ACS Chemical Neuroscience

Review

pubs.acs.org/chemneuro

Identifying Novel Radiotracers for PET Imaging of the Brain: Application of LC-MS/MS to Tracer Identification

Vanessa Barth* and Anne Need

Eli Lilly and Co., Lilly Research Laboratories, Indianapolis, Indiana 46285, United States

ABSTRACT: Nuclear medicine imaging biomarker applications are limited by the radiotracers available. Radiotracers enable the measurement of target engagement, or occupancy in relation to plasma exposure. These tracers can also be used as pharmacodynamic biomarkers to demonstrate functional consequences of binding a target. More recently, radiotracers have also been used for patient tailoring in Alzheimer's disease seen with amyloid imaging. Radiotracers for the central nervous system (CNS) are challenging to identify, as they require a unique intersection of multiple properties. Recent advances in tangential technologies, along with the use of iterative learning for the purposes of deriving in silico models, have opened up additional opportunities to identify radiotracers. Mass spectral technologies and in silico modeling have made it possible to measure and predict in vivo characteristics of molecules to indicate potential tracer performance. By analyzing these data alongside other measures, it is possible to delineate guidelines to increase the likelihood of selecting compounds that can perform as radiotracers or serve as the best starting point to develop a radiotracer following additional structural modification. The application of mass spectrometry based technologies is an efficient way to evaluate compounds as tracers in vivo, but more importantly enables the testing of potential tracers that have either no label site or complex labeling chemistry which may deter assessment by traditional means; therefore, use of this technology allows for more rapid iterative learning. The ability to differentially distribute toward target rich tissues versus tissue with no/less target present is a unique defining feature of a tracer. By testing nonlabeled compounds in vivo and analyzing tissue levels by LC-MS/MS, rapid assessment of a compound's ability to differentially distribute in a manner consistent with target expression biology guides the focus of chemistry resources for both designing and labeling tracer candidates. LC-MS/MS has only recently been used for de novo tracer identification; however, this connection of mass spectral technology to imaging has initiated engagement from a wider community that brings diverse backgrounds into the tracer discovery arena.

KEYWORDS: *Positron emission tomography (PET), small molecule, tracer, liquid chromatography coupled to mass spectrometry (LC-MS/MS), physicochemical properties, lipophilicity, biomarker*

Drug discovery has increasingly become more costly.[1−3] As companies strive to reduce late stage clinical failures, the ability to measure biomarkers has become critical. Biomarkers allow for assessment of target engagement or occupancy, downstream functional changes, and impact on disease pathophysiology.[4] Biomarkers can bridge the preclinical effects measured for a given drug to the parallel clinical readout. They provide confidence that drugs are finding and binding their intended target or influencing an intended biochemical pathway thought to be linked to the disease of interest.

There are a variety of technology platforms to make these measurements, but for many central nervous system disorders such techniques are limited by the samples that one can collect. In these cases imaging is an informative option that can produce a variety of readouts depending on the modality chosen: X-ray, computed tomography (CT), ultrasound, magnetic resonance imaging (MRI) based techniques, and nuclear medicine based techniques. In the latter case, the readouts include target occupancy, endogenous tone of neurotransmitter systems, pathophysiology such as inflammation and amyloid burden, and target expression or target activity and the variation of these in disease states.[5,6] The more common application of nuclear imaging in the CNS is to assess the relationship between target occupancy and exposure. This translational approach is applied in drug discovery to select doses of therapeutic molecules entering Phase 2. This can only be accomplished if a radiotracer exists for the target of interest.[7] A molecule under development can be administered to subjects followed by the radiotracer. These subjects then undergo a scan to quantify how much of the tracer is able to bind the target. The more therapeutic agent that reaches the target, the less target that is available for the tracer to bind. As such, as the occupancy of the drug goes up, the tracer signal decreases. Percent occupancy values can be calculated by comparing a subject's baseline scan, representing zero percent occupancy, to the blocked scan. The occupancy value can be linked to the dose of the drug administered as well as the plasma levels of the drug. For most drug development programs, there is

Received: April 7, 2014
Revised: May 13, 2014
Published: May 14, 2014

a hypothesis regarding the level of target occupancy that needs to be achieved to have an impact on the disease state. A classic example of this is achieving approximately 60% dopamine D_2 occupancy for antipsychotic drugs to achieve positive benefits in schizophrenia.[7] The ability to evaluate a single dose in the clinic for efficacy enables smaller trials with the certainty that the designed clinical trial is testing the hypothesis.[8] Organizations do not have to ask "What if we had just dosed higher?"

In all of these applications there is a single common prerequisite: a radiotracer. Without these unique tool molecules, the applications mentioned are not feasible. The ease with which a radiotracer can be discovered and developed is a function of both the biology and the available/known chemical space.[9] Biological feasibility is a function of target type and level of expression. Some targets are more easily approached than others. This is exemplified in the literature where radiotracers exist in abundance for G protein coupled receptors (GPCRs), followed by intracellular enzymes, and some ligand gated ion channels. Fewer radiotracers exist for nuclear hormone receptors, epigenetic targets, voltage gated ion channels, and kinases, for instance. Chemical space refers to small molecule (molecular weight < 400) starting points with known affinity for the target of interest. While in vitro assays can be employed to discover new chemical matter with target affinity, most tracer discovery projects begin from chemical matter where target activity has already been measured. For a given target, the available chemical space may differ from one scientist to another. An individual's available chemical space is composed of what is in the public domain as well as what may be private chemical matter within the scientist's collaborations, laboratory, or institution. For some targets, successful radiotracer discovery is a lower hurdle because the target is highly expressed, or because a plethora of chemical space is available for tracer design. Conversely, the target may be quite challenging due to low levels of expression or, as is common with novel targets, limited to no known chemical space.

What makes it so difficult to find a radiotracer for a particular target? It appears there is a narrow window within which a balancing act must take place (Figure 1). In the case of CNS radiotracers, this requires selective, high affinity molecules (K_d < 10 nM) that are lipophilic enough to allow for sufficient blood-brain barrier penetration but that do not possess high levels of nonspecific binding. While many properties must come together for a radiotracer to function properly for imaging purposes, the unique defining feature is the ability to differentially distribute toward target rich tissues versus tissue devoid of or expressing lower levels of the target.[10] Tracer binding in the target rich tissue represents the total binding and is composed of the specific binding of the tracer to the target as well as the nonspecific binding to everything else, including other proteins present, whereas the binding in the tissue devoid of target represents the nonspecific binding only. While this sounds rather straightforward, assessing nonspecific binding measures and how they translate in vivo is not. Currently, calculated and measured cLogP/D guides lipophilicity assessment; however, falling within a range (1.5−3.5) in combination with high target affinity does not guarantee success.[11] A relatively new application of an established technique employs assessing plasma and brain unbound fraction.[12] This measure indicates what percentage of the compound is free to cross the blood brain barrier and free to interact with the target. It appears that plasma unbound fractions of greater than 10% and brain unbound fractions of greater than 1% increase the chance for tracer success.[13] While useful in evaluating potential tracers, these measures do not take into account the tightness of the nonspecific interaction, leaving a gap in tracer assessment. Attempts to build on these measures have leveraged the merging of multiple variables such as unbound tracer exposure relative to affinity, and the mouse brain uptake assay (MBUA).[13] More recently, sophisticated modeling has been applied within a target class to select the best compounds to move forward for imaging based on the compounds' predicted time activity curves.[14,15]

In tracer discovery, compounds are assessed in in vitro binding and functional assays to understand potency at the target of interest as well as the degree of selectivity against other targets (Figure 2). Compounds are then evaluated from a perspective of their calculated properties and confirmed with some of those values being measured: molecular weight, cLogP/D, passive permeability, solubility, and others. Compounds that meet established criteria are then traditionally radiolabeled, thus requiring resynthesis of the parent and/or precursors as well as synthetic work for incorporation of a radioisotope (assuming a label site exists). After molecules are labeled, autoradiography can be performed to assess if the tracer distribution matches the target's known expression profile, and estimate nonspecific binding. The next steps typically include PET imaging in rodent and/or nonhuman primate. In many cases, radiolabeled tracers will bypass autoradiography and/or ex vivo analysis and move directly into animals for noninvasive PET imaging.

After the initial preclinical PET imaging study is complete, positive data result in additional studies to replicate and validate specific binding of the tracer followed by evaluation in human. If the data are negative, a variety of issues could have arisen which will require the team to iterate and select a new compound or the same compound with a different labeling site/strategy. Potential outcomes that cause iteration include low brain uptake (SUV),[16] low signal to background (requiring improved K_d (dissociation constant) or reduced nonspecific binding),[17] radiometabolites and/or defluorination,[18] species differences in ABC transporters,[19] and low radiochemical yield.[20] This traditional tracer discovery path requires that institutions have access to certain technologies. In the preclinical discovery space, this generally consists of the ability to radiolabel small molecules with tritium, iodide, or fluoride to assess the molecules using either autoradiography or ex vivo binding. Whether these resources exist in house or through a partner, these techniques require the availability of appropriate precursors, appropriate licensing and training to work with radioactivity, and the time to work out and conduct reactions. In the best cases, these processes yield labeled molecules on the order of weeks and require significant

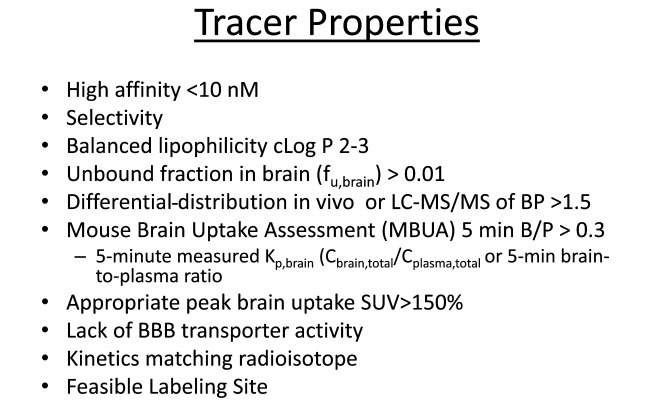

Figure 1. Properties to consider when searching for a tracer.

dx.doi.org/10.1021/cn500072r | ACS Chem. Neurosci. 2014, 5, 1148−1153

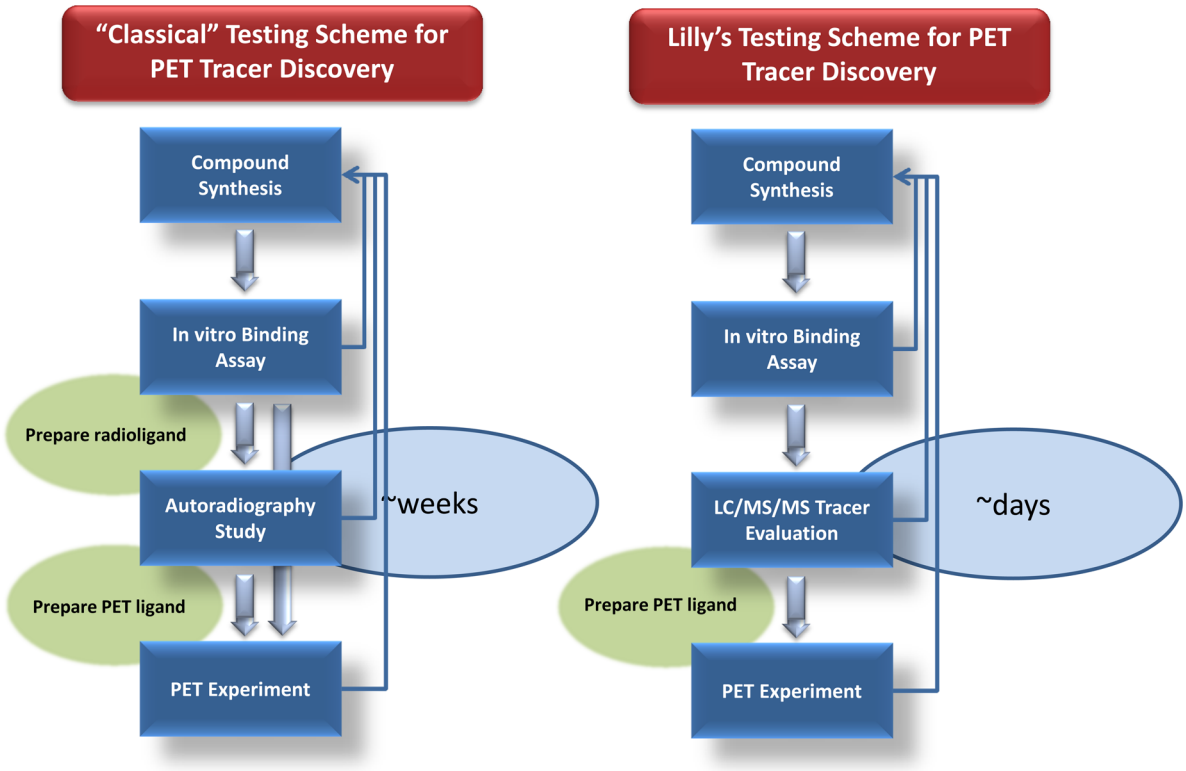

Figure 2. Traditional and LC-MS/MS drive flow schemes for identification of tracers.

chemistry investment from multiple angles as well as access to more expensive equipment. Additionally, compounds of interest that are not easily labeled or do not have a labeling site are passed over and not evaluated. A great opportunity exists and is currently being explored to leverage microfluidic devices which would enable small scale fluorine radiochemistry to take place on the standard benchtop.[21] Such advances would increase the access to labeling technology for experimentation.

In the case of preclinical PET imaging, many institutions begin with rodent PET, followed by nonhuman primate PET evaluation, and then evaluate the tracer in human. The investment for such imaging studies is large with access to a cyclotron, radiochemistry, and a PET camera being the limiting factors. While one cannot circumvent these needs, there is the potential to eliminate unnecessary preclinical PET experiments in an effort to move into human more quickly and in a more cost-effective manner by evaluating potential tracers in vivo in rodent with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).[22−28] Applying LC-MS/MS enables the sampling of chemical space for compounds that provide a signal relative to background or binding potential in vivo. It exploits the same biology that PET measures by comparing levels of the tracer in a total binding region to that of a null or target deficient region representing nonspecific binding. In many cases, across both technologies, the null region ends up being a pseudonull region but still serves its purpose: to identify chemical space with high affinity, selectivity (built in to the molecule or via the biology), and low nonspecific binding.[24,28,29] Herein, lies one of the main values of the initial LC-MS/MS readout on tracer performance: the capacity to quickly assess with minimal resource investment the ability of small molecules to differentially distribute in vivo without artificial wash steps but rather based on clearance and diffusion. An example of LC-MS/MS derived data is seen in Figure 3 using the dopamine D_2/D_3 tracer

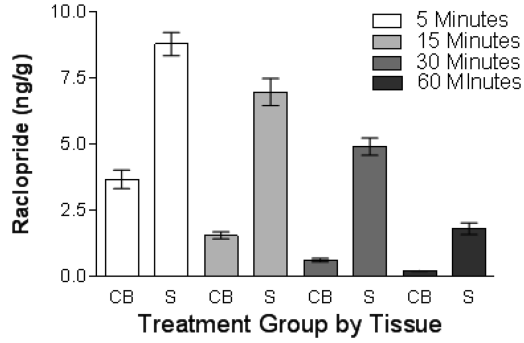

Figure 3. Raclopride measured by LC-MS/MS in rat (n = 4) striatum and cerebellum following intravenous administration.

raclopride.[22] Evaluating a 3 ug/kg dose of raclopride in rat demonstrates the molecule's ability to find and bind the target receptor in the striatum relative to the null tissue, cerebellum, at all time points. The signal to background, or binding potential, grows with time as raclopride clears from the null tissue more rapidly than the total binding tissue until the signal stabilizes, or reaches pseudoequilibrium. Measures of kinetics and uptake are also feasible from this single experiment and align well with what PET data have been published on raclopride.[30] Such an experiment takes a day with results available to analyze the following day as long as the cold parent material is on site. Not depicted here is the pharmacological validation. Such validation can leverage KO mice or the use of well-characterized tool compounds to reduce tracer target specific binding upon administration.[22,24,27,28] This LC-MS/MS technique can also be applied to compounds known to bind irreversibly. The readouts are the same; however, as to be expected the tissue

dx.doi.org/10.1021/cn500072r | ACS Chem. Neurosci. 2014, 5, 1148−1153

kinetics will differ and later time points will be needed to fully characterize the compound.

After the ability of a chemical entity to differentially distribute is established and validated, property optimization and the design of a radiochemistry synthetic pathway should be the next steps. It appears from our own de novo tracer discovery projects it is easier to identify chemical space that yields an in vivo differential distribution first, and then tweak or build in other requirements for PET imaging such as increased brain penetration, a radiolabeling site, improved kinetics, and/or reduction of radiometabolites.[24,28] If initial tracer molecules can satisfy all of these requirements in parallel, that is ideal; however, in some cases, this distracts researchers from finding the best chemical space within which to design tracers yielding the best specific binding window or binding potential.

The mass spectrometers employed in these evaluations are standard triple quadropole instruments that are relatively inexpensive on the spectrum of such equipment (200−300 K). Sensitivity varies with tissue as a function of matrix interference; in the case of brain tissue, most compounds can easily be detected down to 0.1−0.3 ng/g. The typical starting experiment to evaluate a potential molecule as a tracer in vivo is to assess a low (3 ug/kg) and a high (30 ug/kg) dose of the compound coupled with sacrifice intervals of 20 and 40 min post dose. Tissues are harvested, weighed, and kept on ice until probe sonication in 100% acetonitrile with 0.1% formic acid. Homogenized tissue is centrifuged and supernatant is diluted with sterile water. Liquid chromatography is used to separate the analyte of interest for mass spectral detection and quantification. The chromatography consists of using a standard reverse phase column packed with C18, and a mobile phase composed of various ratios of organic to water. Experimental samples are compared to known spiked tissue standards to quantitate the amount of analyte present. In initial screening, rigor is not applied to obtain exact quantities of compound in the tissues, as the more important readout is the ability of the compound to differentially distribute toward the target rich tissue versus the low to no expression tissue. If there is not a tissue present that has no to low expression, one can compare a vehicle treated group to a group pretreated with a centrally penetrant compound with high affinity for the target to determine if the potential tracer's levels are reduced by blockade.

While these tracer doses are higher than those typically used for PET imaging, approximately 0.1−10 ng injected i.v. to a rat, they still permit assessment of the fundamental property of a tracer: the ability to distribute in a pattern matching the expression of the target protein. When translating to PET studies, given increased sensitivity for detecting the tracer, the dose of the tracer administered will decrease relative to the dose administered in the LC-MS/MS assessment. This will enhance the binding potential assuming no species differences in expression levels and/or affinity. Given the higher doses used in the LC-MS/MS technique, there could be concerns that there would be pharmacological effects of the tracer. However, even if the tracer occupies a higher level (<5%)[31] of target site than is traditionally considered acceptable, it is unlikely for an antagonist to produce pharmacological effects. In the case of an agonist, this is something to monitor. It has been shown with labeled and nonlabeled raclopride that the difference in tracer dose did not impact the D_2 occupancy measured for eight antipsychotics.[22]

To validate this technique, multiple clinical PET tracers routinely employed across imaging centers were evaluated by LC-MS/MS in rodent in a manner similar to raclopride: a reverse translation.[13,32] Data exemplified in the raclopride data set are quickly obtained in 24 h for these established ligands. While binding potentials (BP_{ND}) and standardized uptake values (SUV) did not match one to one with the PET data, it is evident that the LC-MS/MS readout can be used to improve the likelihood of successful outcomes by PET. Potential explanations for this include the species differences in B_{max} and K_d when comparing rat to nonhuman primate and human data, the LC-MS/MS readout of the parent tracer does not include effects of radiometabolites, and the data pulled from the publications were from single points on time activity curves. Nonetheless, values generally seem to be within a few fold of each other. After assessing many clinical PET tracers and evaluating de novo tracer identification projects, it appears peak SUVs of greater than 150% at 5 min post i.v. dose and BP_{ND} of greater than 1.5 (ratios of total to nonspecific binding of 2.5) lead to tracers that have adequate specifically bound brain signal.[13] A number of PET tracers have been discovered leveraging LC-MS/MS tracer data including CB1, NOP-1, Kappa, and mGluR1.[23,24,26,28]

While no technique yields all the answers, there are different paths to obtaining a similar output: a tracer to test by PET imaging. Various institutions employ different strategies to achieve success, and these strategies each have their own strengths and weaknesses, and in many cases are driven by access to particular resources. Neither the traditional preclinical PET nor the LC-MS/MS based technique accounts for species differences in expression (B_{max}) or affinity (K_d). This can be partially circumvented by using humanized animals. The LC-MS/MS application has addressed some issues associated with the traditional method of tracer discovery, including the additional time and capabilities required to radiolabel tracer candidates. The application of LC-MS/MS to tracer discovery does have its own short comings. The risks of radiometabolites that contaminate the signal are not well addressed in the LC-MS/MS. In order to assess metabolites, standards need to be synthesized to quantitate against. In many instances, the metabolites generated after a 3/30 ug/kg dose are at such a low level they are challenging to detect and quantitate; however, in some circumstances, this has been viable. That being said, it appears the best way to assess radiometabolite liability is to image higher order primates. With both radiometabolites and defluorination, there are often species differences where preclinical studies do not predict issues in higher order species as seen, for instance, in the development of the mGluR5 tracer, SP203.[33]

Another of the drawbacks of mass spectrometry based tracer identification is that it relies on the ability of the experimenter to recognize and accurately dissect the region of interest in a rodent brain. In some cases, the region of highest target expression is difficult to distinguish from surrounding tissue. In other cases, the region of interest is a relatively small nucleus within a larger, easily dissected brain region. Dissection and analysis of the larger brain region can lead to dilution of the specific tracer signal by increased nonspecific binding. These issues are less problematic in autoradiography due to the images generated. Recent gains in sensitivity, resolution, and ease of use of desorption electrospray ionization (DESI)[34] and matrix-assisted laser desorption (MALDI)[35] may provide a mass spec based solution in these situations. Both of these mass spectrometry ionization techniques have demonstrated the ability to generate images of various molecular species, including small molecules, within tissues slices. They could prove useful in tracer identification as

dx.doi.org/10.1021/cn500072r | ACS Chem. Neurosci. 2014, 5, 1148−1153

these smaller regions could potentially be more easily visualized and identified with these techniques.

Additional shortcomings of the LC-MS/MS technique include the possibility for false negatives and the requirement for the terminal use of animals. For targets with ubiquitous distribution or extremely low expression levels, or for compounds with minimal selectivity relative to other target family members, the LC-MS/MS screen purely for a differential distribution might lead one to overlook a potential tracer candidate. In such circumstances, the use of KO mice to prove on target binding or well characterized blocking agents to validate specific binding are essential. While animals are being used in higher number relative to when advancing directly into preclinical PET imaging studies, autoradiography and ex vivo binding experiments have similar to larger animal usage requirements.

While there are shortcomings, the advantages of the LC-MS/MS method are numerous. Though the LC-MS/MS is expensive, it is much less so than access to a cyclotron and PET camera, and they are typically available at most medium to large institutions. This lower cost and general accessibility could allow for additional contributors to enter the field with their unique chemical libraries, thereby significantly increasing the number of potential tracer compounds evaluated. The LC-MS/MS based method also allows for the ability to test more compounds in vivo more quickly. This could lead to more rapid identification of a potential tracer for human evaluation. It also facilitates an iterative learning process which helps in identifying and linking changes in structure to property space and in turn to in vivo performance (binding potential, kinetics, uptake). Circumventing autoradiography and rodent PET studies and moving straight into nonhuman primate reduces radioactive waste. Application of the LC-MS/MS approach can also aid in deciding if tackling more complex radiochemistry is worthwhile by testing molecules that do not possess a label site or require complex time-consuming radiochemistry. Additionally, there is the option to multiplex and assess multiple tracer candidates in a single animal/experiment.[36]

Some laboratories proceed directly into nonhuman primate PET imaging studies with a radiolabeled tracer, potentially saving time by not performing ex vivo binding and autoradiography (Figure 2). However, the LC-MS/MS application can evaluate more molecules more quickly as potential tracers with respect to uptake, distribution, and kinetics. Logistically, multiple compounds can be assessed daily where this would not be feasible or sustainable for monkey PET studies. By evaluating potential tracers by LC-MS/MS first, PET imaging resources can be focused on testing the more promising tracer candidates.

Regardless of technique or tracer screening paradigm, PET imaging approaches have not been applied as much in the periphery. From a drug discovery perspective, there appears to be little engagement from non-neuroscience therapeutic areas. An opportunity exists to better understand the relationship between target engagement, duration of action, efficacy, and unwanted side effects in these other therapeutic areas with peripheral targets. While PET imaging may be challenging or not feasible due to the location of organs relative to tracer metabolism or movement, there still appears to be the opportunity to influence pharmacokinetic/pharmacodynamic modeling to better predict clinical efficacious dose.

While the mass spectrometry based applications are nontraditional in the tracer discovery space, they enable target engagement assays and tracer discovery within a new population of individuals and organizations. These additional perspectives bring diversity to learning regarding what makes a good tracer and how to most efficiently discover these tools. With technologies evolving, this is an opportunity to leverage different techniques to identify novel tracers for new targets, pathways, and diseases. It is also an opportunity to grow the tracer discovery community and harness diversity to make additional gains in understanding the properties that coalesce to define a small molecule's ability to function as a tracer (Figure 1). This will ultimately lead to the common goal to noninvasively elucidate the roles of more challenging targets and biochemical pathways in healthy and diseased patients.

AUTHOR INFORMATION

Notes

The authors declare the following competing financial interest(s): Both authors are employed by Eli Lilly and Company.

ACKNOWLEDGMENTS

We thank and dedicate this review to Dr. Lee Phebus who invented the idea of applying LC-MS/MS to measure both target occupancy and tracer performance with nonlabeled compounds.

REFERENCES

(1) Suryawanshi, S., Zhang, L., Pfister, M., and Meibohm, B. (2010) The current role of model-based drug development. *Expert Opin. Drug Discovery 5*, 311−321.

(2) FitzGerald, G. A. (2011) Re-engineering drug discovery and development. *LDI Issue Brief 17*, 1−4.

(3) Creeden, J. (2012) Building bridges to the future of medicine: recommendations for boosting development of novel and companion diagnostics. *Pharmacogenomics 13*, 1651−1659.

(4) Kim, E., Howes, O. D., and Kapur, S. (2013) Molecular imaging as a guide for the treatment of central nervous system disorders. *Dialog. Clin. Neurosci. 15*, 315−328.

(5) Zimmer, L., and Luxen, A. (2012) PET radiotracers for molecular imaging in the brain: past, present and future. *NeuroImage 61*, 363−370.

(6) Lister-James, J., Pontecorvo, M. J., Clark, C., Joshi, A. D., Mintun, M. A., Zhang, W., Lim, N., Zhuang, Z., Golding, G., Choi, S. R., Benedum, T. E., Kennedy, P., Hefti, F., Carpenter, A. P., Kung, H. F., and Skovronsky, D. M. (2011) Florbetapir f-18: a histopathologically validated Beta-amyloid positron emission tomography imaging agent. *Semin. Nucl. Med. 41*, 300−304.

(7) Tauscher, J., and Kapur, S. (2001) Choosing the right dose of antipsychotics in schizophrenia: lessons from neuroimaging studies. *CNS Drugs 15*, 671−678.

(8) Ridler, K., Gunn, R. N., Searle, G. E., Barletta, J., Passchier, J., Dixson, L., Hallett, W. A., Ashworth, S., Gray, F. A., Burgess, C., Poggesi, I., Bullman, J. N., Ratti, E., Laruelle, M. A., and Rabiner, E. A. (2014) Characterising the plasma-target occupancy relationship of the neurokinin antagonist GSK1144814 with PET. *J. Psychopharmacol. 28*, 244−253.

(9) Zhang, L., Villalobos, A., Beck, E. M., Bocan, T., Chappie, T. A., Chen, L., Grimwood, S., Heck, S. D., Helal, C. J., Hou, X., Humphrey, J. M., Lu, J., Skaddan, M. B., McCarthy, T. J., Verhoest, P. R., Wager, T. T., and Zasadny, K. (2013) Design and Selection Parameters to Accelerate the Discovery of Novel Central Nervous System Positron Emission Tomography (PET)Ligands and Their Application in the Development of a Novel Phosphodiesterase 2A PET Ligand. *J. Med. Chem. 56*, 4568−4579.

(10) Mintun, M. A., Raichle, M. E., Kilbourn, M. R., Wooten, G. F., and Welch, M. J. (1984) A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann. Neurol. 15*, 217−227.

(11) Waterhouse, R. N. (2003) Determination of lipophilicity and its use as a predictor of blood-brain barrier penetration of molecular imaging agents. *Mol. Imaging. Biol. 5*, 376−389.
(12) Zoghbi, S. S., Anderson, K. B., Jenko, K. J., Luckenbaugh, D. A., Innis, R. B., and Pike, V. W. (2012) On quantitative relationships between drug-like compound lipophilicity and plasma free fraction in monkey and human. *J. Pharm. Sci. 101*, 1028−1039.
(13) Joshi, E. M., Need, A., Schaus, J., Chen, Z., Benesh, D., Mitch, C., Morton, S., Raub, T. J., Phebus, L., Vanessa, B. (2014) Efficiency Gains in Tracer Identification for Nuclear Imaging: Can in Vivo LC-MS/MS Evaluation of Small Molecules Screen for Successful PET Tracers? *ACS Chem. Neurosci.* (submitted).
(14) Guo, Q., Owen, D. R., Rabiner, E. A., Turkheimer, F. E., and Gunn, R. N. (2012) Identifying improved TSPO PET imaging probes through biomathematics: the impact of multiple TSPO binding sites in vivo. *NeuroImage 60*, 902−910.
(15) Gunn, R. N., Summerfield, S. G., Salinas, C. A., Read, K. D., Guo, Q., Searle, G. E., Parker, C. A., Jeffrey, P., and Laruelle, M. (2012) Combining PET biodistribution and equilibrium dialysis assays to assess the free brain concentration and BBB transport of CNS drugs. *J. Cereb. Blood Flow Metab. 32*, 873−883.
(16) Poisnel, G., Oueslati, F., Dhilly, M., Delamare, J., Perrio, C., Debruyne, D., and Barré, L. (2008) 11C]-MeJDTic: a novel radioligand for kappa-opioid receptor positron emission tomography imaging. *Nucl. Med. Biol. 35*, 561−569.
(17) Fujita, M., Zoghbi, S. S., Crescenzo, M. S., Hong, J., Musachio, J. L., Lu, J. Q., Liow, J. S., Seneca, N., Tipre, D. N., Cropley, V. L., Imaizumi, M., Gee, A. D., Seidel, J., Green, M. V., Pike, V. W., and Innis, R. B. (2005) Quantification of brain phosphodiesterase 4 in rat with (R)-[11C]Rolipram-PET. *NeuroImage 26*, 1201−1210.
(18) McCarron, J. A., Pike, V. W., Halldin, C., Sandell, J., Sóvágó, J., Gulyas, B., Cselényi, Z., Wikström, H. V., Marchais-Oberwinkler, S., Nowicki, B., Dollé, F., and Farde, L. (2004) The pyridinyl-6 position of WAY-100635 as a site for radiofluorination—effect on 5-HT1A receptor radioligand behavior in vivo. *Mol. Imaging Biol. 6*, 17−26.
(19) Buiter, H. J., Windhorst, A. D., Huisman, M. C., De Maeyer, J. H., Schuurkes, J. A., Lammertsma, A. A., and Leysen, J. E. (2013) Radiosynthesis and preclinical evaluation of [11C]prucalopride as a potential agonist PET ligand for the 5-HT4 receptor. *EJNMMI Res. 3*, 24.
(20) Nabulsi, N. B., Zheng, M. Q., Ropchan, J., Labaree, D., Ding, Y. S., Blumberg, L., and Huang, Y. (2011) (2011) [11C]GR103545: novel one-pot radiosynthesis with high specific activity. *Nucl. Med. Biol. 38*, 215−221.
(21) Dewkar, G. K., Sundaresan, G., Lamichhane, N., Hirsch, J., Thadigiri, C., Collier, T., Hartman, M. C., Vaidyanthan, G., and Zweit, J. (2013) Microfluidic radiosynthesis and biodistribution of [18 F] 2-(5-fluoro-pentyl)-2-methyl malonic acid. *J. Labelled Compd. Radiopharm. 56*, 289−294.
(22) Barth, V. N., Chernet, E., Martin, L. J., Need, A. B., Rash, K. S., Morin, M., and Phebus, L. A. (2006) Comparison of rat dopamine D2 receptor occupancy for a series of antipsychotic drugs measured using radiolabeled or nonlabeled raclopride tracer. *Life Sci. 78*, 3007−3012.
(23) Donohue, S. R., Krushinski, J. H., Pike, V. W., Chernet, E., Phebus, L., Chesterfield, A. K., Felder, C. C., Halldin, C., and Schaus, J. M. (2008) Synthesis, ex vivo evaluation, and radiolabeling of potent 1,5-diphenylpyrrolidin-2-one cannabinoid subtype-1 receptor ligands as candidates for in vivo imaging. *J. Med. Chem. 51*, 5833−5842.
(24) Mitch, C. H., Quimby, S. J., Diaz, N., Pedregal, C., de la Torre, M. G., Jimenez, A., Shi, Q., Canada, E. J., Kahl, S. D., Statnick, M. A., McKinzie, D. L., Benesh, D. R., Rash, K. S., and Barth, V. N. (2011) Discovery of aminobenzyloxyarylamides as κ opioid receptor selective antagonists: application to preclinical development of a κ opioid receptor antagonist receptor occupancy tracer. *J. Med. Chem. 54*, 8000−8012.
(25) Pike, V. W., Rash, K. S., Chen, Z., Pedregal, C., Statnick, M. A., Kimura, Y., Hong, J., Zoghbi, S. S., Fujita, M., Toledo, M. A., Diaz, N., Gackenheimer, S. L., Tauscher, J. T., Barth, V. N., and Innis, R. B. (2011) Synthesis and evaluation of radioligands for imaging brain nociceptin/orphanin FQ peptide (NOP) receptors with positron emission tomography. *J. Med. Chem. 54*, 2687−2700.
(26) Zanotti-Fregonara, P., Barth, V. N., Liow, J. S., Zoghbi, S. S., Clark, D. T., Rhoads, E., Siuda, E., Heinz, B. A., Nisenbaum, E., Dressman, B., Joshi, E., Luffer-Atlas, D., Fisher, M. J., Masters, J. J., Goebl, N., Kuklish, S. L., Morse, C., Tauscher, J., Pike, V. W., and Innis, R. B. (2013) Evaluation in vitro and in animals of a new 11C-labeled PET radioligand for metabotropic glutamate receptors 1 in brain. *Eur. J. Nucl. Med. Mol. Imaging 40*, 245−253.
(27) Zheng, M. Q., Nabulsi, N., Kim, S. J., Tomasi, G., Lin, S. F., Mitch, C., Quimby, S., Barth, V., Rash, K., Masters, J., Navarro, A., Seest, E., Morris, E. D., Carson, R. E., and Huang, Y. (2013) Synthesis and evaluation of 11C-LY2795050 as a κ-opioid receptor antagonist radiotracer for PET imaging. *J. Nucl. Med. 54*, 455−466.
(28) Pedregal, C., Joshi, E. M., Toledo, M. A., Lafuente, C., Diaz, N., Martinez-Grau, M. A., Jiménez, A., Benito, A., Navarro, A., Chen, Z., Mudra, D. R., Kahl, S. D., Rash, K. S., Statnick, M. A., and Barth, V. N. (2012) Development of LC-MS/MS-based receptor occupancy tracers and positron emission tomography radioligands for the nociceptin/orphanin FQ (NOP) receptor. *J. Med. Chem. 55*, 4955−4967.
(29) Skaddan, M. B., Zhang, L., Johnson, D. S., Zhu, A., Zasadny, K. R., Coelho, R. V., Kuszpit, K., Currier, G., Fan, K. H., Beck, E. M., Chen, L., Drozda, S. E., Balan, G., Niphakis, M., Cravatt, B. F., Ahn, K., Bocan, T., and Villalobos, A. (2012) The synthesis and in vivo evaluation of [18F]PF-9811: a novel PET ligand for imaging brain fatty acid amide hydrolase (FAAH). *Nucl. Med. Biol. 39*, 1058−1067.
(30) Farde, L., Hall, H., Ehrin, E., and Sedvall, G. (1986) Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET. *Science. 231*, 258−61.
(31) Hume, S. P., Gunn, R. N., and Jones, T. (1998) Pharmacological constraints associated with positron emission tomographic scanning of small laboratory animals. *Eur. J. Nucl. Med. 25*, 173−176.
(32) Chernet, E., Martin, L. J., Li, D., Need, A. B., Barth, V. N., Rash, K. S., and Phebus, L. A. (2005) Use of LC/MS to assess brain tracer distribution in preclinical, in vivo receptor occupancy studies: dopamine D2, serotonin 2A and NK-1 receptors as examples. *Life Sci. 78*, 340−346.
(33) Shetty, H. U., Zoghbi, S. S., Siméon, F. G., Liow, J. S., Brown, A. K., Kannan, P., Innis, R. B., and Pike, V. W. (2008) Radiodefluorination of 3-fluoro-5-(2-(2-[18F](fluoromethyl)-thiazol-4-yl)ethynyl)-benzonitrile ([18F]SP203), a radioligand for imaging brain metabotropic glutamate subtype-5 receptors with positron emission tomography, occurs by glutathionylation in rat brain. *J. Pharmacol. Exp. Ther. 327*, 725−735.
(34) Lanekoff, I., Thomas, M., Carson, J. P., Smith, J. N., Timchalk, C., and Laskin, J. (2013) Imaging nicotine in rat brain tissue by use of nanospray desorption electrospray ionization mass spectrometry. *Anal. Chem. 85*, 882−889.
(35) Hsieh, Y., Li, F., and Korfmacher, W. (2010) Mapping pharmaceuticals in rat brain sections using MALDI imaging mass spectrometry. *Methods Mol. Biol. 656*, 147−158.
(36) Need, A. B., McKinzie, J. H., Mitch, C. H., Statnick, M. A., and Phebus, L. A. (2007) In vivo rat brain opioid receptor binding of LY255582 assessed with a novel method using LC/MS/MS and the administration of three tracers simultaneously. *Life Sci.*, 8117−8118.