Published in final edited form as:

Bioorg Med Chem Lett. 2011 April 15; 21(8): 2550–2553. doi:10.1016/j.bmcl.2011.02.013.

Development of Anti-EGF Receptor Peptidomimetics (AERP) as Tumor Imaging Agent

Datta E. Ponde^{§,*}, ZiFen Su[¶], Alan Berezov^{§,†}, Hongtao Zhang⁺, Abbas Alavi[§], Mark I. Greene⁺, and Ramachandran Murali^{§,†,*}

⁺Department of Pathology and Laboratory Medicine, 3620 Hamilton Walk, University of Pennsylvania, Philadelphia, PA 19104.

[§]Department of Radiology, Cyclotron Facility, 420 Curie Blvd, University of Pennsylvania, Philadelphia, PA 19104.

[¶]Institute of Nuclear Science and Technology, Sichuan University, Chengdu, Sichuan 610064, China

Abstract

EGFR is over-expressed in several solid tumors including breast, prostate, pancreas and lung cancers and is correlated to the metastatic potential of the tumor. Anti-EGFR receptor-binding peptidomimetics (AERP) were examined to assess the small molecule's potential use as tumor-specific imaging agents. The aim of this work was to design and characterize the binding specificity of the radiolabeled peptidomimetics to EGFR over-expressing cell lysate and to A431 xenograft tumors. Our newly designed peptidomimetic, AERP, was conjugated to DTPA and labeled with ^{99m}Tc. The *in vivo* tumor accumulation of [^{99m}Tc] DTPA-AERP-2 was 1.6 ± 0.1 %ID/g and tumor to muscle ratio was 5.5. Our studies suggest that this novel peptidomimetic, AERP-2, warrants further development as an EGFR-specific tumor-imaging agent.

Keywords

Radiolabeled peptidomimetics and peptide; AERP-2; technetium-99m; EGFR; c-erbB2; tyrosine kinase receptors; EGFR specific radiotracer

The epidermal growth factor (EGF) family of tyrosine kinase receptors were expressed at high levels in a wide variety of human cancers and had been associated with various features of advanced disease and poor prognosis. The EGFR tyrosine kinases are found to be involved in proliferation, signal transduction, angiogenesis and apoptosis of cancer cells.¹⁻³ The tyrosine kinase family receptors are over-expressed in 20%-30% cancers of the breast,⁴ prostate,⁵ ovary,⁶ and pancreas.⁷ The over-expressed receptors promote the growth and metastasis of cells.⁸

© 2011 Elsevier Ltd. All rights reserved.

* Correspondence should be addressed to: R.M (muralir@csmc.edu) or D.P (pondede@mail.med.upenn.edu) Department of Biomedical Sciences 8700 Beverly Blvd, Los Angeles 90048 Telephone: 1-310-248-7681, fax: 1-310-423-0225.

[§]Current Address: Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA 90048

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Monoclonal antibodies have already shown promise in targeting epidermal growth factor receptor-positive tumors.^{9,10} There are various drugs in the market such as Iressa,¹¹ which are used as anticancer drugs and targets EGFR. Another drug in clinical trial is Tarceva.¹² Molecules such as IMC-C225 (anti-EGFR antibody) can specifically target the receptors on cancer cells to interrupt the transduction of growth signals,¹³ prohibit metastasis,¹⁴ and induce apoptosis¹⁵ of the cancer cells. Because of their receptor-binding specificity, IMCC225 could be used as imaging/therapeutic agents for tumors when radiolabeled.¹⁶

Epidermal growth factor (EGF) is a ~6 KDa polypeptide and first ligand known to bind EGFR. Because of its high affinity to EGFR, investigators radiolabeled EGF and exploit its potential in tumor targeting.^{17,18} Since it is a natural ligand, using EGF as probe carry risk of activating EGFR receptors, and thereby potentiate tumorigenesis. We believe that, molecules that show anti-tumor effects might be better candidate for tumor imaging studies. Antibodies are high affinity molecules that show anti-tumor effects. However, the radiolabeled antibodies, despite the high percentage of injected dose accumulated in tumors after a few days circulation, exhibited slow clearance from blood and therefore caused high background for imaging and excessive toxicity to normal organs in therapy. Smaller molecules with inherent receptor-binding affinity would overcome these problems because clearance of the molecules from the circulation is faster compared to the monoclonal antibody. Recently investigators generated a fully human anti-EGFR Fab that recognizes the extra-cellular domain of EGFR and showed labeled anti-EGFR Fab had reasonable antigen-binding capability and accumulated only in tumors with high or moderate EGFR expression.¹⁹ These and many other investigators employed various other antibody fragments such as nanobody²⁰, single domain antibody format²¹, affibody²² but many of these results are still in very early stage of their research and its not clear if they can be utilized clinically in mapping EGFR receptors.

In recent years, radiolabeled peptides have been successfully developed and used in the clinic for nuclear medicine receptor imaging.^{23,24} In addition, several radiolabeled peptides, such as [^{99m}Tc] neurotensin (NT) for pancreatic tumor imaging,²⁵ bombesin analogues with ^{99m}Tc-labeled for tumor imaging,²⁶ and [⁶⁴Cu]RGD for $\alpha_v\beta_3$ integrin imaging,²⁷ have shown promising results.

In this preliminary work a radiolabeled peptidomimetic (AERP), was evaluated as a tumor-specific agent. The newly designed AERP is a peptide bind to the EGFR with moderate affinity. In order to design EGFR-specific peptidomimetics, we used the deduced structure of the monoclonals (MAb) 225 and 425 resolved by X-ray crystallography (Murali, unpublished). A bioactive Anti-EGF Receptor Peptidomimetic (AERP) has been designed from the three-dimensional information of two anti-EGFR monoclonal antibodies. The newly fashioned AERP mimetics bind to the EGFR, with low nanomolar affinity. This interaction has been demonstrated to impart biological function similar to the interaction of a full-length monoclonal antibody raised against this growth receptor oncoprotein. Two AERP analogs have been resolved. The sequence of the peptide is shown in Figure 1. A control peptide CD4 ([Cys³-Cys¹²]-HABuFCYIGEVEDQCY-OH) was synthesized in our lab. These peptides were cyclized, purified by HPLC.

Suitable peptidomimetics for solution structure by NMR were selected by performing one-dimensional NMR spectra under varying salt, pH and temperature conditions. The optimum conditions for structural characterization of these peptides were found to be in 50 mM acetate buffer, with 100 mM NaCl at pH 4.5 and 14 °C. A pair of 2-dimensional experiments were used to connect protons within a given spin system and determine their sequential assignments. The NOESY and TOCSY spectra for the peptides were collected on a 750 MHz spectrometer. Structural constraints for chemical shift, dihedral angles and through

space distances were collected and the structure calculations were performed with the CNS program²⁸ using the NMR constraints as inputs. A simulated annealing protocol was used to generate a family of structures that satisfied the 217 distance and 10 dihedral experimental constraints. The structure refined to 0.6 Å pair-wise RMSD for the backbone atoms for a set of 17 structures.

The structures of the 15 residue (Figure 2) AERP-2 peptide analogs contain a bend at the N-terminal portion fixed by a disulfide linkage between residues 2 and 8. The middle section contains a 3-10 helix and the C-terminal end is disordered. Three of the four aromatic side chains are clustered on one side of the molecule. A common design feature of the peptides is the positioning of 2 cysteine residues that constrain the structures. Some peptides produced aggregate states through the possible formation of intermolecular disulfide bonds. Other peptides containing proline residues displayed multiple cis/trans isomeric states. The resulting structure of the exocyclic peptide revealed features not predicted by the molecular modeling applied to this peptide such as residues that might influence aggregates and isomerization.

We obtained baculovirus-produced and partially purified ectodomain forms of EGFR species from Mark Lemmon (University of Pennsylvania). The ectodomain construct was further purified by high performance gel filtration chromatography. Kinetic binding characteristics of AERP to the ectodomain of the EGF receptor were studied using biosensor techniques.^{29,30} AERP binds to the EGF receptor with an approximate affinity of 400 nM. At optimum surface density (3600 RU), AERP bound to EGF receptors in a concentration-dependent manner with a dissociation pattern (k_{off}) within an order of magnitude to that of the 225 MAb (data not shown). Importantly, AERP did not show binding to either immobilized Her2/neu or TNF receptors.

The intramolecular cyclization of the peptides (0.1 mg/mL or 0.5~0.7 mM, pH 8–8.5) was carried out at 4 °C as described previously.^{29,30} The advance of the cyclization process was monitored by testing the concentration of the free thiol groups of the peptide by the DTNB method.^{29,30} The testing procedures were: a) 400 µL of the reaction solution was mixed with 100 µL of 0.1M pH 8 phosphate buffer and 5 µL of 10 mM pH 8 of DTNB; b) the mixture was incubated at 4 °C in the dark for 30 min; c) absorption at 412 nm of the mixture was measured with the reaction solution as reference; d) cyclization was considered to be completed when the absorptions at 412 nm of the mixture and the reaction solution became identical. After the completion of the cyclization, the solvents were removed by lyophilization. The purity of the cyclized peptides was tested by HPLC (ODS C18 column, 0.01 M pH 5.2 NH₄OAc/MeOH as mobile phase with gradient of 40% MeOH to 90% MeOH over 25 min) before further use.

In order to do conjugation of cyclized peptide with DTPA (Figure 3), to a 10 mg/mL solution of the cyclized peptide in water was added an equal volume of 0.2 mol/L bicarbonate buffer, pH 8.6. A suspension of the DTPA cyclic anhydride in DMSO (10 mg/mL) was then added drop-wise with agitation to achieve a final DTPA-to-peptide molar ratio of 3:1. The mixture was stirred at room temperature for 1h and the DTPA conjugate was purified by HPLC employing 0.01 M NH₄OAc/MeOH as mobile phase. HPLC purified product containing solvent was evaporated by rotary evaporation at 35°C and lyophilized to get pure DTPA conjugates. It was observed that the cyclization rate of a peptide was hydrophilicity dependent. For example, during our previous work cyclization of 30 mg (17 mmol) of each AHNP took 18 days to complete,^{29,30} whereas in this work, it took 60 days for AERP with the same molarities to complete the cyclization.

In order to explore tumor imaging potential of AERP, we coupled the peptidomimetic with DTPA and labeled with ^{99m}Tc . The conjugations of DTPA to the peptidomimetic were carried out as explained above. The yields of the products ranged from 35%–68% after the purification by Reverse Phase (RP) semi-prep HPLC ($n = 6$). The purpose of introducing DTPA as chelating agent to the peptides was that, with DTPA the peptides can be labeled by ^{99m}Tc . Due to their appropriate emissions, ^{99m}Tc labeled peptides are suitable for *in vitro* cell binding tests and other pharmacologic tests. In terms of availability, ^{99m}Tc should be the first isotope to be considered in the course of developing radio peptidomimetic cancer imaging agents. Technetium-99m was obtained from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator, which was available at University of Pennsylvania Health System's (UPHS) nuclear medicine department.

For radiolabeling of the DTPA conjugate with ^{99m}Tc , DTPA conjugate (1 μg) in 10 μL of water was added to 20 μL of a buffer consisting of 0.5 mol/L sodium bicarbonate, 0.25 mol/L ammonium acetate, and 0.175 mol/L ammonium hydroxide, pH 9.2. To this was added approximately 1–3 mCi (37–121 MBq) $\text{Na}^{99m}\text{TcO}_4$ (10–100 μL) followed immediately by addition of 6–12 μL of a fresh solution of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (1 mg/mL in 10 mmol/L HCl). Reaction mixture was incubated at room temperature for 30 min, the labeled peptidomimetics was analyzed by analytical HPLC on a Zorbax C18 (4.6 $\times$ 250 mm) column with 0.01 M $\text{NH}_4\text{OAc}/\text{MeOH}$, pH 5.2, (gradient 0% MeOH to 90% MeOH over 25 min) as mobile phase. The semi-prep purification was carried out on a size-exclusion (SE) superdex peptide 10/30 column with 0.01 M pH 7.39 phosphate buffer as mobile phase ($n = 6$) (Figure 4). The purified radiolabeled peptides were stable in solution for over 12 h without significant change.

Next, we analyzed the growth characteristics of AERP-treated cells using the standard MTT assay. In A431 cell lines that over-express the EGFR, treatment with the AERP resulted in a dose-dependent 40% inhibition of cell growth driven by recombinant EGF (Figure 5). On the other hand, Jurkat cells which do not over-express EGF receptors and T6-17 (HER2/neu expressing cells) were unaffected by AERP or CD4.M3 (an unrelated anti-CD4 mimetic) treatment, (The % inhibition for CD4 was 1.63 (T6-17) and 0.35 in Jurkat cells). As AERP-2 showed promising inhibitory results than AERP-2A, further bio-distribution study was only performed employing AERP-2.

Approximately one million A431 cells in PBS were implanted in the right flank of each of the 6 nude mice. After the tumor grew to 1 cm in any dimension, each nude mouse bearing A431 xenografted tumors received 3–6 μCi of purified [^{99m}Tc] DTPA-AERP-2. After 3 h, each animal was anesthetized with 0.2 mL of avertin (a mixture of 39.5 mL of saline and 0.5 mL of avertin stock solution prepared by dissolving 25 g of avertin in 15.5 mL hot *tert*-amyl alcohol) and then sacrificed. Blood was sampled and organs were removed and weighed. Radioactivity in the samples was measured in a gamma well counter.

Bio-distribution data (Table 1) showed that the labeled peptidomimetic (in terms of percentage of injected dose per gram organ, %ID/g) accumulated in the tumor was 5.5 fold greater than that found in the normal tissue. Among the organs studied, kidney showed highest radioactivity uptake in terms of %ID/g due to the hydrophilic nature of the peptides. The EGFR specific probe, [^{99m}Tc] DTPA-AERP-2, displayed a 2.5 fold higher accumulation level in blood and 2.5 fold higher in lungs than in normal tissue. Since these tissues do not over-express EGFR, the uptake of the radioactivity may be partially non-specific. In this circumstance, lipophilicity of a radiolabeled peptidomimetic might be an important factor of determining the level of non-specific cellular uptakes. A peptide of greater lipophilicity might exhibit a higher level of cellular uptakes. Accordingly, the tumor accumulation of a labeled peptidomimetic might include a contribution of non-specific

uptake, which could be related to its lipophilicity. It is presumed that lactates around the tumor cells may be able to mask the receptors^{31,32} and prevent the peptide from interacting with tumor receptors. Non-specific tumor accumulation of some other radiolabeled peptides, proteins, and oligonucleotides has been observed.³³⁻³⁵

While peptide based imaging probes showed promise in tumor targeting, its fast clearance (< 3hr) limits their usefulness in tumor detection. It has been observed that in the category of monoclonal antibody (Mab) based imaging agents, blood clearance of imaging agent is inversely related to the size of the injected antibody or its fragments³⁶. Investigators even claim that the *in vivo* tumor uptake of receptor-binding radiopharmaceuticals is mostly controlled by their elimination rate from the blood than by the level of receptor expression on the cancer cells¹⁰, indicating faster clearance of peptide needs to be improved. In this work, we have identified and evaluated peptidomimetics for targeting EGFR, now we are fusing this peptidomimetics with other small protein to improve the pharmacokinetics. We strongly believe that this is way new tumor targeting peptidomimetics will be developed.

In conclusion, as per our initial preliminary analysis, cell study *in vitro* data indicate that AERP, like the anti-EGFR antibody 225, can specifically inhibit cell growth of EGFR-expressing cells. During the bio-distribution study, the ^{99m}Tc labeled DTPA-AERP-2 preferentially accumulated in the tumor compared to the normal tissue and the ratio of the %ID/g is over 5 fold greater accumulation on tumor than normal tissue. The measured blood serum half-life of the labeled mimetic is about 3 hrs. Moreover, the small size (1200 Dalton MW) of peptidomimetics supports the fact that this class of radiolabeled EGFR specific probe AERP-2 might be developed as useful tumor imaging agents. These studies will be basis for developing AERP-2 as a radiopharmaceutical, as an alternate to antibody based therapy.

Acknowledgments

Authors would like to thank Drs. Kathy Valantine and Josh Wand, Department of biophysics and biochemistry of University of Pennsylvania for providing the solution structure of AERP. This work was partly supported by grants from Society for Nuclear Medicine (R. M), NIH (5P01 CA089480-03) to R.M. and M.I.G., and by grants from Susan G. Komen Breast Cancer Foundation to R.M (IMG0201367).

References

1. Pines G, Kostler WJ, Yarden Y. FEBS Lett. 2010; 584:2699–706. [PubMed: 20388509]
2. Yarden Y, Sliwkowski MX. Nat Rev Mol Cell Biol. 2001; 2:127–37. [PubMed: 11252954]
3. Warren CM, Landgraf R. Cell Signal. 2006; 18:923–33. [PubMed: 16460914]
4. Suo Z, Risberg B, Karlsson MG, Villman K, Skovlund E, Nesland JM. Int J Surg Pathol. 2002; 10:91–9. [PubMed: 12075402]
5. Mellinghoff IK, Tran C, Sawyers CL. Cancer Res. 2002; 62:5254–9. [PubMed: 12234993]
6. Smith V, Hobbs S, Court W, Eccles S, Workman P, Kelland LR. Anticancer Res. 2002; 22:1993–9. [PubMed: 12174876]
7. Korc M. Surg Oncol Clin N Am. 1998; 7:25–41. [PubMed: 9443985]
8. Brandt BH, Roetger A, Dittma T, Nikolai G, Seeling M, Merschjann A, Nofer JR, Dehmer-Moller G, Junker R, Assmann G, Zaenker KS. FASEB J. 1999; 13:1939–49. [PubMed: 10544177]
9. Herbst RS, Shin DM. Cancer. 2002; 94:1593–611. [PubMed: 11920518]
10. Reilly RM, Kiarash R, Sandhu J, Lee YW, Cameron RG, Hendler A, Vallis K, Gariepy J. J. Nucl Med. 2000; 41:903–11. [PubMed: 10809207]
11. Herbst RS, Kies MS. Clin Adv Hematol Oncol. 2003; 1:466–72. [PubMed: 16258434]
12. Laack E, Sauter G, Bokemeyer C. Lung Cancer. 2010; 69:259–64. [PubMed: 20646778]
13. Saukville EA, El-Sayed Y, Monga M, Kim G. Annu Pharmacol Toxicol. 2003; 43:199–231.

14. Tol J, Punt CJ. Clin Ther. 2010; 32:437–53. [PubMed: 20399983]
15. Liu B, Fan Z. Oncogene. 2001; 20:3726–34. [PubMed: 11439335]
16. Herbst RS, Hong WK. Semin Oncol. 2002; 5:18–30. [PubMed: 12422310]
17. Cuartero-Plaza A, Martinex-Miralles E, Rosell R, Vadell-Nadal C, Farre M, Real FX. Clin Can Res. 1996; 2:13–20.
18. Capala J, Barth RF, Bailey MQ, Fenstermaker RA, Marek MJ, Rhodes BA. Bioconjug Chem. 1997; 8:289–295. [PubMed: 9177833]
19. Xu N, Cai G, Ye W, Wang X, Li Y, Zhao P, Zhang A, Zhang R, Cao B. Anticancer Res. 2009; 29:4005–12. [PubMed: 19846943]
20. Gaikam LOT, Huang L, Caveliers V, Keyaerts M, Hernot S, Vaneycken I, Vanhove C, Revets H, De Baetselier P, Lahoutte T. J Nucl Med. 2008; 49:788–95. [PubMed: 18413403]
21. Bell A, Wang ZJ, Arbabi-Ghahroudi M, Chang TA, Durocher Y, Trojahn U, Baardsnes J, Jaramillo ML, Li S, Baral TN, O'Connor-McCourt M, MacKenzie R, Zhang J. Cancer Lett. 2010; 289:81–90. [PubMed: 19716651]
22. Nordberg E, Friedman M, Gostring L, Adams GP, Brismar H, Nilsson FY, Stahl S, Glimelius B, Carlsson J. Nucl Med. Biol. 2007; 34:609–618. [PubMed: 17707800]
23. Blok D, Feitsma RI, Vermeij P, Pauwels EJ. Eur J Nucl Med. 1999; 26:1511–9. [PubMed: 10552097]
24. Reubi JC, Maecke HR. J Nucl Med. 2008; 49:1735–38. [PubMed: 18927341]
25. Buchegger F, Bonvin F, Kosinski M, Schaffland AO, Prior J, Reubi JC, Blauenstein P, Tourwe D, Garcia Garayoa E, Bischof Delaloye A. J Nucl Med. 2003; 44:1649–54. [PubMed: 14530481]
26. Lin KS, Luu A, Baidoo KE, Hashemzadeh-Gargari H, Chen MK, Brenneman K, Pili R, Pomper M, Carducci MA, Wagner HN Jr. Bioconjug Chem. 2005; 16:43–50. [PubMed: 15656574]
27. Chen X, Hou Y, Tohme M, Park R, Khankaldyyan V, Gonzales-Gomez I, Bading JR, Laug WE, Conti PS. J Nucl Med. 2004; 45:1776–83. [PubMed: 15471848]
28. Brunger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. Acta Cryst. 1998; D54:905–921.
29. Berezov A, Zhang HT, Greene MI, Murali R. J Med Chem. 2001; 44:2565–74. [PubMed: 11472210]
30. Park BW, Zhang HT, Wu C, Berezov A, Zhang X, Dua R, Wang Q, Kao G, O'Rourke DM, Greene MI, Murali R. Nat Biotechnol. 2001; 18:194–8. [PubMed: 10657127]
31. Bhujwalla ZM, Artemov D, Aboagye E, Ackerstaff E, Gillies RJ, Natarajan K, Solaiyappan M. Novartis Found Symp. 2001; 240:23–38. discussion 38–45, 152–3. [PubMed: 11727932]
32. Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW, Mueller-Klieser W. Int J Radiat Oncol Biol Phys. 2001; 51:349–53. [PubMed: 11567808]
33. Tsai SW, Sun Y, Williams LE, Raubitschek AA, Wu AM, Shively JE. Bioconjug Chem. 2000; 11:327–34. [PubMed: 10821648]
34. Wu AM, Yazaki PJ, Tsai S, Nguyen K, Anderson AL, McCarthy DW, Welch MJ, Shively JE, Williams LE, Raubitschek AA, Wong JY, Toyokuni T, Phelps ME, Gambhir SS. Proc Natl Acad Sci USA. 2000; 97:8495–500. [PubMed: 10880576]
35. Nakamura K, Fan C, Liu G, Gupta S, He J, Dou S, Kubo A, Rusckowski M, Hnatowich DJ. Bioconjug Chem. 2004; 15:1475–80. [PubMed: 15546217]
36. Rosebrough SF, Grossman ZD, McAfee JG, Kudryk BJ, Subramanian G, Ritter-Hrncirik CA, Witanowski LS, Tillapaugh-Fay G, Urrutia E, Zapf-Longo C. J Med Chem. 1988; 29:1212–22.

AERP-2 (Primary structure: dYCASRDYDYDGRCYFD-NH₂)

AERP-2A (Primary Structure: YCRDYDYDGRYFDCY-NH₂)

Figure 1.
Amino acid sequences of two derivatives of AERP

Figure 2. Solution structure of AERP mimetic analog. The overall fold between AERP-2 and AERP-2A is similar (rmsd for back bone structure is 2.9Å) and rmsd for all non-hydrogen atoms is 5.64Å. The disposition of Arg residues in these two structures are different and the results show the effect of introducing a serine in the cyclic ring of AERP.

Figure 3.
The structure of AERP-DTPA-Tc complex

Figure 4.
HPLC chromatogram of labeled AERP

Figure 5.

Biological activity of EGFR specific AERP as determined by a standard MTT assay. Two different species of AERP were tested in EGFR+ A431 cell lines. EGFR specific AERP show comparable activity to anti-EGFR monoclonal antibody C225 at 10 ug/ml. AERP-2 form shows the best activity.

Table 1Bio-distribution of ^{99m}Tc labeled AERP-2 in rodents with xenografted A431 tumors (N=6)

Organs	$^{99m}\text{Tc}(\text{AERP-2})$	
	% ID/g	Standard deviation
Liver	5.3	0.40
Heart	0.49	0.030
Kidney	5.95	0.15
Lung	0.8	0.010
Spleen	28	2.5
Stomach	11	2.7
Small Intestine	0.565	0.16
Large Intestine	2.4	0.10
Muscle	0.31	0.09
Tumor	1.6	0.10
Blood	0.79	0.010
Tumor/Muscle	5.5	1.3
Tumor/Blood	2.0	0.1