

## EDITORIAL COMMENT

# Targeted Molecular Imaging

## Target Significance and Probe Validation\*

William C. Eckelman, PhD

Bethesda, Maryland

In the post-genomic era, drug development and diagnostic imaging have become more closely linked in the search for targets. Since the 1940s, nuclear imaging has been based on physiological targets, some more easily saturable than others. Recently, the search for drug targets has been increasingly based on genomics, primarily on genome-wide association studies, an approach used in genetics research to look for associations between specific genetic variations (most commonly single-

angiotensin-converting enzyme (ACE) inhibitors and matching imaging probes have been developed (6,7).

Given the time constraints on multiple imaging studies, choosing a single target given the genetic complexity of many diseases is a challenge that should be addressed in *iJACC* papers. Imaging single targets would be better suited for Mendelian single-gene disorders. Nevertheless, matching targeted imaging probes with effective drugs targeted to a single-protein expression product such as imatinib mesylate, trastuzumab, lisinopril (ACE inhibitor), and alprenolol (beta-adrenergic receptor) have potential clinical applications. Although a broad rationale for a particular probe is often given in the paper's introduction, identifying a unique clinical application will accelerate the translation to the clinic. The competitive environment should also be recognized. For example, in the case of trastuzumab, an in vitro kit was approved at the time the drug was approved so imaging of the primary disease may not be cost-effective. To monitor the treatment of high blood pressure with beta-adrenergic receptors or ACE inhibitors, a sphygmomanometer was used, and an imaging application had to be chosen carefully. A unique application, such as monitoring a large field of view for disease burden and changes in disease burden, is best suited for diagnostic imaging.

Whether the signal is from a nuclear, magnetic resonance, or fluorescence signaling probe or a multiplex probe (8), validation of the probe(s) is crucial. The technical requirements for validation vary widely among investigators as evidenced by the diversity in information published by the National Cancer Institute-sponsored Molecular Imaging and Contrast Agents Database group's publications on the topic (9,10).

[See page 607](#)

nucleotide polymorphisms) and particular diseases (1). Genomic technology has led to very important therapeutic innovations, including the use of imatinib mesylate (Gleevec, Novartis Oncology, East Hanover, New Jersey) in Bcr-Abl chronic myeloid leukemia and trastuzumab (Herceptin, Genentech, San Francisco California) in Her2-positive breast cancer, but the approach has not been widely successful to date (2). In complicated psychiatric disorders such as schizophrenia and bipolar disorder, many loci have been identified that meet genome-wide significance but have not led to effective drug development (3). More complete links of physiology and disease are being pursued (4,5). This is not to say that targeted drugs and imaging probes were not developed before the sequencing of the human genome; for example, in cardiology, alpha- and beta-adrenoreceptor and

\*Editorials published in *JACC: Cardiovascular Imaging* reflect the views of the authors and do not necessarily represent the views of *JACC: Cardiovascular Imaging* or the American College of Cardiology.

From Molecular Tracer LLC, Bethesda, Maryland. Dr. Eckelman has reported that he has no relationships relevant to the contents of this paper to disclose.

For nuclear reporters radiolabeled with single-photon emission computed tomography and positron emission tomography radionuclides, there is a well-defined list of metrics; this list includes criteria for radiochemical yield, chemical yield, radiochemical and chemical purity, and specific activity that should be applied to magnetic resonance imaging and fluorescent probes as well. For example, specific activity can be interpreted as probes per molecule including antibodies and peptides or per nanoparticle rather than the more traditional radioactivity per mass (MBq/ $\mu\text{mol}$ ). If chiral centers are present, the stereochemistry should be described. For human use, the absence of organic volatiles, pyrogens, and bacteria should be included as well.

More diversity in literature data is seen in the next step of validating the targeting of the desired protein both in vitro and in vivo. In vitro, Motulsky (11) has defined saturation and competitive binding to measure saturable binding, but this approach does not address specificity unless a large number of potential binding sites are also studied. Specificity becomes more critical when validating a probe for a specific receptor subtype, and in this case knock-out mice have been used effectively. In addition, saturability and specificity in vitro do not directly address the relationship between binding constants and target protein density in terms of external imaging potential. There are best-case rules of thumb such as  $B_{\text{max}}/K_i$  (12) and more complicated models of in vivo data by Mintun et al. (13) and other groups (14).

Blocking studies, a term loosely used to define increasing amounts of competing ligand injected either before, with, or after the signal-containing probe, are used most often to demonstrate saturable binding. It is important that this is a single-variable experiment in that the added mass does not alter confounding factors such as blood flow, transport, metabolism, or multidrug resistance pumps. These studies are conclusive if only the target organ concentration of the signal-containing probe decreases with increasing blocking mass. Agonists especially can cause pharmacological effects in higher mass amounts that can change the blood flow, permeability, and metabolism. Another approach that avoids some of the challenges is the use of animal models in which the target protein, including specific subtypes, has been knocked out.

Other investigators use probes (nuclear, magnetic resonance imaging, or fluorescent) that have very low affinities for the target protein as a control, and

others use a target to normal tissue ratio or a tumor containing the target compared with a tumor without the target. Others use active and inactive stereoisomers for small molecules. All methods have challenges because the control probe must have the same pharmacokinetics as the targeted probe, and the control tissue or tumor must have the same blood flow and permeability. A pharmacokinetic analysis of the probe and the control would be the best assurance of this.

This issue of *iJACC* contains an example of this approach. Hara et al. (15) have presented their evidence for molecular imaging of fibrin deposition in deep vein thrombosis using a new fibrin-targeted near-infrared fluorescence (NIRF) imaging strategy. They presented a strong rationale for influencing patient care; namely, the development of a thrombosis-specific molecular imaging agent to detect and monitor thrombogenesis and fibrinolysis in vivo could improve the diagnosis, risk stratification, and treatment of thrombosis syndromes. They validated the saturable and specific binding to thrombin by blocking studies using a 100-fold excess of unlabeled competitor peptide. In the previous issue of *iJACC*, Dilsizian et al. (16) presented an equally focused clinical goal of assessing myocardial ACE-1 up-regulation to monitor ACE as a function of progressive heart failure using external imaging. Because left ventricular ACE expression has been found to be significantly enhanced in the failing human myocardium in proportion to increasing disease severity, monitoring ACE by noninvasive imaging as a function of treatment should have clinical impact. Dilsizian et al. (16) used another approach to demonstrate specific and saturable binding. ACE-1-overexpressing transgenic rats were compared with wild-type Sprague-Dawley rats. There was a significant increase in the uptake of the radiolabeled lisinopril analogue in the heart of the transgenic rats.

These validation steps are necessary but not sufficient to produce a useful diagnostic probe if the goal is to follow changes in the target protein as a function of disease or treatment. The pharmacokinetic distribution must be relatively free from changes in blood flow or permeability as a function of disease or treatment to measure changes in the target protein. If cell counting and the ability to detect small volumes of target tissue is the goal, then the highest affinity possible is ideal, but this is the definition of a flow agent and therefore that confounders must be evaluated in parallel.

Validation on all levels is not a straight-forward study, and thus a wide diversity in the published studies on new probes is inevitable. A recently published text (17) contains the approaches taken by several investigators following the Bench to Bedside paradigm. But the goal is clear even if the path is not. First, choose a disease in which imaging will have an impact, then choose the

target most sensitive to disease progression, and finally validate a targeted molecular imaging probe that delivers unique clinical information by external imaging.

**Reprint requests and correspondence:** Dr. William C. Eckelman, Molecular Tracer LLC, 10432 Snow Point Drive, Bethesda, Maryland 20814. *E-mail:* [wceckelman@verizon.net](mailto:wceckelman@verizon.net).

## REFERENCES

1. Feero WG, Guttmacher AE, Collins FS. Genomic medicine—an updated primer. *N Engl J Med* 2010;362:2001-11.
2. Carden CP, Sarker D, Postel-Vinay S, et al. Can molecular biomarker-based patient selection in Phase 1 trials accelerate anticancer drug development? *Drug Discovery Today* 2010;15:88-97.
3. Ripke S, Sanders AR, Kendler KS, et al. Genome-wide association study identifies five new schizophrenia loci. *Nat Genet* 2011;43:969-76.
4. Dollery CT. Beyond genomics. *Clin Pharmacol Ther* 2007;82:366-70.
5. Schadt EE, Zhang B, Zhu J. Advances in systems biology are enhancing our understanding of disease and moving us closer to novel disease treatments. *Genetica* 2009;136:259-69.
6. Dilsizian V, Eckelman WC, Loreda ML, Jagoda EM, Shirani J. Evidence for tissue angiotensin-converting-enzyme in explanted hearts of ischemic cardiomyopathy using targeted radiotracer technique. *J Nucl Med* 2007;48:182-7.
7. Riemann B, Schäfers M, Law MP, Wichter T, Schober O. Radioligands for imaging myocardial alpha- and beta-adrenoceptors. *Nuklearmedizin* 2003;42:4-9.
8. Kobayashi H, Longmire MR, Ogawa M, Choyke PL. Rational chemical design of the next generation of molecular imaging probes based on physics and biology: mixing modalities, colors and signals. *Chem Soc Rev* 2011;40:4626-48.
9. Chopra A, Shan L, Eckelman WC, Leung K, Menkens AE. Important parameters to consider for the characterization of PET and SPECT imaging probes. *Nucl Med Biol* 2011;38:1079-84.
10. Chopra A, Shan L, Eckelman WC, et al. Molecular imaging and contrast agent database (MICAD): evolution and progress. *Mol Imaging Biol* 2012;14:4-13.
11. Motulsky HJ. *Analyzing Data with GraphPad Prism*. San Diego, CA: GraphPad Software Inc, 2009. Available at: [www.graphpad.com](http://www.graphpad.com). Accessed April 21, 2012.
12. Eckelman WC. Design criteria for targeted molecules: muscarinic cholinergic systems biology. In: Welch MJ and Eckelman WC, editors. *Targeted Molecular Imaging*. Boca Raton, FL: CRC Press, Taylor & Francis Group, 2012:201-15.
13. Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann Neurol* 1984;15:217-27.
14. Carson RE. Tracer kinetic modeling, positron emission tomography. In: Valk PE, Bailey DL, Townsend DW, Maisey MN, editors. *Basic Science and Clinical Practice*. London, UK: Springer-Verlag, 2003:147-79.
15. Hara T, Bhayana B, Thompson B, et al. Molecular imaging of fibrin deposition in deep vein thrombosis using fibrin-targeted near-infrared fluorescence. *J Am Coll Cardiol Img* 2012;5:607-15.
16. Dilsizian V, Zynda TK, Petrov A, et al. Molecular imaging of human ACE-1 expression in transgenic rats. *J Am Coll Cardiol Img* 2012;5:409-18.
17. Welch MJ, Eckelman WC, editors. *Targeted Molecular Imaging*. Boca Raton, FL: CRC Press, Taylor & Francis, 2012.

**Key Words:** clinical impact ■ disease control points ■ personalized medicine ■ quality control ■ targeted probes.