In vivo radioimaging of bradykinin receptor B1, a widely overexpressed molecule in human cancer

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ABSTRACT

The bradykinin receptor B1R is overexpressed in many human cancers where it might be used as a general target for cancer imaging. In this study, we evaluated the feasibility of using radiolabeled kallidin derivatives to visualize B1R expression in a preclinical model of B1R-positive tumors. Three synthetic derivatives were evaluated in vitro and in vivo for receptor binding and their ability to visualize tumors by positron emission tomography (PET). Enalaprilat and phosphoramidon were used to evaluate the impact of peptidases on tumor visualization. While we found that radiolabeled peptides based on the native kallidin sequence were ineffective at visualizing B1R-positive tumors, peptidase inhibition with phosphoramidon greatly enhanced B1R visualization in vivo. Two stabilized derivatives incorporating unnatural amino acids (68Ga-SH01078 and 68Ga-P03034) maintained receptor-binding affinities that were effective, allowing excellent tumor visualization, minimal accumulation in normal tissues and rapid renal clearance. Tumor uptake was blocked in the presence of excess competitor, confirming that the specificity of tumor accumulation was receptor-mediated. Our results offer a preclinical proof of concept for non-invasive B1R detection by PET imaging as a general tool to visualize many human cancers.
INTRODUCTION

The bradykinin receptor family is constituted of two subtypes, B1 and B2, which are involved in the response to tissue damage and are important mediators of pain and inflammation. These G-protein-coupled receptors bind bradykinin and kallidin, which are produced by enzymatic cleavage of kininogens (1). The overexpression of the B1 and B2 receptors (B1R and B2R) has been documented in many cancers (2). While B2R is ubiquitously expressed, B1R has low endogenous expression in normal tissues. Molina et al. showed that B1R was expressed in a high proportion of ductal breast carcinomas in situ as well as invasive ductal breast carcinomas (3). Increased B1R expression was also noted in prostate carcinomas (4). Activation of B1R and/or B2R has been reported to promote the phosphorylation of extracellular regulated kinases (3), upregulate matrix metalloproteinases (5), and stimulate cell invasion and metastasis (6, 7). Crosslinked dimers of bradykinin analogs have also been proposed as therapeutic agents for prostate cancer and small cell lung cancers (8, 9).

Due to low expression in normal tissues, B1R is an attractive target for the development of imaging probes to visualize B1R positive (B1R+) tumors. Furthermore, since bradykinin receptors are important mediators of pain and inflammation, radiopharmaceuticals targeting these receptors might be useful to understand the role of the kinin-kallikrein system in human tumors in vivo and explore the relationship between these receptors, the immune system and cancer progression. While fluorescent derivatives have been reported for microscopy
and flow cytometry (10, 11), there have been so far few successful attempts to visualize B1R expressing tissues non-invasively, in vivo. Stahl et al. described a $^{99m}$Tc labeled peptide (HOE 140) to visualize B2R expression, and evaluated the biodistribution of this compound in healthy mice (12). Fuchs et al. reported the visualization of chronic cutaneous delayed type hypersensitivity reaction in mice using a $^{11}$C-labelled sulfonamide targeting B1R, but high, non-displaceable background activity was observed with the radiotracer (13). The purpose of this study was to develop and evaluate radiolabeled peptides to non-invasively visualize B1R expression in vivo.

**MATERIALS AND METHODS**

All chemicals and solvents were obtained from commercial sources, and used without further purification.

**Peptide synthesis**

B1R targeting peptides were synthesized on solid phase and the methods are further described in the supplementary data section. A semi-preparative (Phenomenex C18, 5µ, 250×10 mm) and an analytical column (Eclipse XOB-C18, 5µ, 150×4 mm) were used for HPLC purification and analysis. To obtain gallium conjugates, a solution of the DOTA-conjugated peptide (4 µmol) and GaCl$_3$ (20 µmol) in 500 µL sodium acetate buffer (0.1 M, pH 4.0) was incubated at 80 °C for 15 min. The methods for conjugation with non-radioactive gallium are described in the supplementary data section. We
prepared 3 conjugated peptides: Ga-DOTA-Ahx-[Leu\(^9\),desArg\(^{10}\)]kallidin (hereafter referred as P03083), Ga-DOTA-Ahx-[Hyp\(^4\),Cha\(^6\),Leu\(^9\),desArg\(^{10}\)]kallidin (SH01078) and Ga-DOTA-PEG2-[Hyp\(^4\),Cha\(^6\),Leu\(^9\),desArg\(^{10}\)]kallidin (P03034). The chemical structures of the peptides are shown in the Supplementary data.

**Radiochemistry**

An Eckert & Ziegler IGG100 \(^{68}\)Ga generator was used to obtain \(^{68}\)Ga. Radioactivity of \(^{68}\)Ga-labeled peptides was measured using a Capintec CRC-25R/W dose calibrator. The \(^{68}\)Ga generator was eluted with a total of 4 mL of 0.1 M HCl. The elution which contained the activity was mixed with 2 mL concentrated HCl. The mixture was passed through a DGA resin column and the column was washed by 3 mL 5 M HCl. After the column was dried by passage of air, \(^{68}\)Ga was eluted off with 0.5 mL water. The purified \(^{68}\)Ga solution was added to a 4-mL glass vial preloaded with 0.7 mL of HEPES buffer (2 M, pH 5.0) and the DOTA-conjugated precursor. All labeling procedures were performed in a conventional microwave oven, using a 1-minute reaction time. Microwave labeling was used due to previous experience achieving high yields in a short time using similar peptides – it is likely that similar results can be obtained with conventional heating. This step was followed by HPLC purification to ensure high specific activity for preclinical imaging. For \(^{68}\)Ga-P03083 50 µg of peptide was used. The reaction mixture was purified by HPLC using the semi-preparative column eluted with 17/83
CH$_3$CN/PBS (pH 7.1) at a flow rate of 4.5 mL/min. For $^{68}$Ga-P03034, 50 µg of precursor was used and the reaction mixture was purified by HPLC using the semi-preparative column eluted with 18/82 CH$_3$CN/PBS (pH 7.1) at a flow rate of 4.5 mL/min. $^{68}$Ga-SH01078 was synthesized using 100 µg of the radiolabeling precursor and purified using the same conditions as $^{68}$Ga-P03034. The quality control was performed by HPLC on the analytical column eluted with 16/84, 18/82, and 19/81 CH$_3$CN/PBS at a flow rate 2 mL/min, for $^{68}$Ga-P03083, $^{68}$Ga-P03034 and $^{68}$Ga-SH01078, respectively. The retention times of $^{68}$Ga-P03083, $^{68}$Ga-P03034 and $^{68}$Ga-SH01078 were 6.8, 7.7 and 6.1 min, respectively. The specific activity of the $^{68}$Ga-labeled peptides was measured using the analytical HPLC system. It was calculated by dividing the injected radioactivity (~37 MBq) of final products by the mass of the peptides in the injected solution. The mass of $^{68}$Ga-labeled peptides was estimated by comparing the UV absorbance obtained from the injection with a previously prepared standard curve.

**Stability in mouse plasma**

Balb/c mouse plasma for stability studies was obtained from Innovative Research. Aliquots (100 µL) of the $^{68}$Ga-labeled peptide (P03083, P03034 and SH01078) were incubated with 400 µL of balb/c mouse plasma for 5, 15, 30, and 60 minutes at 37°C. At the end of each incubation period, samples were quenched with 500 µL 70% CH$_3$CN and centrifuged for 20 min. The metabolites were measured using a semi-preparative HPLC system (Agilent).
The suspension was loaded onto the HPLC and eluted with 17/83 CH$_3$CN/PBS (pH 7.1), 23% MeCN (0.1% TFA), and 23% MeCN (0.1% TFA) at a flow rate of 4.5 mL/min, for $^{68}$Ga-P03083, $^{68}$Ga-P03034 and $^{68}$Ga-SH01078, respectively. The retention times of $^{68}$Ga-P03083, $^{68}$Ga-P03034 and $^{68}$Ga-SH01078 were 16.6, 12.1, and 12.5 min, respectively.

**LogD$_{7.4}$ measurements**

Aliquots (2 μL) of the $^{68}$Ga-labeled peptides were added to a vial containing 3 mL of octanol and 3 mL of 0.1 M phosphate buffer (pH 7.4). The mixture was vortexed for 1 min and then centrifuged at 5000 rpm for 10 min. Samples of the octanol (1 mL) and buffer (1 mL) layers were taken and counted in a well-counter. LogD$_{7.4}$ was calculated using the following equation: LogD$_{7.4}$ = log$_{10}$[(counts in octanol phase)/(counts in buffer phase)].

**Creation of a stable B1R expression cell line**

We used human embryonic kidney cells HEK293T transduced with both GFP and B1R for this study, using separate vectors. HEK293T cells were obtained from Clontech Laboratories. For green fluorescent protein (GFP) transduction, we used the Lenti-XTM Expression System (Clontech Laboratories), using the pGIPz(TurboGFP) cloning vector. For B1R overexpression, GFP-positive HEK293T cells were transduced using pre-made inducible lentiviral particles at 1×10$^7$ IFU/mL, obtained from GenTarget (cat. no. LVP291). The B1R open reading frame was constitutively expressed under a suCMV promoter. An
antibiotic blasticidin (Bsd) – RFP (red fluorescence protein) fusion marker under RSV promoter was present in the expression vector to allow for selection and verification of transduced cells. The presence of both GFP and RFP expression was confirmed by fluorescence microscopy using a Nikon Eclipse TE2000 E confocal microscope.

**Receptor binding assays**

The affinity of the peptides for B1R was measured using competitive binding assays. CHO cell membranes overexpressing the recombinant human B1R receptor (PerkinElmer) were used for those assays. Briefly, 96-wells MultiScreen plates with glass fiber filter and PVDF support (Millipore) were pre-soaked with 0.5% of cold polyethyleneimine (Sigma-Aldrich) for 30 minutes. Afterward, wells were washed once with 50 mM of Tris-HCl pH 7.4. The wells were loaded with the assay buffer containing 50 mM of Tris-HCl pH 7.4 and 5 mM of MgCl₂. Varying concentration of non radioactive control Lys-(Des-Arg⁹)-Bradykinin (Bachem) or the peptides of interest were added in the presence of 4.8 nM of [³H]-(des-Arg¹⁰,Leu⁹)-Kallidin (PerkinElmer). B1R membranes were added to each well to a final protein concentration of 50 µg/well. The MultiScreen plate was incubated at 27°C for 15 minutes with gentle agitation at 300 rpm. The assay was stopped by aspirating the reaction solution through the PVDF membrane filter, followed by washing with ice-cold 50 mM Tris-HCl pH 7.4. The filter membranes were dried prior to adding scintillation liquid, and the activity in the plates was measured using a 1450
MicroBeta Counter (PerkinElmer). Data analysis was performed with GraphPad Prism 5, using a one-site competitive binding model. The $K_i$ was calculated from the IC$_{50}$ using the Cheng-Prusoff equation.

The successful transduction of B1R was also measured by a saturation assay on B1R+ HEK293T cell membranes. Briefly, the cells were disrupted using a Dounce homogenizer, and cell membranes were isolated by sequential centrifugation. Protein concentration was determined using a Bradford assay. 50 μg of membrane protein per well was used for the saturation assay. The assay conditions were similar to the competitive binding assays with the exception that progressively higher concentrations of radioactive $[^3H]$-(des-Arg$^{10}$,Leu$^9$)-Kallidin were used (range 0.05 – 20 nM), with and without the presence of excess competitor (30 μM Lys-(Des-Arg$^9$)-Bradykinin, Bachem). GraphPad Prism 5 was used to calculate the binding affinity ($K_d$) and receptor concentration ($B_{max}$), normalized in fmol/mg of protein.

**Biodistribution experiments**

Animal experiments were approved by the University of British Columbia Animal Care Committee. Male immunodeficient NOD.Cg-Prkdc$^{scid}$ $Il2rg^{tm1Wjl}$/SzJ (NSG) mice were obtained from an in-house breeding colony at the Animal Resource Centre of the BC Cancer Agency Research Centre. B1R+ and negative (B1R-) HEK293T tumors were inoculated by subcutaneous injection of $1 \times 10^6$ cells on each dorsal flank of the mice. Thus, each mouse had
a positive and negative control. After growth period ranging from 10-14 days, palpable tumors measuring approximately 7 mm in diameter were obtained. Mice (n = 4 to 7 per group) were injected with either 0.37 MBq or 3.7 MBq of \(^{68}\)Ga-labeled peptides, depending on whether the mice were used solely for biodistribution or for imaging followed by biodistribution. For blocking experiments, the radioactive compound was co-injected with 100 μg of the same non-radioactive (e.g. natGa) compound. After 60 minutes of uptake, the mice were anesthetized by isoflurane inhalation, then euthanized by CO\(_2\) inhalation. Blood was promptly withdrawn, and the organs of interest were harvested, rinsed with normal saline, blotted dry and inserted into pre-weighted tubes. The tubes were weighed again to obtain the exact organ weight. The radioactivity of the collected mouse tissues was counted using a Cobra II gamma counter (Packard), normalized to the injected dose using a standard curve and expressed as the percentage of the injected dose per gram of tissue (%ID/g).

**Preclinical imaging**

PET imaging experiments were conducted using a Siemens Inveon microPET/CT scanner. Mice bearing B1R+ and B1R- tumors, as described above, were used for the experiments. In some mice, dynamic scanning was performed to determine the time-activity course of the radiopharmaceuticals in organs of interest. The mice were sedated with 2% isoflurane inhalation and positioned in the scanner. A baseline CT scan was obtained for localization and
attenuation correction prior to radiotracer injection, using 60 kV x-rays at 500 μA, 3 sequential bed position with 33% overlap and 220 degree continuous rotation. The mice were kept warm by a heating pad during acquisition. The dynamic acquisition of 60 minutes was started at the time of i.v. injection with 3.7 MBq of $^{68}$Ga-labeled peptide, with or without the presence of 100 μg of excess unlabeled natGa-peptide. The list mode data was rebinned into time intervals (12×10 sec, 6×30 sec, 5×60 sec, 6×300 sec, 2×600 sec) to obtain tissue time-activity curves. As we noticed higher renal accumulation and lower urinary excretion in these mice due to the effects of prolonged isoflurane sedation, these animals were not used for biodistribution experiments or for the final analysis of tumor uptake by imaging at 60 minutes. Static imaging was done in the other mice. The mice were briefly sedated for i.v. injection of the radiotracer, then allowed to recover and roam freely in their cages for 55 minutes. At that point, the mice were sedated with 2% isoflurane inhalation, placed in the scanner, and an attenuation correction CT scan was obtained as described above. A single static emission scan was acquired for 10 minutes. The mice were euthanized and the organs harvested for biodistribution.

Peptidase inhibition with phosphoramidon or enalaprilat

To determine the effects of peptidase inhibition on radiotracer uptake in target tissues, groups of mice injected with $^{68}$Ga-P03083 were co-injected with either 0.3 mg of phosphoramidon or 0.25 mg of enalaprilat in normal saline. Imaging and/or biodistribution were performed 55-60 minutes later, as described above.
Statistical analysis

Statistical analysis was performed using the Prism 6 software (GraphPad). Biodistribution data was analyzed by two-way ANOVA, with the organs of interest as a factor, and the radiotracers with or without peptidase inhibition as a second factor. Tukey’s multiple comparison test was used to compare the uptake in the tumors and organs between the following groups: P03083 injected without peptidase inhibition, P03083 injected with enalaprilat, P03083 injected with phosphoramidon, $^{68}$Ga-SH01078 and $^{68}$Ga-P03034. An adjusted $p$ value of less than 0.05 was considered significant.

RESULTS

Radiochemistry and plasma stability

The radiochemistry and plasma stability data is reported in Table 1. Due to the use of HPLC, all radiopeptides were obtained in good yield, at high specific activities, suitable for use in mice for receptor imaging. The stability tests performed in vitro using mouse plasma showed relatively good stability for all peptides in mouse plasma, with the best results obtained with $^{68}$Ga-SH01078.

Cellular expression of B1R and binding affinity

Following transduction with the hB1R, successful gene transfer from the lentiviral construct was confirmed by the expression of red fluorescent protein (Figure 1). No red fluorescence was observed in the control HEK293T cell line.
Saturation assays performed with $[^3\text{H}]-\text{(des-Arg}^{10},\text{Leu}^9)$-kallidin showed a maximal binding capacity ($B_{\text{max}}$) of 451 ± 88 fmol/µg of protein for the B1R+ HEK293T cell membranes and 56 ± 48 fmol/µg of protein for the control HEK293T cells. The binding affinities ($K_d$) of $[^3\text{H}]-\text{(des-Arg}^{10},\text{Leu}^9)$-kallidin for those cell membranes were 1.9 ± 0.5 and 1.6 ± 0.2 nM, respectively. By competitive binding assays, the inhibition constant ($K_i$) of P03083 was 2.6 ± 0.7 nM. The $K_i$ of SH01078 and P03034 were 27.8 ± 4.9 nM and 16.0 ± 1.9 nM, respectively.

**Biodistribution and imaging**

The results of the biodistribution experiments are presented in Figure 2, and the entire data can be found in Supplementary data. The peptides cleared rapidly by urinary excretion, with accumulation in the bladder and kidneys. No significant uptake was seen in other normal organs, with minimal background activity. When $^{68}$Ga-P03083 was injected without peptidase inhibitors, tumor uptake was 4-fold higher in the transduced B1R+ compared to the control B1R- tumor. This did not improve significantly when $^{68}$Ga-P03083 was co-injected with enalaprilat. Enalaprilat increased renal uptake of $^{68}$Ga-P03083, but did not improve tumor accumulation. However, when co-injected with phosphoramidon, a significant increase ($p < 0.0001$) in radiotracer uptake was noted in the B1R+ tumors, with 9-fold higher uptake compared to the B1R- tumors (Figure 3). Similarly, the metabolically stable peptides ($^{68}$Ga-SH01078 and $^{68}$Ga-P03034) led to significantly higher accumulation in B1R+ compared
to B1R- tumors (Figure 4). With $^{68}$Ga-P03083 co-injected with phosphoramidon, the average ratios of B1R+ tumor to plasma, B1R+ tumor to muscle and B1R+ to B1R- tumors were 9.2 ± 3.7, 35 ± 3 and 9.2 ± 3.7, respectively. With $^{68}$Ga-SH01078, the average ratios were 5.2 ± 2.3, 28 ± 5 and 4.4 ± 1.0, respectively. Similar results were obtained using a PEG linker, as evidenced by the high tumor-to-background ratios obtained with $^{68}$Ga-P03034. Thus, while the absolute tumor uptake remained moderate due to rapid renal clearance of the radiolabeled peptides, excellent contrast was achieved using either $^{68}$Ga-P03083 co-injected with phosphoramidon, or with the stable derivatives $^{68}$Ga-SH01078 and $^{68}$Ga-P03034. Blocking experiments were performed for $^{68}$Ga-P03034. The uptake of $^{68}$Ga-P03034 in the B1R+ tumor was completely blocked by the co-injection with 100 µg of P03034 (Figure 5). Time activity curves of the radiolabeled peptides showed progressive clearance from the blood and non-target tissues, with retention in B1R+ tumors and high activity in the kidneys (Figure 6).

**DISCUSSION**

Bradykinin receptors are overexpressed in several human cancers (2). While B2R is expressed in normal tissues, normal physiological expression of B1R is low (1). Thus, B1R is a potentially attractive target for breast and prostate cancer detection. B1R expression is stimulated by the presence of bradykinin, tumor necrosis factor alpha and interleukin-1β (14). The soluble receptor of the globular heads of C1q (sgC1qR) has also been shown to induce B1R expression.
in endothelial cells (15). The cause of B1R overexpression in cancers is not known. The presence of tissue kallikreins (notably kallikrein-2 and kallikrein-3, also known as the prostate specific antigen) might play a role in the overexpression of B1R (4). In particular, kallikrein-2 might release bradykinin locally from low and high-molecular weight kininogens, which could chronically stimulate the overexpression of B1R in cancer cells. Alternatively, the immune system might contribute to enhance B1R expression due to leukocyte infiltration associated with secretion of cytokines.

In this study, we observed that despite the addition of a chelator and radiometal at the N-terminus of [Leu^9,desArg^10]kallidin, receptor binding affinity was maintained using a short spacer. We also showed that replacing proline and phenylalanine by hydroxyproline and cyclohexylalanine at positions 4 and 6 deteriorated binding affinity to B1R but improved metabolic stability in vitro. More importantly, we demonstrated that it is possible to visualize non-invasively B1R in vivo, by positron emission tomography. We found that using the native antagonist sequence did not lead to good tumor visualization, and confirmed that this was due to peptidase activity. In vitro plasma stability assays did not predict such low in vivo stability for ⁶⁸Ga-P03083, suggesting that the peptidase inactivating this peptide was not circulating in plasma.

Peptidase inhibition with phosphoramidon, but not enalaprilat, led to improved tumor uptake in vivo. The beneficial effects of phosphoramidon parallels the recent finding of Nock et al, who observed higher levels of circulating intact
radiopeptides and improved tumor visualization following phosphoramidon co-administration (16). Enalaprilat is an inhibitor of the angiotensin conversion enzyme (ACE), while phosphoramidon inhibits endopeptidase 24.11 in addition to being a potent inhibitor of ACE and other peptidases (15). ACE, carboxypeptidase N and endopeptidase 24.11 have been implicated in the degradation of bradykinin (7, 17). We did not see a statistically significant effect of enalaprilat on tumor accumulation of the antagonist radiopeptides in this study. However, enalaprilat administration led to slightly higher renal accumulation of ⁶⁸Ga-P03083. Whether this was caused by the effects of enalaprilat on kidney glomerular filtration rate, or due to a change in the nature of peptide fragments from ⁶⁸Ga-P03083 degradation remains uncertain.

Poor tumor uptake of the ⁶⁸Ga-P03083, a peptide derived from the native sequence of bradykinin, led us to design radiopeptides, ⁶⁸Ga-SH01078 and ⁶⁸Ga-P03034, with unnatural amino-acids to improve stability. Despite lower affinity to B1R than P03083, images obtained using these modified peptides had significantly improved tumor uptake and tumor:background ratios when used in the absence of peptidase inhibitors. All three peptides evaluated in this study presented significant renal accumulation. It is known that small peptides are filtered by glomerular filtration, but can be reabsorbed by endocytic receptors in proximal tubular cells, notably megalin. The exact mechanism by which the B1R peptides presented in this study accumulate in the kidneys remain unknown, but likely follow similar mechanisms (18).
We obtained high contrast images with minimal background activity and excellent tumor visualization, despite moderate absolute tumor uptake values. It is worth emphasizing that the absolute tumor uptake is in part dependent on the clearance rate of a radiopharmaceutical – typically much higher uptake is observed with radiotracers that remain in circulation for many days, such as antibodies, compared with lower molecular weight radiotracers. Since higher tumor uptake values have been achieved with other radiopeptides with similar molecular weight (19), it is likely feasible to improve tumor visualization by further improvements in metabolic stability, receptor-binding affinity, or by imaging at later time points to optimize the tumor-to-background ratio.

While the overexpression of B1R has been well documented in studies conducted on resected human tumor specimen, there are no established models of a B1R expressing tumor for preclinical studies. We thus used a transfected tumor model to avoid the confounding factor of potentially limited target expression on various commonly used in vitro cancer cell lines. This model allowed us to directly compare, in the same animal, a tumor overexpressing the target with the same tumor that expresses low endogenous levels of B1R. Such models are commonly used in preclinical studies for some radiotracers such as somatostatin receptor ligands (20), but may not reflect the actual expression levels that may be encountered in the clinical setting. Further studies will be required to identify a suitable model with endogenous overexpression of B1R to more closely reflect the clinical situation.
In conclusion, we demonstrated in this study that bradykinin receptor imaging was possible using metabolically stable derivatives of \([\text{Leu}^9,\text{desArg}^{10}]\text{kallidin}\), a bradykinin receptor antagonist sequence. Good receptor binding affinities to B1R were observed after the addition of a radiometal chelator at the N-terminus of these peptides. Protection from peptidase activity was critical to achieve good tumor visualization in \textit{vivo} for non-invasive imaging of B1R expression. These radiolabeled peptides can readily be translated for non-invasive imaging in human subjects. \(^{68}\text{Ga}\), a radioisotope that is readily available from a long-lived \(^{68}\text{Ge}\) generator, does not require an on-site cyclotron and provides excellent image quality with low radiation exposure.

The causes and significance of B1R expression in human cancers will require further investigations. B1R imaging could be a potential surrogate biomarker of the activity of some tissue kallikreins (notably kallikrein 2), reflect an immune response to cancer cells due to the known relationship between B1R expression and tumor necrosis factor alpha and interleukin-1β, or be intrinsically driven by other cellular processes. Potential clinical applications of B1R imaging peptides in oncology include the early detection of breast and prostate cancers, and the non-invasive assessment of B1R expression to predict potential benefits from drugs targeting this receptor. Provided that renal accumulation can be mitigated or reduced, other potential applications include the possibility of using this receptor for the delivery of peptide-drug conjugates, or for radionuclide therapy, using peptides labeled with therapeutic alpha or beta emitters.
AKNOWLEDGEMENTS

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REFERENCES


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FIGURE LEGENDS

Figure 1 – Fluorescent images of successful B1R transduction. The cells were previously transduced by green fluorescent protein (GFP, middle panel). Red fluorescent protein (RFP) was transduced in the same lentiviral vector as B1R (right panel).

Figure 2 – Biodistribution data showing organ or tumor uptake (expressed as %ID/g) for selected organs, and tumors with or without B1R expression.

Figure 3 – Maximum intensity projection (MIP) positron emission tomography images obtained with $^{68}$Ga-P03083 without (left), with enalaprilat (middle) and with co-injection of phosphoramidon (right). The B1R+ tumor (red arrow) was located on the right shoulder (the animal is viewed on a coronal projection, ventral viewpoint). The B1R- tumor (blue arrow) had no significant uptake. The greyscale bar to the right of each image is set in units of %ID/g.

Figure 4 – MIP (left) and fused PET/CT images (right) of $^{68}$Ga-SH01078, showing high tumor accumulation in the B1R+ tumor (red arrow), and negligible accumulation in the negative control (B1R-, blue arrow) tumor. The greyscale bar is set in units of %ID/g.

Figure 5 – MIP images of $^{68}$Ga-P03034 images with (right) and without (left) injection of competitor. Uptake in the B1R+ tumor is blocked in the presence of excess unlabeled P03034, confirming receptor-mediated uptake. The greyscale bar is set in units of %ID/g.
**Figure 6** – Time activity curve of $^{68}$Ga-P03034 using regions-of-interest located around the tumors, heart and kidney (A: linear scale, B: log scale).

There was rapid clearance from the blood, fast renal excretion and sustained tumor accumulation.
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