HHS Public Access

Author manuscript

Circ Cardiovasc Imaging. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:

Circ Cardiovasc Imaging. 2018 February ; 11(2): e005355. doi:10.1161/CIRCIMAGING.117.005355.

MOLECULAR IMAGING IN DRUG DISCOVERY AND DEVELOPMENT

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Abstract

Non-invasive imaging has played an increasing role in the process of cardiovascular drug development. This review focuses specifically on the use of molecular imaging which has been increasing applied to improve and accelerate certain pre-clinical steps in drug development including the identification of appropriate therapeutic targets, evaluation of on-target and off-target effects of candidate therapies, assessment of dose response, and the evaluation of drug or biologic biodistribution and pharmacodynamics. Unlike the case in cancer medicine, in cardiovascular medicine molecular imaging has not been used as a primary surrogate clinical endpoint for drug approval. However, molecular imaigng has been applied in early clinical trials, particularly in Phase 0 studies, to demonstrate “proof of concept” or to explain variation in treatment effect. Many of these applications where molecular imaging has been used in drug development have involved the “retasking” of technologies that were originally intended as clinical diagnostics. With greater experience and recognition of the rich information provided by in vivo molecular imaging, it is anticipated that it will increasingly be used to address the enormous time and costs associated with bringing a new drug to clinical launch.

Keywords

imaging; drug discovery; molecular imaging

Justification for Changes to the Drug Development Paradigm

Recent breakthroughs in applied molecular biology have potentiated the processes of drug discovery and stepwise drug development to the stage of clinical launch. In particular, drug discovery has been accelerated by the maturation of high-throughput technologies for rapid and automated candidate molecule screening, the pharmaceutical application of “omics” approaches to understand the basis of health and disease-related targets, and the use of

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DISCLOSURES

Dr. Lindner has investigator-initiated grants from GE Healthcare and Pfizer, Inc..

"virtual screens" for understanding structure.[1,2] Nonetheless, the healthcare industry is currently facing unprecedented obstacles to the development of truly innovative therapeutics. One problem that has become increasingly apparent through media channels is the erosion of trust in pharmaceutical companies based on real or perceived lapses in business ethics, scientific rigor, and scientific reporting. It is unlikely that the scientific community alone will solve these problems.

An equally important challenge is the business model on which drug discovery and both pre-clinical and clinical testing is based. For largely financial reasons, over the last two decades there has been a progressive decline in the proportion of drugs approved that are considered first-in-class, or what the pharmaceutical industry terms "new molecular entities" (NMEs).[3–5] The majority of first-in-class therapeutics over the past decade has been in the category of cancer therapeutics or biologics, which have not impacted cardiovascular disease as much as cancer, inflammatory or rheumatologic disease.[4,6]

Non-invasive imaging has played an increasing role in the process of cardiovascular drug testing. Just a few tangible examples of biologic readouts that have been assessed by conventional imaging in pre-clinical models and humans include the assessment of left ventricular function, pulmonary artery pressure, myocardial ischemia, and arterial morphology (e.g. plaque size by intravascular ultrasound) which have each been used in preclinical and clinical studies to assess drug efficacy or to provide proof-of-mechanism information vital to drug approval. Newer molecular imaging techniques that are able to characterize tissue phenotype have been developed for essentially all forms of non-invasive *in vivo* imaging.[7] Their development is based on the premise that they will improve patient outcomes and healthcare efficiency by providing: (i) earlier diagnosis, (ii) more definitive diagnosis, and (iii) information helpful for selecting the most appropriate therapy. The central theme of this article is focused on how molecular imaging has yet unrealized potential to increase the efficiency of bringing a drug to approval at several different stages of development of new drugs.

Scope of the Problem

Patterns and trends in the activities of the pharmaceutical industry reveal that the development of innovative first-in-class drugs is in decline. In 2016, the United States Food and Drug Administration (FDA) approved only 22 new drugs classified as NMEs or biologics that were approved through the Investigational New Drug (IND) or Biologic License Applications processes.[4] Of note, a relatively large proportion (41%) of these new therapeutics were for rare or "orphan" disease applications. None were targeted to cardiovascular disease. Even when considering "same-class" drug approvals, which represent modifications of previously approved drugs, the majority do not necessarily provide any substantial improvement in care. A study examining drugs approved by the European Medicines Agency between 1999 and 2005 found that only around 10% of new drugs had some incremental clinical benefit over existing medications, other than cost or convenience.[8]

There have been many descriptive models that have been generated to explain the increasing hurdles for pharmaceutical research and development. Understandably, most of these models have been based on industry metrics that do not always take into account basic science efforts to uncover “druggable” processes, which is often performed by academia. One such model is illustrated in Figure 1, which highlights the benchmarks in the process of bringing a drug to market.[3] Again, in this model the important and often costly initial process of target identification, which can occur in either the industry or non-industry setting, is not included. A notable feature of this model is that the out-of-pocket expenses from discovery-to-launch approach $0.9 billion for a new drug. Even more concerning is the cost to launch which is over $1.7 billion if one takes into account capitalized costs which were assumed to be 11% in the model but are variable based on industry infrastructure and the cycle time for each development stage. The capitalized cost figures for developing a first-in-class drug have been consistent between models and industry surveys. It is reasonable to assume that the development of NMEs may not be aligned with pharma business pressures to rapidly produce “hits” in order to meet near-term financial expectations of investors.

From the model in Figure 1, it is clear that there are several opportunities for improving the efficiency of drug discovery and development based on the model descriptors. These include reductions in cycle time, reduced cost per phase, and increased probability of success for transitioning to the next stage, which is heavily influenced by the identification of impactful molecular targets and rapid evaluation of on-target and off-target effects. In an effort to address some of the hurdles, the FDA has implemented a variety of facilitated regulatory pathways such as the Fast Track, Breakthrough Therapy, and Accelerated Approval pathways which expedite the pre- and post-IND phases. These pathways have been estimated to shorten the time for approval by 40–50%.[9] The majority of the new FDA drug approvals for 2016 benefited from fast tracking programs.

Molecular imaging has the potential to positively impact the efficiency of drug discovery and development. There are many steps involving both pre-clinical and clinical investigation where molecular imaging has already been used to accelerate new drug investigation (Figure 2). At the current time, application of molecular imaging has been most appreciated in the development process for cancer therapeutics. There is increasing evidence that cardiovascular drug development will be impacted in a similar fashion. In fact, molecular imaging can be particularly impactful in cardiovascular drug development since, unlike in cancer medicine, tissue is not routinely obtained from humans and imaging may be able to provide a “virtual biopsy” of drug effects on the heart or vascular tissues.

Molecular Imaging in Medicine and Science

Methods for assessing specific cellular or molecular processes have been developed for all forms of non-invasive imaging used in cardiovascular medicine and science.[7,10,11] These techniques are used to assess disease-related processes such as protein synthesis or trafficking, gene expression, metabolic activity, cell migration, enzymatic activity, and receptor availability. The development of *in vivo* molecular imaging techniques has been predicated on the ability to provide unique quantitative spatial and temporal information that can be used for a variety of purposes in patients and in pre-clinical models of disease. For

drug development, molecular imaging has been used to: (i) identify new “druggable” targets, (ii) evaluate biodistribution and appropriate dosing strategies, (iii) test efficacy and off-target effects, (iv) to select appropriate patient cohorts for initial testing, and (v) to serve as a surrogate endpoint in pre-clinical and clinical studies. The role of molecular imaging is likely to increase given trends in academia and industry to focus more on humans or non-rodent animal models that more clearly resemble humans for the early stages of drug development.[12]

From a technical standpoint, molecular imaging relies on one of several strategies. A commonly used approach is to engineer contrast agents that are selectively retained by the biologic process of interest. Contrast agents for radionuclide imaging, magnetic resonance imaging (MRI), ultrasound, optical imaging and computed tomography (CT) have all been modified (e.g. conjugation of a targeting ligand) to alter their kinetics and be used for molecular imaging.[7,10,11] Targeting moieties used to promote binding to a disease process can exist in a direct linear ratio with the signal-generating contrast agent, which is commonly the case with radionuclide single photon emission tomogaphy (SPECT) or positron emission tomography (PET) agents; or many ligands can be conjugated in a multivalent fashion to the surface of a particle-based contrast such as those used in ultrasound or MRI. A critical factor in the evaluation of the relative value of the different contrast imaging approaches is the biodistribution of the contrast agents (e.g. diffusible versus confinement to the vascular compartment).

Another approach for molecular imaging is to design contrast agents that leverage natural processes for uptake or retention. Examples of this strategy include uptake of ^{18}F-fluorodeoxyglucose (FDG) during PET imaging to detect processes with increased energy expenditure such as advanced atherosclerosis or sarcoidosis, or the detection of inflammation through opsonization and uptake of particle-based contrast agents which are recognized as foreign by immune cells, and may even reveal specific monocyte subtypes in atherosclerosis.[13–17] Other forms of molecular imaging rely on the “activation” of contrast agents by the disease-related process of interest. This strategy is most commonly used in optical imaging by development of fluorophores that produce either a change in photon emission or a spectral shift after interaction with a pathogenic pathway such as protease activity or abnormal redox state.[18,19] Finally, some forms of molecular imaging do not require exogenous contrast agents but instead are able to detect molecular environment through endogenous signal production, such as MRI-based blood oxygen level dependent (BOLD) imaging,[20] or autofluorescence.

Selection of the most appropriate targeting approach and the most appropriate imaging modality to use within the scope of drug development is based on similar considerations as when applying the technology in the clinical realm (Figure 3). It is worth noting that experience in cancer molecular imaging indicates that an imaging modality used to assess pre-approval drug efficacy is more likely to be adopted by clinicians for patient selection or for assessing therapeutic response. Technical deliberations include need for high sensitivity, spatial resolution, temporal resolution, target specificity, and biodistribution of contrast agent, when applicable, which determines likelihood for accessing the biologic process of interest. Some of the biggest technical concerns are to assure that a signal reflects tissue

phenotype rather than primarily reflecting blood flow, vascular permeability, or other variables that can influence tracer uptake. Practical considerations for both academia and pharma include cost, availability, and safety.

Identification of New Targets for Therapy with Imaging

The identification of molecular pathways previously unknown to be involved in the pathophysiology of a disease is often a catalyst for the development of new treatments. Information on newly recognized pathways, proteins or genes allows for the generation of lead candidates for therapy which can be tested by a variety of molecular biology approaches, most commonly in the form of automated high-throughput or focused compound screening processes.[1,21] Another approach is that of “rational drug design” or whereby structural proteomics provides knowledge of the detailed structural and biochemical properties of a target molecule (protein, channel, enzyme) in order to design a modifier compound.[21] This method has benefited from advances in high resolution techniques for characterizing protein structure by x-ray diffraction crystallography, and the use of computer-based methods for designing small molecule therapeutics and predicting their success through virtual ligand screening.

Non-invasive *in vivo* molecular imaging has been tasked in the research setting to create new insight into pathobiology in a wide range of disease categories. This application of molecular imaging has helped identify new “druggable” targets. In addition to simply uncovering pathophysiology, the opportunity to temporally evaluate a disease-related process non-invasively can provide critical knowledge of how a drug can be used to its greatest effect, or when it is likely to be of little benefit. Moreover, molecular imaging is often paired with more conventional forms of cardiovascular non-invasive imaging in order to match a molecular phenotype to standard measurements of anatomy, flow, or function.

In the field of atherosclerosis biology, most molecular imaging studies have simply demonstrated feasibility of assessing a pathologic process that is already well characterized. Yet, there are examples where molecular imaging has aided in the discovery of a modifiable disease-related process. Molecular imaging of phosphatidylserine expression on the outer leaflet of the cell membrane with annexin-V probes has been used to detect macrophage apoptosis in unstable plaques, but also has been used to demonstrate that this process can occur from non-apoptotic ischemic pathways.[22,23] Imaging events that occur at the endothelial-blood interface has been valuable for discovering that abnormal regulation of self-associated Von Willebrand factor multimers at the endothelial surface is largely responsible for platelet-endothelial interactions that play a role in early atherogenesis (Figure 4A).[24] Optical and SPECT imaging probes that reveal the release and the enzymatic activity of matrix metalloproteinases (MMPs) and cathepsins have been useful for co-localizing protease activity with macrophages or elastin degradation (Figure 4B and 4C).[19,25,26] In vivo PET imaging with the nitroimidazole derivative ^{18}F-fluoromisonidazole which is reduced and retained by cells in hypoxic environments has yielded important information on hypoxic microenvironment in atherosclerosis.[27] The folate receptor-β, CX_3CR-1 (fractalkine receptor), the mannose receptor, and α5-integrins have all been targeted to examine the roles of specific inflammatory cell types (monocyte subclasses,

macrophages) in atherosclerosis and ischemia-related vascular remodeling.[28–32] These studies have done more than simply show feasibility of imaging the innate immune response. They have provided support to the notion that altering the balance of monocyte cell type may be a therapeutic target in certain conditions such as atherosclerosis and LV remodeling.[33] Even in valve disease, molecular imaging of endothelial cell adhesion molecules such as VCAM-1 has contributed to the notion that progression to aortic stenosis can be modified by altering pro-inflammatory signaling.[34] These examples are just a few that highlight the use of targeted imaging probes to reveal new therapeutic targets.

In vivo Testing of Candidate Efficacy

The full characterization of on-target effects and completion of proof-of-mechanism studies are critical steps in drug development. It is a logical assumption that any molecular imaging technique that has been used to uncover modifiable disease-related biology can also be used to non-invasively quantify response to therapy. More commonly, pathophysiology that is not discovered through molecular imaging has also been targeted for evaluation of new therapeutic agents in pre-clinical models and in even in human proof-of-concept trials.

The investment in molecular imaging for pre-clinical *in vivo* testing of candidate efficacy and off-target effects has been justified in situations where it provides incremental benefit. Conventional forms of non-invasive cardiovascular imaging without recourse to molecular imaging protocols provides information on morphology or function including but not limited to: (i) plaque size, volume, and content in atherosclerosis; (ii) left ventricular volumes, systolic function, diastolic properties, and scar area in heart failure; (iii) myocardial perfusion imaging and metabolism in ischemic heart disease. In this context, the rationale for using molecular imaging is often based on its ability to assess modification of a specific targeted biologic pathway early before a structural or functional outcome in order to select the most appropriate of several candidate agents. Aligning with the concept of “precision medicine”, molecular imaging can be used in diseases that have wide phenotypic variation to predict benefit based on the specific molecular or cellular characteristics or the stage of disease. For example, molecular imaging may be particularly valuable when evaluating potent anti-inflammatory therapies in established atherosclerosis where anatomic imaging is limited, or for selecting only those with high cardiac sympathetic activity in order to test drug/device therapies for ventricular arrhythmias. Imaging data can also provide valuable insight when new therapies fail by demonstrating lack of effect on the intended biologic pathway, thereby avoiding an incorrect assumption that a certain pathway is not a suitable for therapeutic targeting.

In atherosclerosis drug development, probably the most recognized use of molecular imaging that has been applied is ^{18}F-FDG imaging with PET.[35] Widespread recognition and acceptance of this technology is based on clinical trials that have used it as an outcome measure for drug efficacy (discussed later) and its documented ability to predict major adverse cardiovascular events.[7,10,36] As reviewed elsewhere, molecular imaging with targeted contrast agents has been used to image atherosclerosis and aneurysm formation including inflammation (endothelial adhesion molecule expression, monocyte recruitment, scavenger receptors, phagocytic activity, matrix proteases, etc.), prothrombotic endothelial

phenotype (VWF, platelet adhesion, fibrin), oxidative stress (phospholipid or protein oxidized epitopes), lipoprotein accumulation, angiogenesis (integrins and proteases) and matrix content.[7,10,11,16,37] For ischemic heart disease and heart failure, molecular imaging of the recruitment of specific inflammatory cell subtypes, matrix remodeling, protease activity, and endothelial and monocyte markers of vascular remodeling have been used to spatially assess tissue ischemia, ventricular remodeling, and endogenous and therapeutic angiogenesis (Figure 5).[7,10,32,38–41] For rhythm disorders, imaging of pre- and post-synaptic cardiac sympathetic function with ^{123}I-meta-iodobenzylguanidine activity and 11C-meta-hydroxyephedrine can provide information on susceptibility to life-threatening ventricular arrhythmias.[42–44] The practicality of using molecular imaging in pre-clinical drug evaluation or dose ranging has benefited from studies that applied these techniques not only in rodents, but also in more relevant canine or porcine models of post-MI remodeling, and non-human primate models of atherosclerosis.[41,45,46]

Illustration that a molecular imaging readout is altered by a therapy already known to be effective in a disease state is often used as a step towards clinical translation of a new diagnostic. These types of studies are also useful when considering whether a molecular imaging technique is suitable for assessing pre-clinical efficacy of a new drug. Statin therapy has been shown in animal models of atherosclerosis, and then eventually in humans to reduce the arterial ^{18}F-FDG signal on PET imaging or signal from uptake of ultra-small superparamagnetic iron oxide (USPIO) nanoparticles (<100 nm in size).[13,47] Statins have been used in gene-targeted murine models of atherosclerosis to confirm a therapeutic reduction in the expression of adhesion molecules such as VCAM-1 by targeted MRI, PET, optical and ultrasound molecular imaging.[48–50] In heart failure, combinations of clinically-established therapies that modify angiotensin and aldosterone signaling have been used to alter post-MI ventricular remodeling in mice which was correlated with radionuclide imaging of myofibroblast activity.[39]

The application of molecular imaging to obtain unique information not provided by anatomic or functional imaging regarding the efficacy of new therapies is slowly increasing. In atherosclerotic disease, molecular imaging has provided valuable information for demonstrating efficacy and in some circumstances optimal dosing for investigational agents such as new potent anti-oxidants (e.g. NOX-2 modifiers), HDL-mimetics, and MMP inhibitors.[16,40,51] These studies have generally employed molecular imaging in models of advanced disease and examined non-morphologic changes in specific processes such as endothelial cell adhesion molecule expression (selectins, VCAM-1), monocyte recruitment, platelet adhesion, oxidized lipoprotein uptake, and intra-plaque protease activity. The notion that new therapies may be able to prevent atherosclerosis altogether hinges on the ability to identify subjects at exceptionally high risk for accelerated disease at a very early stage. It is unlikely that existing risk assessment paradigms will suffice since they are most commonly used to estimate 10 year risk and are heavily influenced by age, leading to a likely underestimation of lifetime risk in young individuals.[52] Molecular imaging has been shown to detect the earliest atherogenic events that occur before histologic evidence for fatty streaks such as endothelial activation, platelet adhesion and oxidized lipid accumulation.[24,46,53] Accordingly, these techniques have begun to be used to establish therapeutic benefit of new drugs that prevent atherosclerosis progression.[51]

Molecular imaging has also been used to provide important proof-of-mechanism information on new therapies that act through vascular remodeling. Targeted imaging of angiogenesis-releated endothelial integrins (α_v-integrins), other adhesion molecules, and markers of pro-angiogenic monocytes has been used as an *in vivo* readout for pro-angiogenic therapies in ischemic heart and limb disease, and for anti-angiogenic therapies intended to reduce plaque neovascularization.[32,33,40,54] For pro-angiogenic cell therapy, molecular imaging has been used to assess phenotypic changes that reflect their intended purpose. For example, *in vivo* optical imaging of a luciferase reporter under the control of a Tie-2 reporter has been used to temporally and spatially assess the transformation to a more "endothelial phenotype" of mesenchymal stem cells injected in mice with myocardial infarction.[55] Molecular imaging has been used as an investigative technique for characterizing paracrine effects of cell therapy on host cells such as the activation of a pro-angiogenic innate immune response stimulated by mesenchymal stem cell therapy in limb ischemia (Figure 5C).[32]

In summary, some of the examples discussed above illustrate how molecular imaging has been used as a readout for the intended or "on-target" drug effects. With the maturation, further validation, and increased penetration of these techniques in research labs, it is highly likely that molecular imaging will play an increasing role in the early evaluation of candidate NMEs.

Biodistribution and Pharmacokinetics

Pharmokinetic and pharmacodynamic profiling is a critical process in understanding drug dosing, toxicity, and likelihood for therapeutic success. Non-invasive imaging has played a role in this process and has been vital in the conduct of many FDA Phase 0 exploratory "microdosing" studies designed to expedite drug approval. In particular, radionuclide imaging with PET and SPECT has been useful for temporally evaluating whole body biodistribution of therapeutic candidates that can be labeled without changing their properties and administered by intravenous route. Another strategy for imaging drug biodistribution is to employ imaging agents that are "activated" or produce signal in the presence of a drug or by a specific interaction of two proteins. This strategy, which has been reviewed elsewhere,[56] often involves two separate molecular moieties or a split protein each of which are labeled with an optical reporter system so that in the presence of a therapeutic agent there is a change in confirmation and rearrangement of the reporters to produce a light signal.

The use of pharmacokinetic imaging of radiolabeled drug candidates has been most impactful in situations where drug uptake at a specific site is desired or when access of drugs to target is in question.[57–59] In neurologic diseases, PET and SPECT imaging have been used to evaluate the transport of drugs across the blood-brain barrier, and have been paired with drug-tracers, including ^{11}C-raclopide ^{123}I-ioflupane, to spatially evaluate dopamine transport and receptor occupancy in the striatum in a variety of motion disorders and neurocognitive processes (Figure 6A).[57,58] Imaging data on brain receptor occupancy is of critical importance since it differs vastly from blood levels, leading to appropriate dosing strategies which would not have been possible with plasma kinetics alone.[58] In cancer

medicine, concerns regarding anti-tumor drug delivery, entry, and retention has justified the use of imaging to assess biodistribution in primary and metastatic solid tumors. This application is particularly important for the assessment of macromolecular biologic therapies that are used not only in cancer but other conditions where site-targeted uptake is important. Use of a common agent with diagnostic and therapeutic properties has been quite helpful in understanding not only whether selective cancer targeting has been effective, but also for predicting therapeutic response and explaining variation in response. For example a recent trial in patients with metastatic HER-2-positive breast cancer used ^{89}Zr-labled trastuzamab PET imaging together with FDG PET to document that poor clinical response could be expected in those with low trastuzamab uptake despite positive biopsy results (Figure 6B).[59]

In cardiovascular drug development, we are not aware of any situations where molecular imaging has been critical for understanding pharmacokinetics for drugs that currently approved. However, molecular imaging has the potential to confirm the penetration and retention of drugs targeted to atherosclerotic plaques, thrombus, and ischemic myocardium. In particular major advances have been made in combining a therapeutic and diagnostic agent into a single nanoparticle moiety, commonly referred to as “theranostics” and which have been reviewed elsewhere.[60,61] For example gadolinium-labeled nanoparticles loaded with anti-angiogenic compounds such as fumagillin have been used to simultaneously assess the presence of functional plaque neovessels, the selective uptake of the delivery system, and response to therapy.[40] Non-invasive imaging with nanoparticle agents that also have direct anti-inflammatory activity has been achieved by targeting macrophage markers and using phototherapy or controlled release of statins, anti-mitogenic compounds, or prostanoids.[60,62–64] The premise of the entire field of theranostics is that imaging the biodistribution of a therapeutic agent will enhance the optimization process and accelerate approval.

Another area where molecular imaging has been used extensively to study biodistribution has been to study stem cell therapy. Strategies for labeling and detecting stem cells have been developed for essentially all forms of non-invasive imaging.[65] There are substantial practical differences in these techniques with regards to: (a) ability to provide quantitative information on number of cells present, (b) duration of detectability, (c) ability to detect daughter cells after division, (d) toxicity or other effects on stem cell function, and (e) transference of tracer to phagocytic cells involved in stem cell immune clearance. Optical, radionuclide, and MRI imaging have all revealed that the residence time of viable adult mesenchymal and bone marrow-derived stem cells is usually temporary, lasting days to weeks,[32,55,65–68] thereby supporting the notion that these cells act primarily through paracrine signaling of endogenous cells. Imaging has also been useful for understanding the failure or unpredictability of cell therapy. For example, PET detection of mesenchymal stem cells has been critical for establishing the concept that the beneficial effects of cell therapy on LV remodeling or angiogenesis are most pronounced when tissue retention of labeled cells is high.[69] Molecular imaging has also been useful for evaluating mechanisms for rapid loss of stem cells from the target tissue.[66,67] It is hoped that non-invasive imaging of stem cell activity and location will provide insight into the rather small cardiovascular benefits of cell therapy in clinical trials when compared to pre-clinical animal models.

Selection of Patients and Endpoints in Clinical Trials

The use biomarkers as surrogate endpoints in clinical trials has been a topic of lively debate for decades. Both pharma and the regulatory agencies that oversee drug approval have accepted the concept that biomarkers, including imaging biomarkers, have an important role for accelerating and reducing cost of the drug development and approval process. The only biomarker that has been consistently accepted for cardiovascular disease drug approval by the FDA has been serum cholesterol levels.

The application of molecular imaging as a potential biomarker in clinical trials hinges on several requirements. The technique must be quantitative and provide temporal information. High sensitivity for detecting the targeted molecular process is advantageous, although equally important is a complete understanding of how specific the target is to the pathway of interest. For example, α_v-integrin-targeted contrast agents have been applied to detect neovascular endothelial phenotype in angiogenesis, but signal from these agents are just as likely to reflect α_v-integrin expression present on activated monocytes which are also involved in vascular remodeling.[70] For contrast agent-based approaches, a solid understanding of in vivo kinetics is vital. For example, for tracers with high first-pass retention, tissue uptake is not simply dependent on target molecule expression but is also influenced by the relative blood flow. This issue is of critical importance when temporally assessing angiogenesis where changes in perfusion over time must be accounted for when registering tracer intensity.[71] Ideally, a targeting approach should not alter the process that is being targeted for drug therapy. For example, there has been demonstration that agents that rely on monocyte/macrophage phagocytosis such as USPIOs may influence monocyte phenotype.[72]

The most common molecular imaging technique that has been used as an endpoint in cardiovascular clinical trials is ^{18}F-FDG PET to assess atherosclerotic disease activity. This approach has been used to assess the effects of many different inflammation-targeted therapeutics which have been reviewed elsewhere.[35] Because of the complexity of ^{18}F-FDG detection in the coronary arteries which are adjacent to the metabolically-active epicardial surface, ^{18}F-sodium fluoride PET has been also for coronary atherosclerosis imaging and could detect early disease based on its ability to detect microcalcification.[73] To our knowledge,, though, molecular imaging biomarker has not been used as a primary surrogate outcome measure for drug approval and instead has been used early in clinical experience for for “proof-of-concept”.

The situation is very different in cancer medicine. Clinical trials in breast cancer, gastrointestinal stromal tumors, lung cancer, etc., have definitively shown that changes in primary and metastatic tumor activity on ^{18}F-FDG PET precedes changes in anatomic tumor burden.[58] Accordingly, PET imaging has been used as a critical component for the approval of a variety of tyrosine kinase receptor inhibitors and immunotherapy in cancer.

Aside from its use as surrogate endpoint, molecular imaging has the potential to select appropriate cohorts for initial testing of new drugs in clinical trials. As mentioned previously, “high-impact” cohorts could include patients with atherosclerosis who have high

inflammatory burden or patients with cardiomyopathy who have high myocardial sympathetic activity for anti-arrhythmic therapy. A barrier to this approach in the pre-NDA phase has been business-related concerns that it could adversely affect return-on-investment by reducing the pool of potential candidates for therapy. However, the use of molecular imaging may be particularly helpful in post-marketing Phase IV clinical studies that are often designed to test drug effectiveness based on dosing or in specific populations of patients, or drug toxicities. There have been examples of this application, such as the use of ^{18}F-FDG PET to compare effectiveness of low and high-dose statin therapy.[13]

Summary

Instead of providing a comprehensive list of molecular imaging techniques that have been used in cardiovascular science, this review has instead attempted to focus on how this technology can be used in drug development at several stages to accelerate the process and reduce cost. Molecular imaging technologies that have been developed for clinical diagnostics have successfully been re-tasked to understand efficacy of candidate therapeutics in pre-clinical and even in early clinical studie**s.** The lack of history using molecular imaging as a surrogate clinical endpoint for drug approval in cardiovascular medicine has not, however, dampened the prospects of using molecular imaging to make “go or no-go” decisions early in cardiovascular clinical trials or for assessing dosing strategies.

Acknowledgments

The authors are grateful for the helpful comments from Kenneth Krohn, PhD.

SOURCES OF FUNDING

Dr. Lindner is supported by grants R01-HL078610 and R01-HL120046 from the National Institutes of Health (NIH), Bethesda, MD. Dr. Lindner is also supported by grant 14-14NSBRI1-0025 from the National Space Biomedical Research Institute. Dr. Link is supported by the National Cancer Institute (NCI) grant P01CA042045

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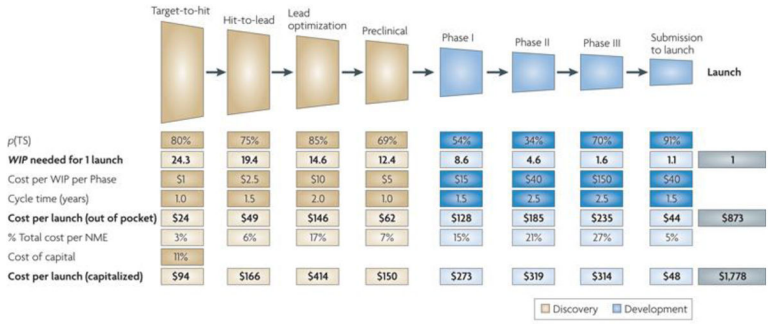

Figure 1.

Model illustrating research and development costs and time for a new molecular entity (*NME*) at the different phases of drug development from lead compound “hit” to drug launch. The model is based on both assumptions and data from industry benchmarks which include the probability of transition to the next stage (*p*[TS]), and the average number of works-in-process (*WIP*) at each stage needed to culminate in a single successful NME launch. Lighter shade boxes are values that are based on assumed inputs, including capital cost rates which are assumed at 11%. Reproduced with permission.[3]

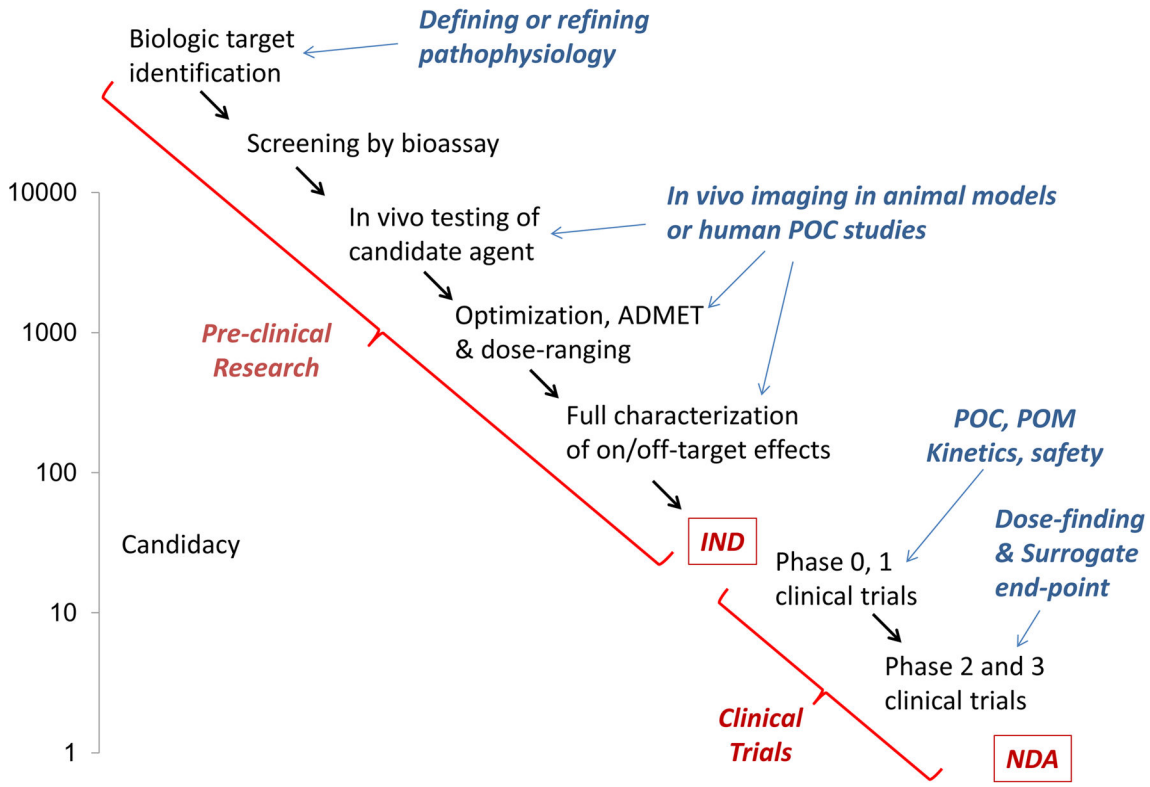

Figure 2.

Schematic illustrating pre-clinical research stages leading to investigational new drug (*IND*) application and clinical stages leading to new drug application (*NDA*); and potential roles of molecular imaging for discrete stages. Left y-axis schematically depicts the number of candidate new molecular entities that at screening stage (top) and each subsequent stage to culminate in a single NDA. ADMET = absorption, distribution, metabolism, excretion and toxicity; POC = proof-of-concept; POM = proof-of-mechanism.

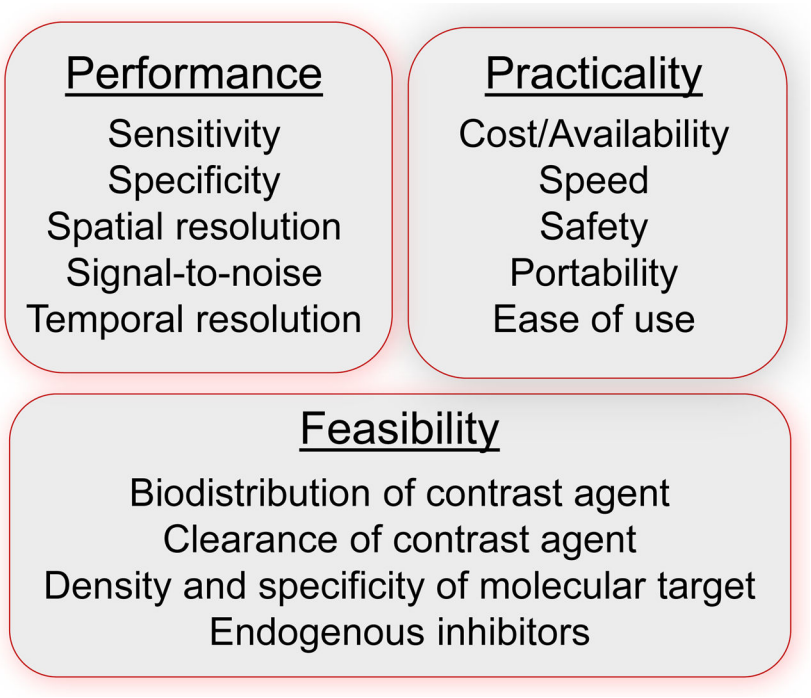

Figure 3.
Imaging technology characteristics commonly considered when selecting an approach for molecular imaging in science and in medicine.

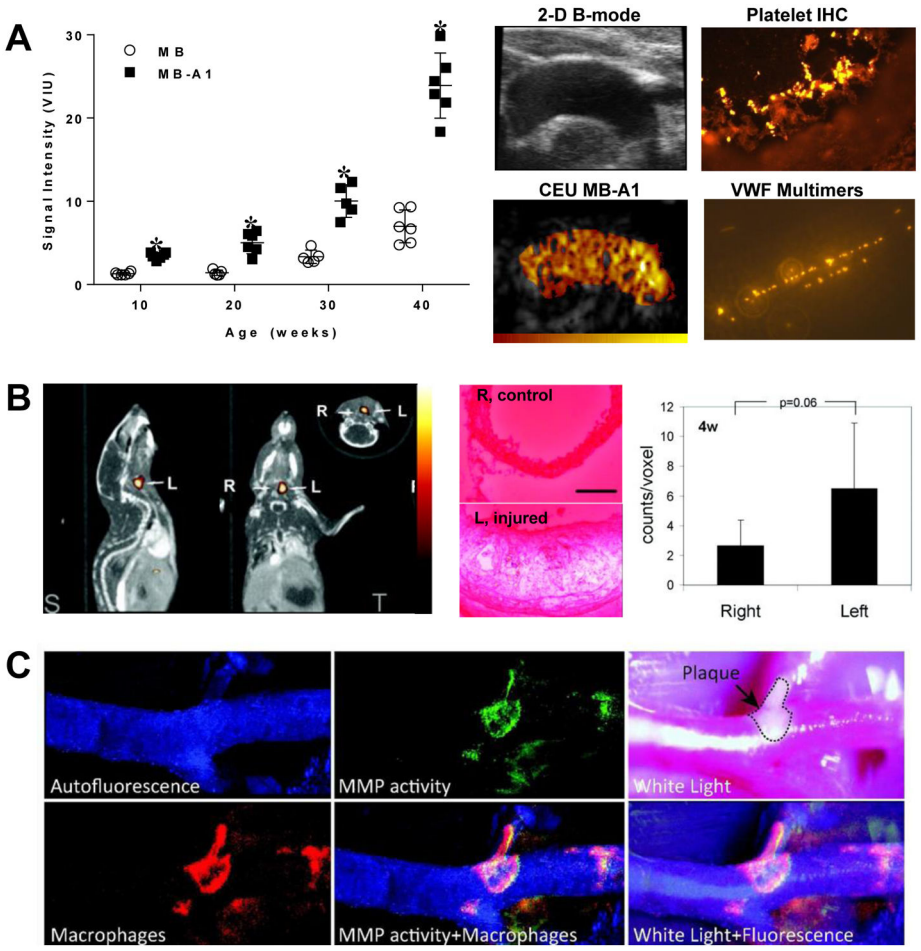

Figure 4.

Molecular imaging studies examining pathobiology of atherosclerosis. (**A**) Ultrasound molecular imaging showing age-dependent increase in signal from platelet-endothelial interactions (*MB-A1*) in the thoracic aorta of LDL-R−/− and Apobec-1−/− atherosclerotic mice (*MB*=control agent); and images illustrating the proximal thoracic aorta by B-mode and platelet-targeted contrast-enhanced ultrasound (CEU MB-A1), platelet-endothelial attachment on immunohistochemistry (*IHC*), and presence of endothelial VWF multimers by *ex vivo* GPIba-nanobead flow chamber. Modified with permission.[24] (**B**) MicroCT and SPECT fusion molecular imaging images and quantitative data in an apoE−/− mouse 3 weeks after left carotid injury with an ^{111}In-labeled agent targeted to MMPs, and *ex vivo* zymography showing protease activity of the left carotid. Modified with permission.[26] (**C**) Intravital optical imaging of the carotid artery of an apoE−/− mouse on atherogenic diet illustrating co-localization of signal from an activatable MMP-13 fluorophore, macrophage-targeted fluorescent nanoparticles in a region of plaque. Modified with permission.[19]

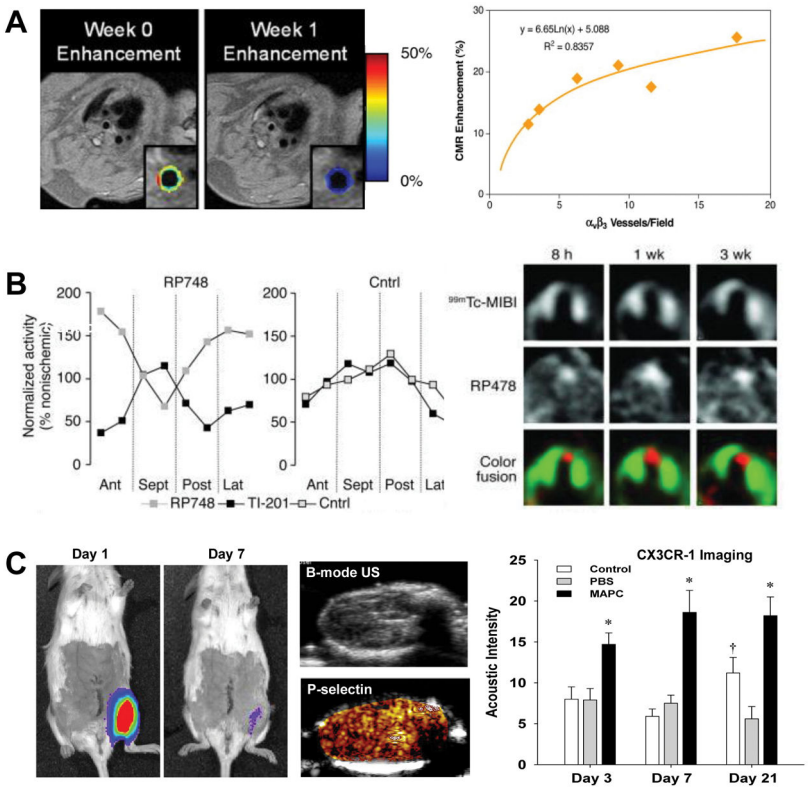

Figure 5.

Molecular imaging of angiogenesis. (**A**) MRI with color-coded signal enhancement from gadolinium-labeled $\alpha_v\beta_3$-integrin-targeted nanoparticles showing a treatment-related reduction in plaque angiogenesis at 1 week in the thoracic aorta of an atherosclerotic rabbit model, and correlation between $\alpha_v\beta_3$-positive vessels on histology with nanoparticle signal (*CMR enhancement*). Modified with permission.[40] (**B**) Quantitative spatial relationship between perfusion by SPECT (*Tl-201 or* ^{99m}*Tc-MIBI*) and regions of angiogenesis with an ^{111}In-labeled $\alpha_v\beta_3$-integrin-targeted SPECT probe (*RP748*) in a canine model of myocardial infarction illustrating highest area of angiogeneic activity in the infarct area. Modified with permission.[41] (**C**) Imaging of therapeutic angiogenesis with multipotential adult progenitor cells (*MAPC*) in a murine model of hindlimb ischemia illustrating rapid clearance of cells by optical imaging of luciferase-transfected MAPCs, ultrasound images (B-mode and P-selectin-targeted imaging) of endothelial adhesion molecule expression in a MAPC-treated hindlimb, and quantitative data from CX_3CR-1 molecular imaging to detect sustained increased recruitment of pro-angiogenic monocytes in MAPC-treated over control conditions. Modified with permission.[32]

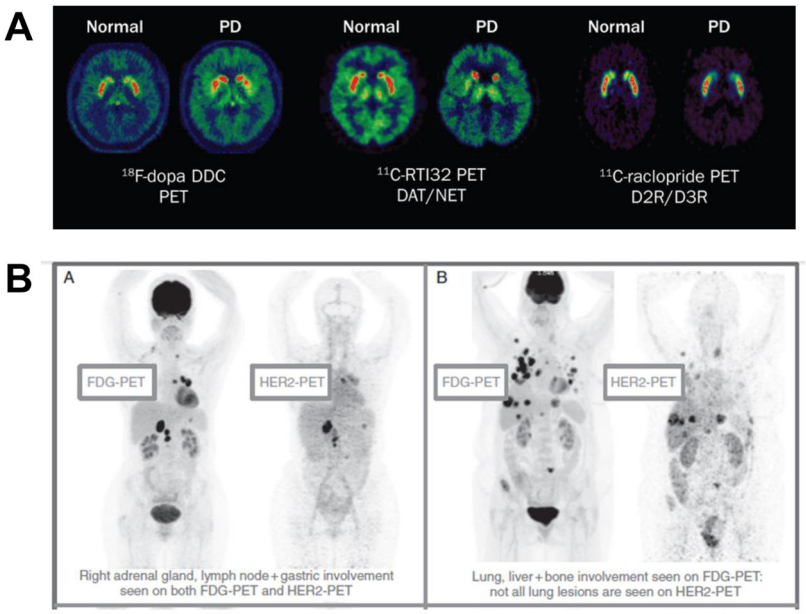

Figure 6.

Imaging to understand drug biodistribution. (**A**) PET brain imaging in Parkinson's disease (PD) to assess dopamine storage (^{18}F-DOPA), transporter function (*^{11}C-RT132*), and relative dopamine D2 and D3 receptor binding (*^{11}C-raclopide*). Modified with permission.[57] (**B**) PET imaging of 89Zr-trastuzamab (*HER2-PET*) illustrating variable uptake of the theapeutic in biopsy-proven HER-2-positive tumor metastases which are all detected by FDG-PET. Reproduced with permission.[59]