Outcome of Grant report for DESC0008432

Prepared by Zhude Tu, PhD.
2017-08-030

Abstract

The goal of this proposal was to provide critical interdisciplinary research training for the next generation of radiochemists and nuclear medicine physicians through a collaboration between basic science and clinical faculty who are actively involved in the development, application, and translation of radiopharmaceuticals. Following the four year funding support period, the 10 postdocs, graduate students, as well as clinical physicians who received training have become faculty members, or senior radiochemists at different academic institutes or industry. With respect to scientific accomplishments, 26 peer-reviewed articles have been published to date as well as numerous poster and oral presentations. The goals of all four scientific projects were completed and several promising radiotracers identified for transfer into clinical investigation for human use. Some preliminary data generated from this training grant led several successful NIH grant proposals for the principal investigators.

Scientific report

The main goal of this training project was to provide critical interdisciplinary research training for the next generation of radiochemists and nuclear medicine physicians through a collaboration between basic science faculty and clinical faculty members who are actively involved in the development, application, and translation of radiopharmaceuticals. Our PET program in Washington University integrates basic radiochemistry science with nuclear medicine clinical training that is carefully designed to provide a breadth and depth of experience sufficient to prepare a new generation of research scientists and clinical investigators to become leaders in radiochemistry and nuclear medicine. With the financial support of this training grant, trainees received superb multidisciplinary training in radiochemistry and medical imaging which will enable them to maintain their vitality in this field and make advances in nuclear medicine research and clinical practice. During the 4-year period of financial support, as shown in the appendix, we trained ***10 radiochemists and nuclear medicine physicians***. These trainees have become very active investigators in the radiopharmaceutical chemistry and nuclear medicine fields. As examples, Ephraim Parent, MD/PhD, is now is Assistant Professor of Radiology at Emory University School of Medicine, Ahlem Bouhlel, PhD, is Assistant Professor of Pharmacy in Aix-Marseille University, Cerimed, France, Adam Rosenberg, PhD, is a Senior Scientist in the PET program of Vanderbilt University, Brain Wright, PhD, is Senior Radiochemist in University of Alabama at Birmingham, and Iljung Lee, PhD, is a Senior Scientist at the Institute of Radiological & Medical Sciences in Korea.

For scientific accomplishments, we have published 28 peer-reviewed articles to date and presented our research findings at numerous international conferences as either oral presentations or posters. The goals of all four scientific projects were well accomplished. With this financial support, we identified several promising radiotracers that target different biomarker proteins with high potency and high specificity in vivo in animal models of human disease. A couple of radiotracers evaluated through this training grants have been transferred into clinical evaluation for human use or are ready for transferring into clinical investigations. We summarize the accomplishments of each scientific project below.

Project 1: *Fluorine-18 radiolabeling of ligands for nuclear receptors that are important drivers of breast and prostate cancer growth, and diagnostic, prognostic, and predictive biomarkers and therapy targets: Monitoring gene expression driven by the estrogen receptor.* Two radiochemists were trained and five articles published (see the appendix).

Project 2: *Development of radiolabeled antibodies targeting different epitopes of HER2 for breast cancer imaging.* Two radiochemists have been trained and four articles published. Based on the *in vitro* and *in vivo* characterization of several human epidermal growth factor receptor 2 (Her2) imaging agents labeled with ^{64}Cu or ^{89}Zr, we received *a NIH/NCI R21 award (#R21CA182945) led by Dr. Farrokh Dehdashti to test the feasibility of imaging HER2 receptors in breast cancer using ^{89}Zr-trastuzumab.* This will facilitate the clinical implementation of quantifying the HER2 receptors in breast cancer and other cancers.

Project 3: *Radiosynthesis and biological evaluation of novel radio-halogenated α,α-disubstituted amino acids for cancer imaging and therapy.* One radiochemist and one nuclear medicine physician were trained in this project; both trainees are now faculty in academic institutes (see appendix). Five articles were published. *This project resulted in a NIH/NCI R01 award (#R01CA200979) led by Dr. Suzanne Lapi to develop radiolabeled antibodies targeting L-type amino acid transporter-1 (LAT1) for imaging and therapy of prostate cancer*.

Project 4: *Development of PET radiotracers for neuroimaging*. During Year 1, Project 4 was led by Dr. Robert Mach to develop dopamine D3 receptor PET agents. After Dr. Mach left Washington University in July 1, 2017, project leadership was transferred to Dr. Zhude Tu as PI of a project to develop a sphingosine 1-phosphate receptor 1 (S1P1) PET tracer for neuroimaging. A total of four radiochemists were trained and 13 articles were published.

For the D3 receptor PET agents project, *two radiochemists* were trained and *five articles* published. The radiotracer ^{18}F-LS-4134 was approved by FDA for human use and it was transferred into clinical investigation for assessment of the D3 receptor expression in human brain. *The findings of the D3 receptor project contributed a R01 NIH/NIA award (#R01DA029840) led by Dr. Mach to develop and evaluate of PET radiotracers for imaging the dopamine D3 receptor*. For the S1P1 PET tracer project, *two radiochemists* were trained and *eight articles* published. Importantly, in this innovative project, we successfully C-11 labeled a S1P1 radiotracer which was tested in three rodent models of inflammatory disease. The promising results suggest that high feasibility for the use of PET in quantifying S1P1 expression in inflamed tissues as a biomarker of inflammatory response in disease. We also explored diverse structures of new analogues with high binding potency (IC_{50} value < 20 nM for S1P1 and high selectivity for S1P1 (>100-fold for S1P1 versus S1P2-5). These new analogues can be F-18 labeled using a conventional F-18 radiochemistry for further in vivo evaluation. *The outcome of the S1P1 radiotracer project also contributed a new R01 NIH/NINDS&NIA award (#R01NS103988) led by Dr. Tu to developing S1P1 imaging agents for multiple sclerosis. One patent application titled "Compounds for sphingosine-1-phosphate receptor 1 (S1P1) and imaging S1P1 using PET" with application number of # 62/527,259, was filed June 12, 2017.*

Appendix

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Prepared by Zhude Tu

08/01/2012-07/31/2017

Title: Training in Techniques and Translation: Novel Nuclear Medicine Imaging Agents for Oncology and Neurology

The goal of this proposal is to provide critical interdisciplinary research training for radiochemists and nuclear medicine physicians. The training projects draw upon the extensive and diverse expertise of faculty from both of the Dept. of Radiology at Washington University in St. Louis and the Dept. of Chemistry at the University of Illinois at Urbana-Champaign. This faculty team consists of basic science and clinical faculty who are actively involved in the development, application, and translation of radiopharmaceuticals for cancer imaging and neuroimaging.

A. Trainees information

PI's Name	**Trainee's Information**		
	Trainee Name	**Resource Covering Time**	**Current Position and Institute or Company**
Suzanne E. Lapi	Brian Wright	2013.10-2015.10	Postdoctoral Research Associate at University of Alabama at Birmingham
	Lihui Song	2016.5-2016.9	Senior Research Associate University of Missouri at Columbia
John A. Katzenellenbogen/ Dong Zhou	Matt MacDonough	2013.10-2016.8	Technology Specialist at Stradley Ronon Stevens & Young LLP
	Norio Yasui	2012.8-2015.6	Postdoc, University of Alabama at Birmingham
Jonathan E. McConathy	Ahlem Bouhlel	2013.4-2015.10	Assistant Professor of Pharmacy, Aix-Marseille University, CERIMED
	Ephraim Parent	2013.4-2016.11	Assistant Professor of Radiology, Emory University School of Medicine
Robert H. Mach	Xin Peng	72012.8-2013.5	ORISE Fellow - Technology Development Specialist at National Institute of Allergy and Infectious Diseases (NIAID)
	Iljung Lee	2013.2-2013.6	Senior Scientist-Korea Institute of Radiological & Medical Sciences
Zhude Tu	Adam Rosenberg	2013.9-2016.3	Senior Scientist, Vanderbilt University
	Derek Rensing	2016.7-2017.7	Postdoc, Washington University

B. Publications and patents

PI Name	Publications
Project 1	
Suzanne E. Lapi /David Reichert	1. Wright, B., Lapi, S.E, (2013) Designing the Magic Bullet? The Advancement of Immuno-PET into Clinical Use *Journal of Nuclear Medicine* 54:1171-1174 2. Marquez, B.M., Ikotun, O.F., Zheleznyak, A., Wright, B., Hari-Raj, A., Pierce, R.A., Lapi, S.E. (2014) Evaluation of 89Zr-Pertuzumab in breast cancer xenografts, *Molecular Pharmaceutics*;11(11):3988-95 3. Wright, B.D., Whittenberg, J., Desai, A., DiFelice, C., Kenis, P.J.A, Lapi, S.E., Reichert, D. (2016) Microfluidic Preparation of an 89Zr-Labeled Trastuzumab Single Patient Dose *Journal of Nuclear Medicine* 57 (5) 747-52 4. Laforest, R., Lapi, S.E., Oyama, R., Bose, R.,Tabchy, A., Marquez-Nostra, B.V., Burkemper, J., Wright, B.D., Frye, J., Frye, S. Siegel, B.A., Dehdashti, F. (2016) [89Zr]Trastuzumab: Evaluation of Radiation Dosimetry, Safety, and Optimal Imaging Parameters in Women with HER2-Positive Breast Cancer. *Mol Imaging and Biology*. 18(6):952-959.
Project 2	
John A. Katzenellenbogen	1. H. Xing, L. Tang, X. Yang, K. Hwang, W. Wang, Q. Yin, N. Y. Wong, L. W. Dobrucki, N. Yasui, J. A. Katzenellenbogen, W. G. Helferich, J. Cheng, Y. Lu Y. Selective Delivery of an Anticancer Drug with Aptamer-Functionalized Liposomes to Breast Cancer Cells in Vitro and in Vivo. *J Mater Chem B Mater Biol Med.* 2013, *1*:5288-5297. PMID: 24159374; PubMed Central PMCID: PMC3800741. 2. S. E. Royston, N. Yasui, A. G. Kondilis, S. V. Lord, J. A. Katzenellenbogen, M. M. Mahoney. ESR1 and ESR2 Differentially Regulate Daily and Circadian Activity Rhythms in Female Mice. *Endocrinology*, 2014, *155*, 2613-2623. PMID: 24735329 3. D. Zhou, M. Lin, N. Yasui, M. H. Al-Qahtani, C. S. Dence, S. Schwarz, J. A. Katzenellenbogen. Optimization of the Preparation of Fluorine-18-Labeled Steroid Receptor Ligands 16alpha-[^{18}F]Fluoroestradiol (FES), [^{18}F]Fluoro Furanyl Norprogesterone (FFNP), and 16beta-[^{18}F]Fluoro-5alpha-Dihydrotestosterone (FDHT) as Radiopharmaceuticals. *J Labelled Comp Radiopharm.*, 2014, *57*, 371-377. PMID: 24861984 PMCID: PMC4138509 4. S. M. Moore, A. J. Khalaj, S. Kumar, Z. Winchester, J. Yoon, T. Yoo, L. Martinez-Torres, N. Yasui, J. A. Katzenellenbogen, S. K. Tiwari-Woodruff. Multiple functional therapeutic effects of the estrogen receptor β agonist indazole-Cl in a mouse model of multiple sclerosis. *Proc Natl Acad Sci USA,* 2014, *111*, 18061-18066. PMID: 25453074 PMCID: PMC4273334 5. N. Yasui, C. G. Mayne, J. A. Katzenellenbogen. Preparation of o-Fluorophenols from Nonaromatic Precursors: Mechanistic Considerations for Adaptation to Fluorine-18 Radiolabeling. *Org. Lett.*, 2015, *17*, 5540-5543. PMID: 26536250

Project 3	
Jonathan E. McConathy	1. Gauvain K, Ponisio MR, Barone A, Grimaldi M, Parent E. Leeds H, Goyal M, Rubin J, McConathy J. ^{18}F-FDOPA PET/MRI for monitoring early response to bevacizumab in children with recurrent brain tumors. Neuro-Oncology Practice. 2017, In Press. 2. Bouhlel A, Alyami W, Li A, Yuan L, Rich K, McConathy J. Effect of alpha-Methyl versus alpha-Hydrogen Substitution on Brain Availability and Tumor Imaging Properties of Heptanoic [F-18]Fluoroalkyl Amino Acids for Positron Emission Tomography (PET). J Med Chem. 2016;59(7):3515-31. doi: 10.1021/acs.jmedchem.6b00189. PubMed PMID: 26967318; PMCID: PMC4957663. 3. Bouhlel A, Zhou D, Li A, Yuan L, Rich KM, McConathy J. Synthesis, Radiolabeling, and Biological Evaluation of (*R*)- and (S)-2-Amino-5-[^{18}F]fluoro-2-methylpentanoic Acid ((*R*)-, (*S*)-[^{18}F]FAMPe) as Potential Positron Emission Tomography Tracers for Brain Tumors. J Med Chem. 2015;58(9):3817-29. doi: 10.1021/jm502023y. PubMed PMID: 25843369; PMCID: PMC4476065. 4. Fraum TJ, Fowler KJ, McConathy J, Parent EE, Dehdashti F, Grigsby PW, Siegel BA. PET/MRI for the body imager: abdominal and pelvic oncologic applications. Abdom Imaging. 2015;40(6):1387-404. doi: 10.1007/s00261-015-0390-3. PubMed PMID: 25805557.

Project 4	
Robert H. Mach	1. Zhou D, Chu W, Xu J, Jones LA, Peng X, Li S, Chen DL, Mach RH. Synthesis, [^{18}F]radiolabeling, and evaluation of poly (ADP-ribose)polymerase-1 (PARP-1) inhibitors for in vivo imaging of PARP-1 using positron emission tomography. *Bioorg. Med. Chem*. 2014; 22: 1700-1707. 2. Zhou D, Chu W, Peng X, McConathy J, Mach RH, Katzenellenbogen JA. Facile purification and click labeling with 2-[^{18}F]fluoroethylazide using solid phase extraction cartridges. *Tetrahedron Letters* 2015; 56: 952-954. 3. Peng X, Wang Q, Mishra Y, Xu J, Reichert DE, Malik M, Taylor M, Luedtke RR, Mach RH. Synthesis, pharmacological evaluation and molecular modeling studies of triazole containing dopamine D3 receptor ligands. *Bioorg. Med. Chem. Lett*. 2015; 25: 519-523. 4. Chu W, Zhou D, Gaba V, Liu J, Li S, Peng X, Xu J, Dhavale D, Bagchi DP, D'Avignon DA, Shakerdge NB, Bacskai BJ, Tu Z, Kotzbauer PT, Mach RH. Design, synthesis and characterization of 3-(Benzylidene)indolin-2-one derivatives as ligands for α-synuclein fibrils. *J. Med. Chem*. 2015; 58: 6002-6017. 5. Lee I, Lieberman BP, Li S, Hou C, Makvandi M, Mach RH. Comparative evaluation of 4 and 6-carbon spacer conformationally flexible tetrahydroisoquinolinyl benzamide analogues for imaging the sigma-2 receptor status of solid tumors. *Nucl. Med. Biol*. 2016; 43: 721-731.

Zhude Tu	1. Rosenberg A.J., Liu H., and Tu Z. (2015) A practical process for the preparation of [^{32}P]S1P and binding assay for S1P receptor ligands. *Applied Radiation Isotopes. 102: 5-9.* 2. Yue X., Jin H., Liu H., Rosenberg A.J., Klein R.S. and Tu Z. (2015) A potent and selective C-11 labeled PET tracer for imaging sphingosine-1-phosphate receptor 2 in the CNS demonstrates sexually dimorphic expression. *Organic & Biomolecular Chemistry. 13, 7928-7939.* 3. Rosenberg A.J., Liu H., Jin H., Yue X., Riley S., Brown S.J., and Tu Z. (2016) Design, synthesis and *in vitro* and *in vivo* evaluation of an ^{18}F-labeled sphingosine 1-phosphate receptor 1 ($S1P_1$) PET tracer. *Journal of Medicinal Chemistry. 59(13):6201–6220.* 4. Liu, H., Jin, H., Yue, X., Luo, Z., Liu, C., Rosenberg, A.J., and Tu, Z. (2016) PET imaging study of S1PR1 expression in a rat model of multiple sclerosis. *Molecular Imaging and Biology*. *18(5): 724–732.* 5. Han J., Liu H., Liu C., Jin H., Perlmutter JS., Egan T.M., and Tu Z. (2017) Pharmacologic characterizations of a P2X7 receptor-specific radioligand, [^{11}C]GSK1482160, for neuroinflammatory response. *Nuclear Medicine Communications. 38(5):372-382.* 6. Liu H., Jin H., Yue X., Han J., Baum P., Abendschein D.R., and Tu Z. (2017) PET study of sphingosine-1-phosphate receptor 1 expression in response to vascular inflammation in a rat model of carotid injury. *Molecular Imaging*, *16:1-7.* 7. Jin H., Yang H., Liu H., Zhang Y., Zhang X., Rosenberg A.J., Liu Y., Lapi S.E., and Tu Z. (2017) A promising carbon-11 labeled sphingosine-1-phosphate receptor 1 specific PET tracer for imaging vascular injury. *Journal of Nuclear Cardiology. 24(2):558-570.* 8. Liu H., Jin H., Han J., Yue X., Yang H., Zayed M.A., Gropler R.H., and Tu Z. (2017) Upregulated sphigosine-1 phosphate receptor 1 expression in human and murine atherosclerotic plaques. *Molecular Imaging and Biology*. *DOI: 10.1007/s11307-017-1141-3.*