

Development and first clinical experiences of a phage display derived bicyclic peptide for EphA2-specific PET imaging

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Background and Objective

Erythropoietin-producing hepatocellular receptor A2 (EphA2) is a tyrosine kinase receptor overexpressed in multiple solid tumors including pancreatic, bladder, head and neck, breast, colon, prostate, and lung cancers. EphA2 is associated with aggressive phenotypes. Following successful preclinical optimization of a phage display-derived EphA2-specific bicyclic peptide¹ (Figure 1), this work outlines the first in-human use of EphA2-targeting [⁶⁸Ga]Ga-BCY18469 in PET/CT imaging.

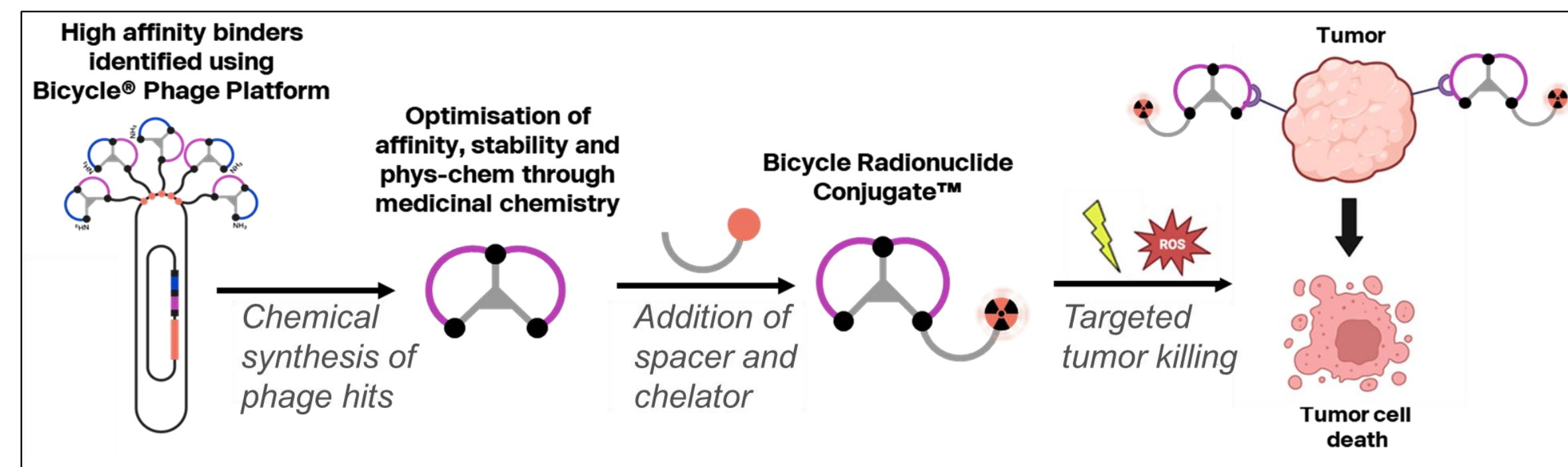


Figure 1: Overview of the identification and design of Bicycle® Radionuclide Conjugates (BRCs).

Materials & Methods

Preclinical characterization of BCY18469 included stability, binding affinity, internalization, biodistribution, and μ PET/MR imaging in EphA2+ HT1080 and EphA2- MCF-7 xenograft tumor-bearing nude mice. For clinical translation, seven patients with histologically confirmed PDAC (5 after chemotherapy, 2 at initial staging) underwent [⁶⁸Ga]Ga-BCY18469-PET/CT (172 ± 42 MBq). The radiopharmaceutical was administered under § 13(2b) of the German Medicinal Products Act (Arzneimittelgesetz, AMG) permitting individualized radiopharmaceutical preparation for selected patients. Four patients were scanned at 15, 30, 45, 60, and 180 min p.i., three at 45 min p.i. Dosimetry calculations were performed using IDAC-Dose software based on organ-specific time-activity curves from whole-body PET acquisitions. Imaging findings were compared with contrast-enhanced CT or MRI (interval: 9–50 days) (ethical approval # 25-1444-S1-retro).

Results

[⁶⁸Ga]Ga-BCY18469 demonstrated EphA2-specific binding and internalization, proteolytic stability up to 72 hours, and rapid background clearance with high tumor uptake, thereby enhancing imaging contrast within 30 minutes in mice (Figure 2).

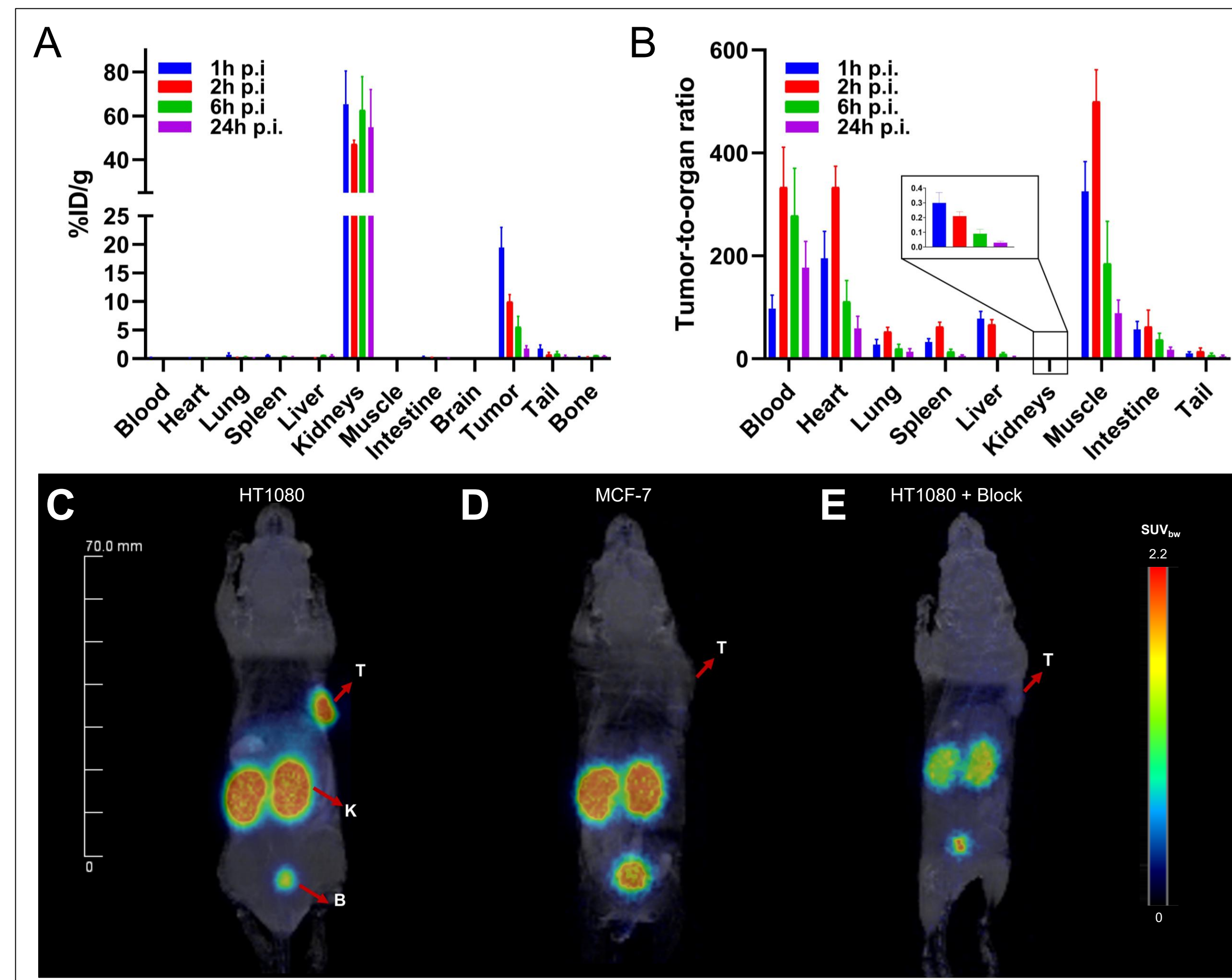


Figure 2: Preclinical *in vivo* evaluation of BCY18469 in tumor xenografts. (A) Organ distribution of 150 pmol ¹⁷⁷Lu-labeled BCY18469 at 1, 2, 6 and 24 h p.i., and (B) corresponding tumor-to-organ ratios in HT1080-tumor bearing BALB/c nu/nu mice. Data are expressed as mean % ID/g tissue ± SD (n=3). Whole-body maximum intensity projections of 150 pmol ⁶⁸Ga-labeled BCY18469 in HT1080- (C, E) and MCF-7- (D) tumor-bearing BALB/c nu/nu mice (right flank) 2h 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 clinical cases, [⁶⁸Ga]Ga-BCY18469 demonstrated rapid tumor uptake and was predominantly excreted via the kidneys (Figures 3-5). Notably, hepatic uptake remained favorably low (SUV_{mean} 0.9 ± 0.3 at 45 min p.i.). Mean absorbed doses were 0.31 ± 0.02 mGy/MBq (kidneys), 0.14 ± 0.08 mGy/MBq (salivary glands), and 0.016 ± 0.003 mGy/MBq (liver). EphA2-targeted PET imaging successfully detected 13 liver metastases (SUV_{max} 6.9 ± 3.4), 13 lymph node metastases (SUV_{max} 5.0 ± 1.1), 2 bone metastases (SUV_{max} 6.1 ± 0.5), and 2 peritoneal metastases (SUV_{max} 5.1 ± 0.8). Primary tumor uptake was observed in 6 of 7 patients, albeit with lower intensity compared to liver metastases (SUV_{max} 4.8 ± 1.6). Two pulmonary foci and 7 liver lesions identified on CT as morphologically consistent with metastases showed no uptake on EphA2-PET. Of 45 total lesions identified by any modality, PET detected 36 lesions (25 concordant with CT/MRI, 11 PET-only), while 9 lesions were detected only by CT/MRI.

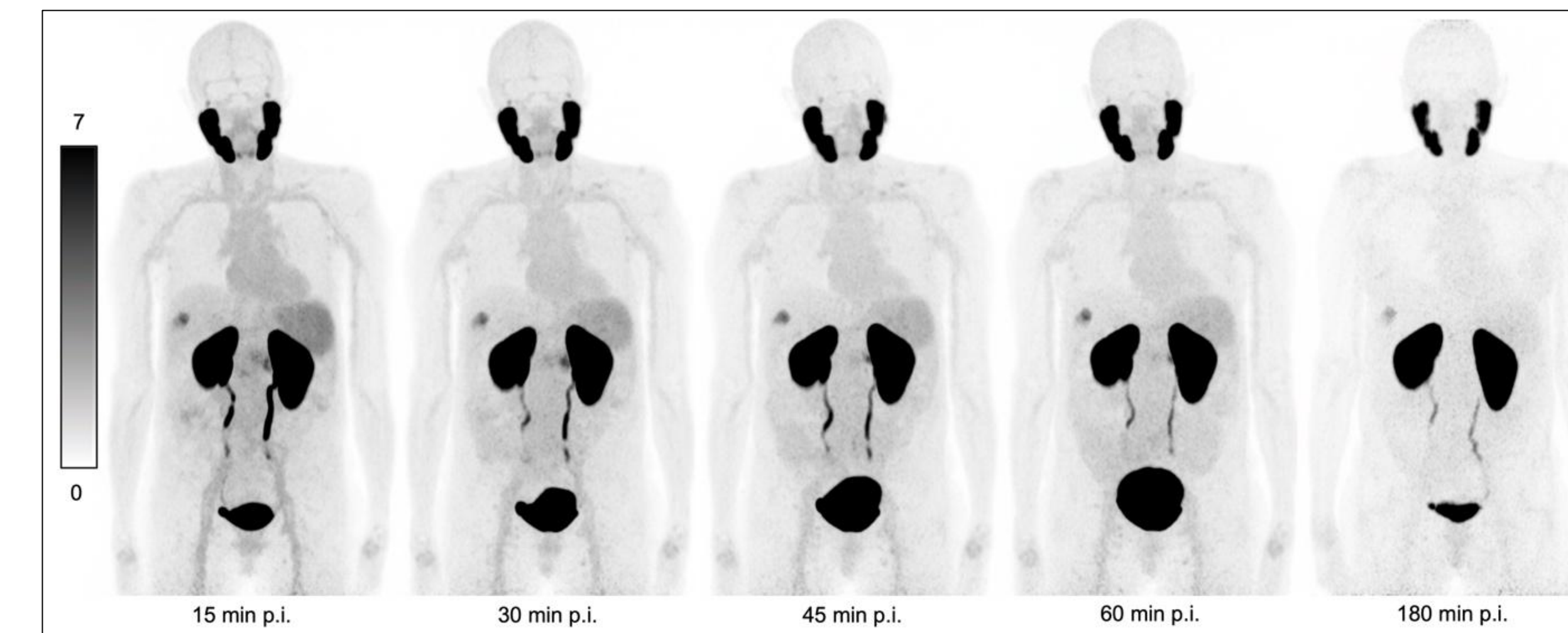


Figure 3: EphA2-specific PET/CT imaging. Maximum intensity projections (MIPs) at five timepoints (15, 30, 45, 60, 180 mins) after injection of [⁶⁸Ga]Ga-BCY18469 in a patient with pancreatic ductal adenocarcinoma and singular liver metastasis.

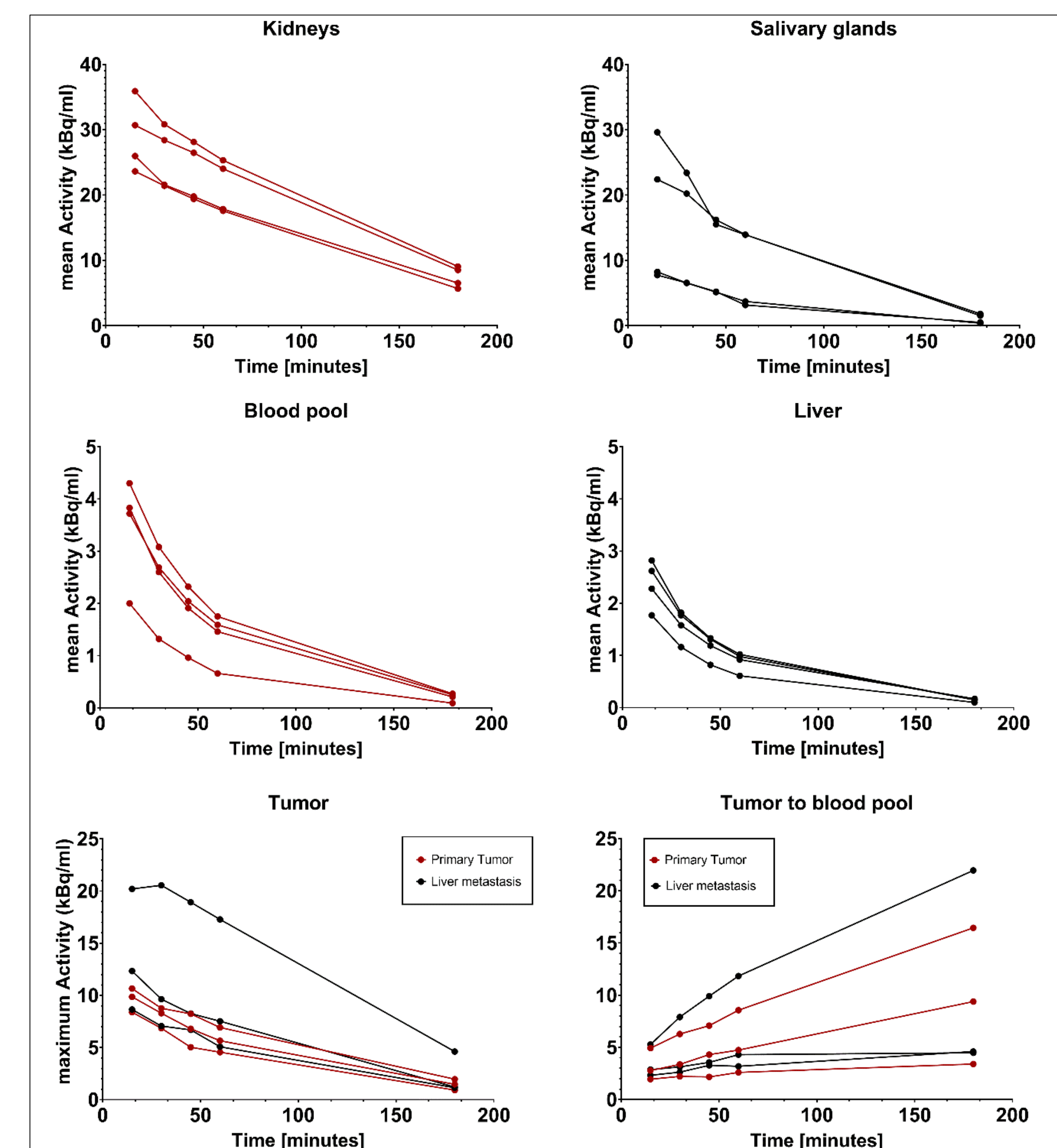


Figure 4: Time-Activity Curves of [⁶⁸Ga]Ga-BCY18469 in Key Organs and Tumor. TACs for kidneys, salivary glands, blood pool, liver, and tumor were derived at 15, 30, 45, 60, and 180 min post-injection (n=4). Mean activity concentrations were used for physiological organs; maximum values for tumor lesions. Where no primary tumor was available, the liver metastasis with highest uptake was analyzed as surrogate.

The 11 PET-only lesions comprised 8 lymph node metastases, 2 peritoneal lesions, and 1 liver metastasis. Potential clinical benefit from PET imaging was demonstrated in 2 of 7 patients with newly diagnosed PDAC: one was upstaged due to metastatic disease, another had a small liver lesion not evident on prior CT. Patients without prior therapy tended to show higher primary tumor uptake than pretreated patients. No adverse events or clinically noticeable pharmacologic effects were observed in any patient.

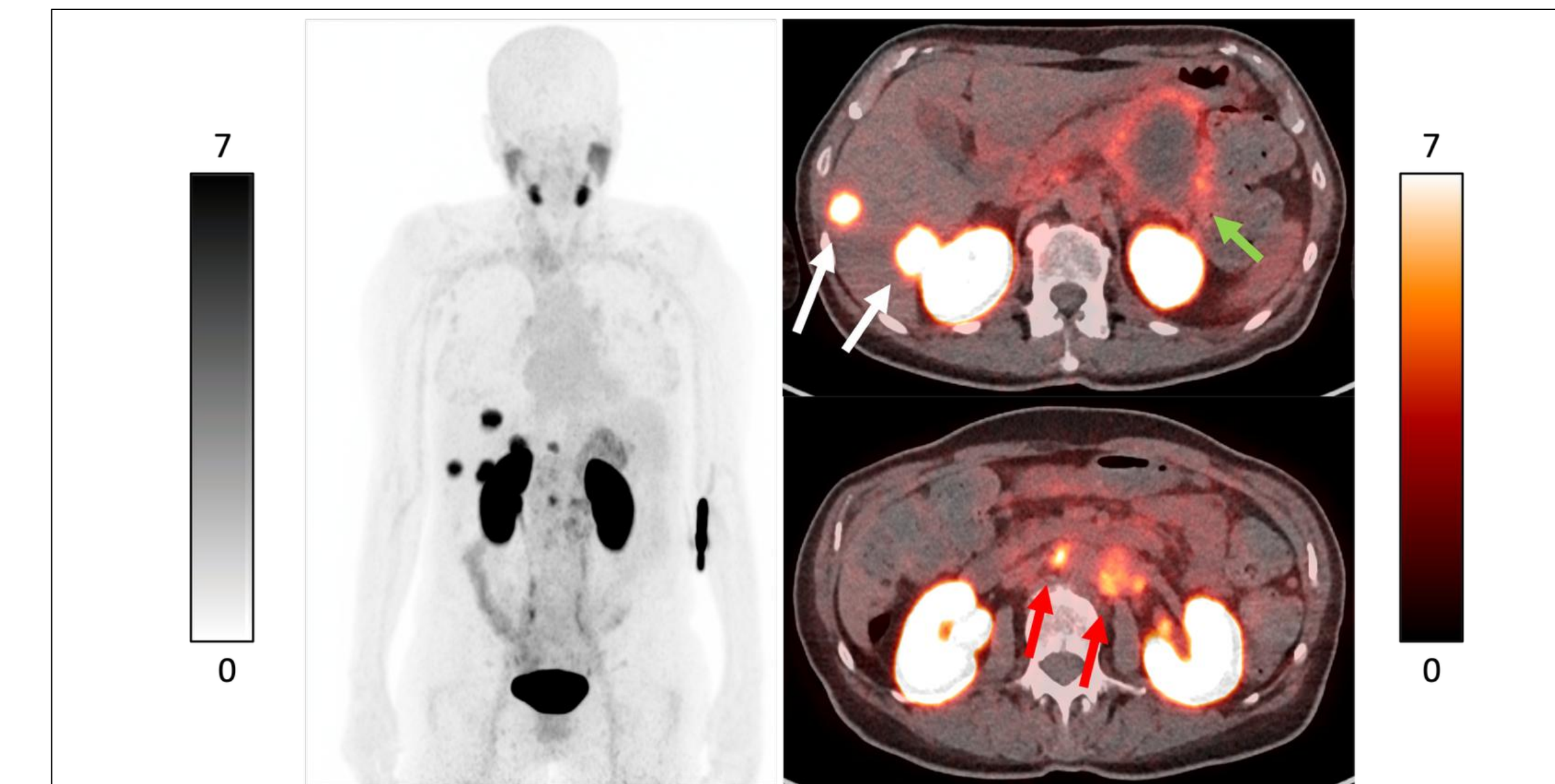


Figure 5: EphA2-PET/CT imaging in lymphonodal and hepatic metastasized pancreatic ductal adenocarcinoma. Maximum intensity projection (on the left side) acquired 45 minutes after injection of [⁶⁸Ga]Ga-BCY18469. Fused axial PET/CT images (on the right side) 45 minutes after injection of [⁶⁸Ga]Ga-BCY18469 showing pancreatic tumor mass (green arrow), hepatic metastases (white arrows) and lymphonodal metastases (red arrows).

Conclusion

This first-in-human application of EphA2-targeting [⁶⁸Ga]Ga-BCY18469 demonstrates the feasibility for visualization of EphA2-expressing primary tumors and metastases, which is in line with the preclinical findings. These initial clinical results support further investigation of [⁶⁸Ga]Ga-BCY18469 as a diagnostic tool with potential to improve tumor characterization and patient management strategies in EphA2-positive cancers.

Disclosure

The preclinical evaluation was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) DFG-grant 423813989/GRK2606 and DFG-grant 430609257/INST 39/1165-1, funding was also received from BicycleTx Limited. GM and AR are named inventors on patent applications relating to compounds described in this work. For clinical imaging BCY18469 precursor was provided by Bicycle Therapeutics.