient with two malignancies (invasive ductal breast cancer G2 and high-grade urothelial cancer G3), the MT1-MMP-targeted PET scan revealed higher tracer uptake

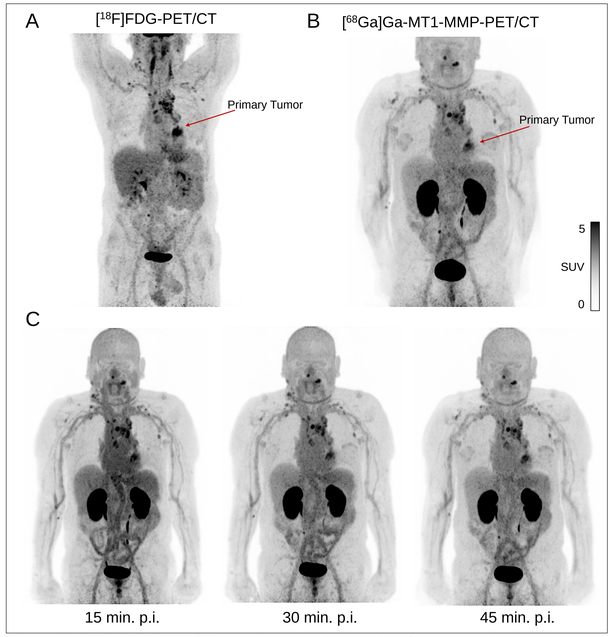

Figure 3: MT1-MMP-PET/CT imaging in advanced pulmonary adenocarcinoma. Maximum intensity projections of [^{18}F]FDG-PET/CT (A) and [^{68}Ga]Ga-BCY25286 PET/CT at 60 mins (B) and at early time points (C) post injection.

compared to local background in the primary breast cancer (SUV_{max} 4.5 g/ml) and bladder cancer (SUV_{max} 6.6 g/ml) (Figure 4). One right iliac lymph node metastasis (SUV_{max} 6 g/ml), bone lesions in the left sacral bone and skull (SUV_{max} 10.8 and 4.2 g/ml), and a left-sided adrenal mass (SUV_{max} 6.4 g/ml) were detected. Within the last 1.5 months, the sacral lesion (from 20 x 21 mm to 34 x 35 mm) and the adrenal mass (from 22 x 15 mm to 31 x 25 mm) showed rapid growth, suggesting malignancy (most likely bladder cancer metastases). Physiological tracer distribution was dominated by vascular activity (left ventricle SUV_{mean} 2.4 g/ml) and renal excretion of the tracer. Liver SUV_{mean} was 2.1 g/ml. Palliative cystectomy confirmed urothelial carcinoma of the bladder with regional lymph node metastasis, MT1-MMP-specific immunohistochemistry verified membranous or stromal expression aligning with PET findings.

To date, 12 out of 14 patients with various indications, including lung, CUP head and neck, and urothelial cancers, undergoing MT1-MMP PET imaging have demonstrated increased tracer uptake in primary and/or metastatic lesions (defined as SUV_{max} in tumor or metastasis > SUV_{mean} left ventricle + 2 standard deviations). Ongoing analysis focuses on more detailed analysis and correlation of findings to IHC.

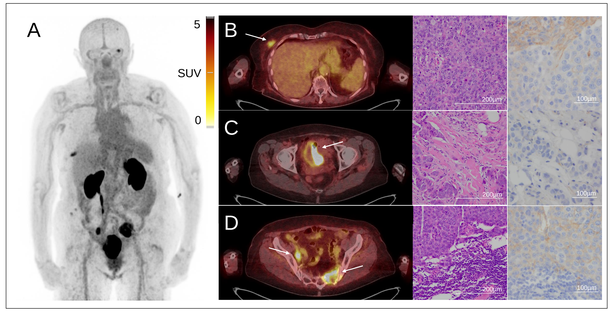

Figure 4: MT-1-MMP-PET/CT imaging in breast and urothelial cancer. Maximum intensity projection of [^{68}Ga]Ga-BCY25286 PET imaging (A) with representative axial PET/CT fusion slices (B-D) and corresponding immunohistochemistry staining (H&E, MT1-MMP-specific) showing the primary breast cancer (B) and bladder cancer (C) with both lymph node and bone metastases in the left sacral bone (D; white arrows). Immunohistochemistry confirmed membranous or stromal MT1-MMP expression in the primary breast cancer, bladder cancer and lymph node metastasis.

Conclusion

This first-in-human application of MT1-MMP-targeting [^{68}Ga]Ga-BCY25286 demonstrates the feasibility for visualization of MT1-MMP-expressing primary tumors and metastases, which is in line with the preclinical findings. These initial clinical results support further investigation of [^{68}Ga]Ga-BCY25286 as a diagnostic tool with potential to improve tumor characterization and patient management strategies in MT1-MMP-positive cancers.

Disclosure

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