Advances in Cardiovascular Imaging

Imaging Biomarkers in Atherosclerosis Trials

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Atherosclerosis and its thrombotic complications are the leading cause of morbidity and mortality in developed countries, and the burden of atherosclerotic disease is expected to increase even further in the coming decades due to soaring obesity rates that feed the diabetes epidemic. There is, therefore, a clear need for new drugs targeting atherosclerosis to add to our current therapeutic armamentarium. Drug approval currently is based on multicenter, randomized, placebo-controlled trials with long-term follow-up in thousands of patients to demonstrate clear benefits in mortality and cardiovascular events and to allow adequate assessment of safety. Cardiovascular drug development has become a hostage to its own success. New drugs must be compared to placebo but on the background of highly effective standard therapy that lowers event rates and necessitates huge sample sizes and long follow-up. Failures of promising new cardiovascular drugs in large clinical trials have had catastrophic consequences for the sponsoring pharmaceutical and biotechnological companies. These conditions are inhibiting new drug development and are stimulating a search for alternate methods to assess new compounds. Cardiovascular imaging techniques have been used to fill this need. Cardiovascular imaging trials are shorter and require only a fraction of the patients needed for a large events trial because all patients who complete an imaging trial contribute to the end point. Thus, imaging trials are less expensive. By themselves, they are not sufficient for drug approval by regulatory agencies partly because of the limited safety data that can be generated with the study drug given the number of patients involved and duration of exposure. Nevertheless, imaging studies can provide evidence to inform the decision about whether a large outcome trial should proceed. They are therefore currently best suited in phase 2 of drug development. Ideally, imaging studies also should provide useful data regarding the mechanism of benefit of the new drug. In addition to human studies, atherosclerosis imaging plays a multifaceted role in preclinical studies in which the efficacy of new therapies can be evaluated in realistic physiopathological conditions.

Biomarkers are objectively measured and evaluated indicators of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. Conventional atherosclerosis imaging techniques provide quantitative and morphological data that have been useful in predicting whether an experimental compound may yield clinical benefit. Future drug therapies are expected to modify plaque biology directly, often in subtle ways that may benefit from emerging molecular and cellular imaging techniques. This review discusses in detail imaging biomarkers of atherosclerosis, including molecular imaging, by different modalities (Table 1); their strengths and limitations; and their application to drug trials (Table 2) and clinical patient evaluation.

Pathophysiology of Atherosclerosis Related to Potential Imaging Targets

Atherosclerosis, a systemic disease of the vessel wall involving the aorta and coronary, carotid, and peripheral arteries, commonly manifests clinically as myocardial infarction, stroke, or sudden death. These clinical consequences of atherosclerosis are often caused by a thromboembolic event at the site of or downstream from the atherosclerotic plaque. Vulnerable plaques, however, are no longer considered the only culprit factor for an acute coronary syndrome. It is now recognized that vulnerable patients prone to thrombosis and vulnerable myocardium prone to arrhythmia play a role in the outcome.

In acute coronary syndromes, thrombosis occurs either from plaque rupture, from erosion, or at the site of calcified nodules. However, most events are caused by plaque rupture and the exposure of thrombogenic plaque constituents to flowing blood. Plaques most prone to rupture have thin fibrous caps overlying a thrombogenic lipid core. The fibrous cap is a dynamic structure where collagen synthesis is modulated by positive and negative factors and can be degraded by metalloproteinases derived from activated macrophages. Upregulation of angiogenesis can lead to erosion of the extracellular matrix and its replacement with physically fragile neovascular beds, thus weakening the arterial wall and promoting rupture. Intraplaque hemorrhage also is believed to lead to abrupt progression in atherosclerosis. Plaque fractures usually occur at the shoulder region of the fibrous cap of eccentric lesions where the cap often is thinnest and the collagen content lowest. Larger lipid cores increase the risk of plaque rupture. Inflamed plaques with soft lipid cores that
are more vulnerable to rupture are measurably hotter than adjacent arterial segments.13 Many of the underlying cellular processes can serve as suitable targets for molecular imaging biomarkers.

Distinct from conventional imaging technologies that rely on anatomic, physiological, or metabolic heterogeneity to provide image contrast, molecular imaging uses targeted imaging agents that can exploit specific molecular targets, pathways, or cellular processes to provide image contrast.14 Molecular imaging techniques are available for most imaging modalities, including CT,15 integrated nuclear CT imaging,16–21 optical imaging,22–27 and MRI.28–34 Although major advances have been made in molecular imaging techniques, the clinical emergence of targeted molecular probes is slower. Nonspecific agents developed for other purposes are still mainly used (fluorodeoxyglucose [FDG], ultrasmall paramagnetic particles of iron oxide [USPIO]) instead of targeted imaging compounds. This is in part due to the safety studies necessary to bring these agents to the clinic and the potential toxicity of many of the promising agents used in animal

<table>
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<tr>
<th>Imaging Targets</th>
<th>Resolution</th>
<th>Radiation</th>
<th>Invasiveness</th>
<th>Advantages and Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Coronary lumen</td>
<td>0.4 mm</td>
<td>Yes</td>
<td>Yes</td>
<td>Two-dimensional silhouette of lumen only, associated with cardiovascular events, accepted surrogate for expanded indication</td>
</tr>
<tr>
<td>Coronary arterial wall</td>
<td>0.08–0.10 mm</td>
<td>No</td>
<td>Yes, catheter</td>
<td>Tomographic, volumetric assessment of changes in plaque burden and vascular remodeling with excellent resolution</td>
</tr>
<tr>
<td>cIMT and plaque</td>
<td>0.15 mm</td>
<td>No</td>
<td>No</td>
<td>Wide availability, wide clinical trial experience, low cost, technically demanding; further validation of 3D plaque assessment required</td>
</tr>
<tr>
<td>Coronary lumen and wall</td>
<td>0.4 mm</td>
<td>Yes</td>
<td>No</td>
<td>Rapid acquisition, limited image resolution for plaque burden, radiation exposure</td>
</tr>
<tr>
<td>Carotid lumen and wall</td>
<td>0.5–1.0 mm</td>
<td>No</td>
<td>No</td>
<td>Plaque characterization possible, no validation with clinical events</td>
</tr>
<tr>
<td>Metabolic activity in carotids and aorta</td>
<td>4 mm</td>
<td>Yes</td>
<td>No</td>
<td>Limited image resolution, limited ability to visualize coronary arteries, no validation with clinical events</td>
</tr>
<tr>
<td>Coronary arterial lumen and wall</td>
<td>Excellent, 0.01 mm</td>
<td>No</td>
<td>Yes, catheter</td>
<td>Best image resolution, limited depth of view Requires flushing of lumen</td>
</tr>
<tr>
<td>Tissue spectral contrast (chemical assessment)</td>
<td>1 mm</td>
<td>No</td>
<td>Yes, catheter</td>
<td>Link to plaque status not clear, images difficult to interpret, limited spatial registration</td>
</tr>
<tr>
<td>Exogenous, molecular contrast</td>
<td>1 mm</td>
<td>No</td>
<td>Yes, catheter and injection of molecular compound</td>
<td>Limited depth of view due to blood absorption, may require flushing; high potential for molecular imaging; depends on targeted compound approval for humans</td>
</tr>
</tbody>
</table>

| Imaging Assessments of Antiatherosclerosis Drugs in Clinical Studies |
|-------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Coronary Angiography | IVUS | cIMT | Coronary CT | MRI | FDG-PET |
| Statins | Slowing of progression in multiple studies | Slowing of progression-regression-several statins | Slowing of progression-regression-several statins | Lack of effect on coronary calcium | Disease regression, effects on plaque composition and inflammation | Reduction in FDG uptake |
| ACAT inhibitors | No beneficial effects | Deleterious effects of 2 agents | Deleterious effects | Not studied | Not studied | Not studied |
| ACE inhibitors | No beneficial effects | Favorable effects of ramipril | Favorable effects of ramipril | Not studied | Not studied | Not studied |
| Niacin | Slowing of progression | Not studied | Slowing of progression-regression | Not studied | Regression of disease | Not studied |
| CETP inhibitors | No benefit with torcetrapib | No benefit on primary end point of torcetrapib | No benefit with torcetrapib | Not studied | Ongoing with dalcetrapib | Ongoing with dalcetrapib |
| PPAR agonists | Slowing of progression with fenofibrate | Slowing of progression with pioglitazone | Slowing of progression with pioglitazone, inconsistent with PPARα | Not studied | Regression with bezafibrate | Not studied |
| HDL infusions | Slowing of progression | Regression vs baseline, change in plaque composition | Not studied | Not studied | Not studied | Not studied |

CETP indicates cholesteryl ester transfer protein; PPARα, peroxisome proliferator-activated receptor α.
models (eg, iron oxide particles at large doses necessary to create acceptable contrast). Furthermore, the molecular imaging techniques used in animals sometimes cannot be immediately translated in humans because of technical difficulties (eg, light attenuation in optical imaging). Another limitation is the fact that targets developed for animal models may not readily translate to humans.

**Coronary Arteriography**

Fifty years after its introduction, coronary angiography remains the standard technique for the clinical evaluation of coronary artery stenosis (Figure 1). The advantages of this technique include familiarity, widespread accessibility, and documented correlation with future coronary events. A rapid, computer-assisted angiographic analysis system, quantitative coronary angiography (QCA), can reproducibly and accurately define the site and severity of coronary stenoses and provide objective measurements of lumen dimensions that have proven to be particularly useful in clinical trials.

Angiographic evidence of atherosclerosis progression has been shown to predict future cardiovascular outcomes. QCA uses videodensitometry with automated vessel-edge detection to estimate the extent to which the atherosclerotic plaque intrudes on the vessel lumen. Technical limitations to this methodology include the inherent limitations of x-ray imaging with reduced contrast and sharpness, image digitization noise, and quantization errors in the analog-to-digital conversion process. Biological limitations include differences in vasomotor tone, vessel motion, myocardial blush, filling of the vasa vasorum, and insufficient blood-contrast mixing.

Although coronary artery lumen stenoses of 70% or more often cause myocardial ischemia and are targets for revascularization, acute coronary and cerebrovascular syndromes frequently are caused by the rupture of plaques that cause stenosis less than 50% and are not flow limiting. These smaller culprit plaques are underestimated by angiography, which relies on measuring the size of the vessel lumen alone. This underestimation occurs partly because of positive arterial remodeling, the process by which the artery initially only increases its external diameter in response to accommodate an enlarging atherosclerotic plaque. This adaptation allows the arterial lumen to remain unchanged until at least 40% of the total vessel is obstructed by atheroma. Angiographic
underestimation of atherosclerotic plaques also is caused by diffuse atherosclerosis, which in the majority of cases involves the so-called reference segment used to assess stenosis severity. The inability to directly visualize plaque and to determine its composition is a significant limitation of coronary angiography in the context of atherosclerosis progression-regression studies. Nevertheless, the ability to assess all major coronary arteries, with their distal bed, with standard invasive angiography is an advantage and constitutes a rationale to complete this assessment in the setting of clinical studies using other invasive imaging modalities, such as intravascular ultrasound (IVUS). Coronary angiographic studies were used to obtain indications of slowing atherosclerosis progression for several statins in the era of truly placebo-controlled trials. QCA results also have been shown to correlate significantly with both on-treatment high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol levels. Furthermore, QCA has been able to detect rapid changes in lumen dimensions induced by infusions of reconstituted HDL in 1 study.

**IVUS**

IVUS produces cross-sectional images of both the lumen and the coronary artery wall in vivo using catheter-based miniaturized 20- to 45-MHz ultrasound transducers. The transducer is withdrawn along the artery at 0.5 mm per second as images are recorded at 30 frames per second and provides excellent axial resolution, reaching approximately 100 μm with high-frequency catheters. IVUS delineates the intima and media within the area circumscribed by the external elastic membrane (Figure 1). Unlike coronary angiography, IVUS is able to clearly detect atherosclerotic plaques not protruding into the lumen as well as positive and negative vascular remodeling. IVUS is able to demonstrate changes in plaque lesion size with time and treatment along with accompanying vascular remodeling and has thus emerged as the new gold standard for atherosclerosis imaging of the coronary arteries in drug trials. Clearly, IVUS has allowed physicians to realize that we have entered an era in which atherosclerosis regression is achievable, including randomized trials of test drugs in which the placebo arm involved the intensive use of statins or 1-arm studies like ASTEROID (A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden).

IVUS remains an invasive imaging modality with potential procedure-related complications, particularly coronary spasm, which is reversible with nitroglycerin. The short- and long-term safety of IVUS in clinical studies of atherosclerosis progression has been documented to be excellent. No myocardial infarction has been reported in a large multicenter registry when IVUS was performed for atherosclerosis assessment (not related to a therapeutic intervention). The longer-term safety also has been demonstrated by confirming the absence of accelerated atherosclerosis at 2 years of follow-up in the coronary artery interrogated by IVUS. In addition to its use as a research tool, IVUS has been shown to be of clinical value for the assessment of ambiguous lesions, particularly those located in the left main coronary artery or at bifurcation sites.

IVUS can be used to stop the development of new chemical entities early in drug development. The lack of benefit of 2 different acyl-coenzyme A:cholesterol acyltransferase (ACAT) inhibitors assessed in separate multicenter studies analyzed by 2 different IVUS core laboratories has greatly reduced the interest toward this class of agents.

We have learned since then that a large amount of unsterified cholesterol in the arterial wall may have paradoxical effects on atherosclerosis progression. This is a very important indication of IVUS in clinical studies that allows reallocation of resources toward other compounds that show evidence of biological efficacy in phase 2 biomarker studies. In contrast, 2 relatively small IVUS studies have suggested that HDL infusions may induce rapid regression of coronary atherosclerosis. These results have created the impetus for additional clinical studies with reconstituted HDL in patients with recent acute coronary syndromes. IVUS also has been used to assess the effects of the cholesteryl ester transfer protein inhibitor torcetrapib in the ILLUSTRATE (A Coronary IVUS Study to Compare Torcetrapib/Atorvastatin to Atorvastatin Alone in Subjects With Coronary Heart Disease) study. Despite a large increase in HDL cholesterol (>60%) and a substantial reduction in LDL cholesterol (>20%) over that obtained with statin therapy, torcetrapib did not demonstrate a significant effect on the primary end point of change in percent atheroma volume. It was later found that torcetrapib also activated the renin-angiotensin-aldosterone pathway. Although IVUS imaging did not demonstrate significant benefits of torcetrapib on atherosclerosis in ILLUSTRATE, it could not predict the clear clinical harm that was observed in the ILLUMINATE (A Study Examining Torcetrapib/Atorvastatin and Atorvastatin Effects on Clinical CV Events in Patients With Heart Disease) clinical outcomes trial.

The major limitation of IVUS is the paucity of data directly linking changes in coronary atheroma and future cardiovascular outcomes. An indirect link has been made between the benefits observed with more intensive lipid lowering (with atorvastatin 80 mg daily) compared with moderate lipid lowering (with pravastatin 40 mg daily) in both an IVUS study (REVERSAL [Reversal of Atherosclerosis With Lipitor]) and a clinical outcomes trial (PROVE-IT [Pravastatin or Atorvastatin Evaluation and Infection Therapy]). This is limited, however, by the fact that these results were obtained in different trials involving patients with different characteristics (all patients were recruited early after an acute coronary syndrome in PROVE-IT). Changes over time on IVUS also have been shown to correlate significantly, albeit weakly, with QCA measurements in another study. Although IVUS has shown that patients with greater severity of recognized risk factors have more severe coronary atheroma, the demonstration that atherosclerotic changes correlate with clinical outcomes would clearly constitute a major element favoring the use of this imaging modality in drug development. The Canadian Atherosclerosis Imaging Network (CAIN), funded by the Canadian Institutes of Health Research and Canada Foundation for Innovation, is presently conducting a large clinical study of patients undergoing serial coronary angiograms, coronary IVUS, and carotid ultrasound.
examinations for up to 2 years and in whom clinical events are collected for 5 years (www.canadianimagingnetwork.org). This study will add to the existing knowledge about rates of atherosclerosis changes (progression or regression) over time in different vascular beds and will establish links with future cardiovascular outcomes. IVUS also will be used in that CAIN study as a fine phenotyping modality to identify novel circulating biomarkers based on omic approaches.

Processed 2D IVUS images allow for the assessment of atheroma burden with excellent image resolution, but their ability to determine plaque composition is more limited. Indexes have been created to characterize plaques with standard IVUS images. More recently, an algorithm (called virtual histology) that provides information on the types of plaques present based on spectral analysis of ultrasound backscatter and mathematical transformation of radio-frequency data into a color-coded representation (including for lipid plaque, fibrous tissue, calcification, and necrotic core) has been used at baseline and compared with clinical events. However, more studies (like CAIN and the High-Risk Plaque Initiative) need to be conducted to link changes over time in plaque characterization with future cardiovascular outcomes.

**Coronary CT**

Electron beam CT (EBCT), a technique specifically developed to image the heart, uses an electron sweep of stationary tungsten rings to generate serial transaxial x-ray images at a slice thickness of 3 mm for the purpose of detecting coronary calcium. The score of coronary calcium present provides an assessment of cardiovascular risk that has been shown to add prognostic information to the Framingham risk score. Detection of arterial calcification confirms the presence of atherosclerosis, but the absence of calcium does not entirely exclude its presence. Furthermore, acute coronary syndrome and fatal coronary occlusion can occur at sites of immature, lipid-laden plaque without detectable calcification. The interest in using EBCT to assess the effects of a drug on atherosclerosis decreased after the BELLES (Beyond Endorsed Lipid Lowering With EBT Scanning) study failed to show benefits of intensive statin therapy on coronary calcifications. Nevertheless, it is conceivable that EBCT could be used to identify subgroups of stable patients at higher risk of increased event rates for enrollment in a phase 3 clinical trial to limit sample size.

Multidetector CT (MDCT) uses a continuously rotating x-ray source to obtain a sequence of thin axial slice images (0.3 to 0.75 mm) during a patient’s single breath-hold. Because of technological advances, MDCT has emerged as the noninvasive imaging modality of choice for the detection of significant coronary artery stenoses as well as of coronary artery plaque burden (Figure 1). Our group has shown a very good correlation between plaque volume determined by MDCT and that determined by IVUS, with good interobserver and intraobserver variability in this single-center study. The sensitivity and specificity of MDCT for the detection of significant stenosis (>50%) in our study when compared to invasive angiographic examination were 100% and 91%, respectively. Recent controversy about the limitations of MDCT was generated principally by a multicenter study that reported a specificity of only 64% for detecting the presence of significant coronary stenosis (≥50%) in a patient-based analysis. In another multicenter study, a specificity of 90%, a positive predictive value of 91%, and a negative predictive value of 83% were reported. These results suggest that MDCT cannot routinely replace invasive coronary angiography, but this study had the limitation of minimization of the radiation dose, tube current that was capped at 270 mA for women and 400 mA for men, a factor that would increase image noise. Additionally, only patients with heart rates >70 beats per minute were given β-blockers, a factor that would contribute to more artifact-burdened studies. MDCT image quality obviously is a key to diagnostic accuracy, but other issues in multicenter studies, such as standardization of imaging examination and analysis in a core laboratory with validated standard operating procedures, are of paramount importance.

Vulnerable plaque is predominantly noncalcified, nonsteno-tic, and heterogeneous in composition. The noninvasive detection of such plaques remains a challenge; however, MDCT has shown some potential in this field of imaging. Evaluation of coronary plaque by MDCT is based on different attenuation coefficient values or densities measured in Hounsfield units for different components of the plaque. Generally, low-density lesions are considered lipid laden, whereas high-density lesions are considered calcified, with an overlap of densities from lipid laden, to fibrous, to calcified. The major limitation in identifying different plaque components is the limited spatial resolution of the currently available technology (0.4 mm), which makes it challenging to accurately identify plaque components with subtle differences in tissue contrast and overlapping noncalcified density differences in relatively small nonobstructive lesions. We have recently used 64-slice MDCT to assess the effects of a 5-lipoxygenase inhibitor in a phase 2 multicenter, multifaceted biomarker study of patients with recent acute coronary syndromes. In that substudy, patients underwent 64-slice coronary CT examinations at baseline and after 24 weeks of therapy. Significant reductions in new coronary plaques and in noncalcified plaque volume were observed with the antiinflammatory drug compared with placebo in patients in whom these end points were analyzable. These preliminary data suggest, therefore, that MDCT allows the noninvasive detection of treatment effects in phase 2 studies and that a reduction in leukotriene production may favorably influence atherosclerosis.

The other main drawback to MDCT is the exposure to radiation. This limitation is being addressed by the newer-generation scanners, such as low-dose high-definition technologies and adaptive statistical iterative reconstruction algorithms, which allow radiation exposure as low as 2.3 to 3.6 mSv. In addition to technological developments, novel contrast agents will play a key role in the development of plaque imaging. Macrophages have been implicated in acute plaque destabilization and are found in higher concentrations in vulnerable plaque. A novel iodinated nanoparticulate contrast agent, N1177, designed to detect macrophages by MDCT has shown promise in animal studies, and correlations.
between contrast-enhanced atherosclerotic plaques and intensity of macrophage infiltration in the lipid-rich core of the corresponding histological sections in the aortic wall of atherosclerotic rabbits have been reported.\textsuperscript{15} Continued improvements in technology, better understanding of the limitations and advantages provided by ongoing studies, and advances in contrast agent development appear promising for the future of MDCT. However, even a reduced radiation dose can potentially have biological effects, and further reductions in dose would be desirable for studies involving serial imaging.

**Carotid Ultrasonography**

Atherosclerotic plaque burden also can be assessed with 2D, or B-mode, carotid duplex ultrasound, which provides quantitative measurements of carotid artery stenosis and intima-media thickness. The diagnosis of carotid artery stenosis is important in clinical practice because it determines the need for surgical intervention.\textsuperscript{79} Carotid intima-media thickness (cIMT) measurements have been used in research trials because they provide a quantitative measure of disease.\textsuperscript{76,77} The advantage of cIMT measurement is that ultrasound is noninvasive, readily available, extremely safe, inexpensive, and well accepted by patients. Additionally, high-resolution ultrasonography measures the diseased arterial wall directly (Figure 1) compared with other techniques, such as brachial artery assessment of endothelial function, which are less direct. The validity of using cIMT for identifying patients with current disease as well as for predicting future cardiac and cerebrovascular events has been demonstrated. Ultrasound cIMT measurements correlate well with pathological measurements.\textsuperscript{78} This technique is reproducible, as documented by several groups of investigators,\textsuperscript{79} and the reliability of longitudinal measurements to document the rate of progression over time has been demonstrated.\textsuperscript{80} In population studies in men and women from various age groups, cIMT has shown strong correlations with classic and emerging risk factors.\textsuperscript{81,82} Even after adjustment for other risk factors, cIMT measurements have been shown to be potent predictors of myocardial infarction and stroke.\textsuperscript{83–86} In addition, cIMT measurements have been used as the primary efficacy measure for randomized placebo-controlled trials where treatment reduced the progression of cIMT and reduced cardiovascular events.\textsuperscript{87}

CIMT can be used in phase 2 studies to determine whether an experimental drug appears to have favorable biological effects that would warrant the launch of a large phase 3 clinical outcomes trial or neutral or detrimental effects that would lead to reallocation of resources to other development programs, as was discussed for coronary IVUS. The RADIANCE (Carotid B-Mode Ultrasound Study to Compare Anti-Atherosclerotic Effect of Torcetrapib/Atorvastatin to Atorvastatin Alone) trials did not demonstrate any beneficial effects of torcetrapib on cIMT measurements,\textsuperscript{88} which is line with the detrimental effects observed in the previously mentioned ILLUMINATE mega trial.\textsuperscript{55} Additionally, the disappointing results of the ACAT inhibitor pactimibe in a cIMT study,\textsuperscript{89} combined with similar coronary IVUS results,\textsuperscript{46,52} have led to the termination of the development of several agents of this class. Another use of atherosclerosis imaging is in phase 3b of development to obtain a specific indication of slowing atherosclerosis progression. Using the CAIN framework, we are presently conducting the DAL-PLAQUE-2: Dalcetrapib and plaque imaging in 2 vascular beds (Dalcetrapib on Atherosclerotic Disease in Patients With Coronary Artery Disease) randomized trial to test the hypothesis that dalcetrapib will slow atherosclerosis progression as assessed with coronary IVUS and cIMT in the same patients. This original study design was approved by regulatory agencies before its launch.

Finally, cIMT has been used in phase 4 studies to test medications that are already clinically available. The ARBITER-6 (Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol-6) study has shown that the use of niacin was associated with more favorable cIMT results than ezetimibe in patients already treated with a statin.\textsuperscript{90} The ENHANCE (Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression) trial generated controversy when it was reported that ezetimibe added to simvastatin 80 mg daily did not provide benefit on cIMT measurements when compared to simvastatin alone in patients with familial hypercholesterolemia.\textsuperscript{91} Although different explanations for this result have been proposed, it is likely that the inability to detect benefit with ezetimibe occurred because of the absence of progression of cIMT over time in the statin-alone group, leading to the need to demonstrate regression in the statin-plus-ezetimibe group, something very difficult to achieve given that baseline cIMT was only mildly thickened. The reproducibility of cIMT measurements certainly was not the issue, as the investigators reported excellent results.\textsuperscript{91} The possibility that ezetimibe could actually not yield cardiovascular benefits despite a 16% reduction in LDL cholesterol because of unforeseen effects remains a possibility. The IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial) mega trial will be important to resolve the controversy and establish indirect links with ENHANCE, but the repeated increases in sample size suggest that the results will show at best a modest benefit.

One disadvantage of cIMT as an end point is that it requires relatively large sample sizes because of the small changes that need to be detected.\textsuperscript{92} It has been suggested that this disadvantage could be overcome by 3D carotid ultrasonography with measurements of carotid plaque area or volume\textsuperscript{93–95} because atherosclerotic plaque of the carotid arteries may progress more rapidly along the length of the vessel rather than in thickness.\textsuperscript{96} In addition to measurement of plaque area or volume, the more recent concept of measuring plaque surface variation or plaque surface roughness has been introduced.\textsuperscript{97} This parameter is based on the concept that carotid ulceration on angiography identifies high-risk patients.\textsuperscript{98} Plaque surface roughness is measured with a computer algorithm to reflect the amount of ulceration on the plaque surface.\textsuperscript{99} This relatively sensitive noninvasive technique could permit smaller sample sizes and shorter trial times. However, limitations related to issues of reproducibility and interobserver variability remain to be resolved. The
Ultrasound Molecular Imaging

Ultrasound is sensitive to mechanical properties of tissues and does not report on molecular events. However, the recent combination of ultrasound with intravenously injected gas-filled microspheres has shown the ability of ultrasound to image molecular surrogates. By attaching a receptor-specific ligand to the microspheres, specific endothelial targets have been imaged through a persistent change of contrast at plaque locations.100

MRI

MRI is a noninvasive, nonionizing imaging modality that does not expose the patient to ionizing radiation, is not operator dependent, and provides multiparameter and multiplanar 3D data. In-plane spatial resolution reaches 250×250 μm² for the carotid arteries, 800×800 μm² for the aorta, and 460×460 μm² for the coronary arteries with a slice thickness of 2 to 5 mm.101 MRI has proven most advantageous in the carotid artery because this target vessel is accessible in the carotid endarterectomy. MRI offers the greatest potential for comprehensive structural plaque characterization, and its use in the diagnosis and treatment of carotid atherosclerotic plaque has been validated.102–105 Criteria for unstable plaque consist of active inflammation, a thin cap with a large lipid core, endothelial denudation with superficial platelet aggregation, fissured or injured plaque, and severe stenosis. MRI is highly efficient at detecting all these major criteria for unstable plaques.102 Generally, lipid components appear as isointense regions within the plaque on T1- and proton density-weighted sequences and are hypointense on T2-weighted sequences. The fibrous cap appears strongly hypointense, more so than the hyperintense thrombus, and calcium within the plaque appears hypointense on T1-, T2-, and proton density-weighted sequences. MRI can identify fibrous cap status, including intact, thick, or ruptured cap, the latter being associated with transient ischemic attack or stroke. MRI has the potential to recognize vulnerable plaque before an ischemic event.104,105

Given that it is noninvasive and suited for serial imaging, carotid MRI has already been used as the end point in successful clinical studies.106,107 More recently, another study using carotid MRI has shown that niacin (2 g daily) reduced carotid wall area within 12 months compared with placebo in 71 patients treated with statins.108 Several ongoing randomized studies using carotid MRI will help to better delineate its role in drug development. Most of the potential roles of IVUS and cIMT in phase 2, 3 and 4 studies also may apply to carotid MRI. One of the potential advantages of vascular MRI is its ability to image noninvasively and serially multiple arterial beds in the same patient; this concept is now being tested with a novel drug targeting atherosclerosis.

Coronary MRI

Whether plaques develop similarly in different vascular beds is uncertain, and the imaging criteria for vulnerable plaque are likely to be at least somewhat different in coronary and carotid arteries. Imaging coronary arteries remains technically challenging because of cardiac and respiratory motion, the small caliber and tortuous course of the coronary arteries, and their location deep within the thoracic cavity surrounded by epicardial fat. Recently, a 3D imaging protocol of the entire heart (using optimized techniques for respiratory and cardiac motion compensation, an intrinsically high-contrast imaging sequence, and steady-state free precession) yielded a high diagnostic performance with regard to stenosis detection compared with invasive coronary angiography (Figure 1).109 Applying the 16-segment coronary artery model, 83% of coronary segments could be evaluated with a sensitivity of 78%, specificity of 91%, and diagnostic accuracy of 89%. Using the 7-segment coronary artery model, 99% of the coronary segments were evaluated with a sensitivity of 82%, specificity of 88%, and diagnostic accuracy of 87%.109

Coronary MRI may prove to be clinically useful for the assessment of lumen stenoses, but it is unlikely to play a role in drug trials in the short term because of the previously mentioned technical challenges and the availability of more-established techniques, such as QCA, coronary IVUS, and MDCT. Evaluation of the coronary artery wall, although of great potential utility for research, has proven to be more technically challenging. MRI can detect the location of increased coronary artery wall thickness in patients with coronary artery disease.110 Similar to MDCT and IVUS, coronary MRI can assess arterial remodeling because it is able to detect increased coronary wall thickness in patients without significant coronary artery stenoses.111 Whether MRI can track coronary artery wall thickness over time and correlate progression with clinical events remains to be investigated.

MRI Molecular Imaging

MRI provides very good spatial resolution but is limited by its low sensitivity. This limitation may be overcome with the advent of molecular imaging, which is based on a signal-imaging element attached to a carrier that transports a ligand that is then recognized by the target molecule.112 The advantage of MRI as a molecular imaging approach resides in its ability to provide soft tissue and functional information by providing proton density, perfusion, diffusion, and biochemical contrasts. Because of these imaging capabilities, MRI allows for the coregistration of molecular and anatomic information within a single imaging modality.

Molecular MRI can target specific markers of atherosclerosis, including macrophages, vascular adhesion molecules, fibrin, and plaque neovessels, which can be used for different purposes (eg, early disease development, late risk for events). Superparamagnetic iron oxide nanoparticles (ie, USPIO) exert strong and reversible relaxation effects, thus creating a signal void or hypointense MRI signal. The uptake of USPIO by macrophages in atherosclerotic plaques registers as a decrease in signal on MRI and is indicative of macrophage activity and inflammation suggestive of an unstable
plaque. A recent in vivo study demonstrated predominant accumulation of USPIO in macrophages in ruptured and rupture-prone atherosclerotic lesions of human carotid arteries, which caused signal changes in T2*-weighted MRI sequences acquired 24 hours after intravenous administration of USPIO. The 11 patients in this study were scheduled to undergo carotid endarterectomy. Histological and electron microscopy analyses of the plaques revealed the accumulation of USPIO in plaque macrophages in 10 of the patients. Furthermore, histological analysis of the carotid arteries showed accumulation of USPIO in 75% of the ruptured and rupture-prone lesions but in only 7% of the stable lesions. These results suggest that the identification of macrophages in atherosclerotic plaques, using USPIO, may offer noninvasive risk stratification at the cellular level. However, iron oxide particles have limitations for use in clinical trials, namely because of their relatively small signal loss and difficult quantification capabilities. Nevertheless, the AHEROMA (Atorva- statin Therapy: Effects on Reduction of Macrophage Activity) study demonstrated that atorvastatin 80 mg per day (compared to 10 mg per day) was associated with a significant reduction in USPIO-defined vascular inflammation at 6 and 12 weeks. Cell adhesion molecules are interesting imaging targets because of their involvement in inflammation and the early stages of atherosclerosis. One such marker, vascular cell adhesion molecule (VCAM)-1, is a ligand for 3 integrin and covered with a monolayer of lipid that contains paramagnetic lipids (Gd-diethylene triamine pentaacetic acid-bis-oleate) can be imaged by MRI. A recombinant HDL-like nanoparticle composed of phospholipids and apolipoprotein A-I was developed to convey gadolinium-based contrast to the intima of atherosclerotic plaques in hyperlipidemic apoE-knockout mice. MRI studies showed a degree of enhancement proportional to the composition of plaque, which depends on plaque composition, evidence that this targeting agent shows promise both in detecting and in characterizing atherosclerosis using MRI. Assessment of plaque neovascularization has been considered by targeting markers that are specifically upregulated on the endothelium, such as α/β3 integrin. A perfluorocarbon nanoparticle targeted to α/β3 integrin and covered with a monolayer of lipid that contains paramagnetic lipids (Gd-diethylene triamine pentaacetic acid-bis-oleate) can be imaged by MRI. This complex has shown promise in detecting marked proliferation of neovessels within the aortic adventitia in a rabbit model of atherosclerosis.

The ability to image the biological activity of specific molecules noninvasively in atherosclerotic plaques in vivo has potentially important clinical and research applications. Great progress has been made in this field, but the ongoing challenge for molecular MRI is to identify nontoxic, reliable, readily detectable, and quantifiable agents that can provide pathophysiologic and prognostic information both from a single time point and from serial imaging.

**Intravascular MRI**

The self-contained intravascular MRI (IVMRI) probe takes advantage of the tissue-imaging capabilities of MRI while overcoming limitations inherent in the external coil MRI. The IVMRI probe has superior spatial resolution in the order of 250-μm radial resolution compared to noninvasive MRI at 460-μm planar resolution. The catheter contains an integrated MRI probe with magnets, radiofrequency coil, and electronics at its tip and is connected to a portable control unit. The system has no external magnets or coils, so it can be transported to the cardiac catheterization laboratory where other studies can be performed concurrently. MRI demonstrates differential water diffusion coefficients within the atherosclerotic plaque lipid core compared with the collagenous cap and medial smooth muscle layers. These differences allow IVMRI to delineate the extent and location of increased vascular lipid infiltration. Initial clinical results demonstrate safety and feasibility in the detection of coronary artery lipid content in patients with intermediate lesions undergoing coronary angiography. However, the potential of IVMRI for use in drug development currently is limited because it is invasive and not widely available.

**Nuclear Imaging**

Nuclear imaging provides accurate methods for identification and quantification of metabolic, cellular, and molecular processes. Single-photon emission CT (SPECT) and PET allow noninvasive detection of many cardiovascular pathologic states, including those associated with atherosclerosis. Hemodynamically significant coronary artery stenoses can be demonstrated through SPECT and PET myocardial perfusion imaging, and additional information on the severity of a stenosis can be obtained because PET allows absolute quantification of coronary flow reserve. SPECT and PET have the potential to depict different vascular beds within a single imaging session and can respectively qualify and quantify the signal produced from exogenously administered radionuclides using an external detector. These imaging modalities are highly sensitive to detect radiotracers at picomolar range but are significantly inferior to MDCT and MRI in terms of structural information and spatial resolution. To overcome the limitation of spatial resolution, coregistration with a second imaging modality has been introduced, including SPECT-CT, PET-CT, and PET-MRI approaches. This coregistration of modalities enables an integration of structure and function. PET is showing promise in the metabolic imaging of aortic (Figure 1) and carotid artery atherosclerotic plaque. Because FDG competes with glucose uptake into metabolically active cells, including macrophages found in vulnerable plaque, 18F-radiolabeled FDG (18FDG) has been used to image metabolically active macrophages and inflammation. The uptake of 18FDG has been shown to correlate with the
extent of macrophage infiltration in carotid plaques of patients scheduled for carotid endarterectomy. Furthermore, the effects of treatment on plaque metabolic activity in large arteries have been reported in a small cohort of patients who were not preselected for the presence of vascular disease. A few multicenter $^{18}$FDG-PET clinical studies presently are evaluating the effects of lipid-modulating and antiinflammatory drugs, and a reduction in FDG uptake in the vascular wall will be interpreted as a favorable result. It is presently used as an exploratory approach to detect early signs of biological effects of an experimental drug on the arterial wall of the aorta and carotid arteries. The links between both baseline values and changes over time in FDG uptake and future cardiovascular outcomes, however, remain to be studied and established. Positive associations with clinical events in large studies would greatly increase confidence in the use of $^{18}$FDG-PET imaging in drug development, particularly in phase 2 studies before moving to a later-stage, large outcomes clinical trial. Coronary artery studies are further limited by the low resolution of PET imaging and by the presence of cardiac motion. However, recent technological advances, including dual respiratory-cardiac gating, may permit future development in this vascular bed. Another limitation of $^{18}$FDG-PET for the coronary arteries is the high background myocardial metabolic signal.

Molecular imaging using integrated PET-CT and PET-MRI technology shows promise beyond macrophage activity in atherosclerosis. The cascade of lipid infiltration, inflammatory response, intraplaque hemorrhage, and plaque rupture leading to thrombus formation can potentially be characterized. Novel radioligands developed as molecular imaging probes have been combined to apoptosis (annexin)$^{21}$ and $\alpha\beta$3 integrin expression markers ($^{18}$F-galacto-RGD)$^{20}$ as well as targeted to matrix metalproteinases. Many of these probes can be labeled for SPECT and PET imaging and could detect responses to treatment of vulnerable plaques, thereby providing early insight into the effects, or lack thereof, of new drug therapies in early clinical studies. For the assessment of unstable plaque, labeling of LDL and HDL, both in oxidized and nonoxidized forms, has been evaluated$^{119,120}$; antibodies against proliferating smooth muscle cells in atheroma also have been studied with SPECT using indium labeling. The latter approaches, however, have not been used in clinical trials of novel drugs targeting atherosclerosis. The noninvasive nature and high sensitivity of nuclear imaging make this technology promising for future use both clinically and in research studies. PET also provides quantitative assessment of biomarker biodistribution. The disadvantages of PET imaging as a biomarker of atherosclerosis include the significant patient exposure to radiation and the high cost.

**Optical Imaging**

Optical coherence tomography (OCT) is an invasive catheter-based imaging modality that measures the intensity of reflected near-infrared light from tissue (Figure 2). The advantages of OCT are the very high resolution provided ($\approx 10 \mu m$) and the increasing video rate with the emergence of new optical frequency domain imaging using swept sources. The initial systems operated at 8 frames per second, but the new swept-source techniques can now work at up to 108 frames per second. OCT can be used in the context of percutaneous coronary interventions to assess stent apposition to the vascular wall, arterial dissections, and in-stent neointimal proliferation. However, the limited ability of OCT to see through blood and the limited penetration and depth of view are significant problems for its development as an imaging biomarker of atherosclerosis for the clinical development of antiatherosclerotic drugs. OCT requires saline flushing, occlusion of flow, or both for the visualization of the vascular wall. Although its depth of view can preclude determination of total plaque volume, OCT may allow the assessment of fibrous cap thickness and other plaque characteristics (eg, lipid pools) that would complement IVUS. Extensive work will have to be done to determine the ability of OCT to serially measure different plaque characteristics with excellent reproducibility and to reliably detect treatment effects before it can be used for decision-making in drug development programs.

Near-infrared spectroscopy (NIRS) is potentially an excellent method to identify chemical composition of atherosclerotic plaques (Figure 2). Catheter-based NIRS can generate spectra that may distinguish cholesterol from collagen and may identify chemical fingerprints in coronary plaques in the cardiac catheterization laboratory. Demonstration, both in animals and in patients, of the reproducibility of results in repeated catheter pullbacks in a coronary artery and of the ability of NIRS to detect changes in plaque composition with treatment will be necessary before this invasive method can contribute to the clinical development of drugs. Because the localization of signals generated by the NIRS catheter along the length of the pullback is difficult despite collection of angiographic data, the availability of a combination NIRS-IVUS catheter may be a significant step forward in the development of this technology for potential future use in clinical atherosclerosis trials.

Optical imaging, namely near-infrared fluorescence, is emerging as a molecular technique to assess plaque status. The potential advantages of optical approaches are the ability to use probes that can be activated and multispectral probes simultaneously to target more than 1 process and their high sensitivity. Optical molecular techniques have become an integral component of cardiovascular animal research, but the high absorption of light by hemoglobin and the difficulty in obtaining images that can yield quantifiable results are important limitations. Transillumination fluorescence molecular tomography (FMT) (Figure 2) has been used to study protease activity in atherosclerosis of small animals. A protease-activatable near-infrared probe reporting on cysteine proteases, including cathepsin B, was used in western-type diet-fed apoE and apoE/endothelial NO synthase double-knockout mice, demonstrating the feasibility of protease activity imaging. These probes are optically silent in their quenched state, but significant signal amplification occurs with enzyme-mediated release of the fluorochrome. Cell adhesion molecules as markers of early disease were studied with Cy5.5 conjugated to VCAM and intercellular adhesion molecule antibodies in apoE-knockout mice on a high-cholesterol diet. Detection was also demonstrated by using fluorophore lifetime-gated FMT and confirmed by immunocytochemistry.
Translation of small-animal FMT optical techniques to humans is challenging because of low light penetration. An invasive approach has been developed by using an optical fiber and a simple illumination scheme to provide a local, nontomographic estimate of fluorescence. Recently, the catheter and associated near-infrared probe were further validated in rabbits, providing evidence that the optical system has enough sensitivity to detect fluorescence in vessels the size of human coronary arteries. However, the diffusive nature of light in tissues, the relatively lower spatial resolution, and the heterogeneous nature of light propagation still remain concerns with optical methods. Future developments involving the combination of an optical catheter with IVUS are expected to be a step forward by providing anatomic guidance for the fluorescence signal interpretation and correlation with abnormalities in the coronary artery wall.

An alternative method using optically mediated ultrasound signal is called intravascular photoacoustic imaging. The physical process behind the technique is to first excite absorbers with pulsed light. The injected pulsed light produces rapid electromagnetic heating, which in turn can be detected by a wide-band ultrasonic transducer sensitive to small vibrations. A tomographic image reconstruction technique similar to that of ultrasound is then applied to locate the sources of optical absorption. Detection by both IVUS and photoacoustics can be performed with the same transducer, leading to straightforward coregistration and nearly identical resolution. Other combinations of optical imaging with separate modalities currently are being explored through the development of new multimodal probes that provide functional MRI and optical contrast simultaneously. For example, a multimodal contrast agent based on HDLs was synthesized and shown to provide enhancement of atherosclerotic plaques. Although promising, optical catheter methods based on smart probes are in their infancy, and human applications remain to be explored.

Regulatory Issues

There are several regulatory challenges in accepting the use of imaging in drug development. These challenges include the need for standardized image acquisition protocols and harmonized image analysis procedures. To overcome these
challenges, leading clinical research centers and core laboratories are working toward standardized image acquisition protocols while implementing intense training programs and certification testing for clinical trial sites. With the use of experienced and collaborative imaging sites and central reading facilities, uniform hardware and software, validation with phantoms, and the refinement of appropriate imaging end point definitions and analyses, the data resulting from imaging clinical trials in drug development have steadily become more reliable and consistent. Another important regulatory issue concerns the link between changes on imaging parameters induced by a study medication and future cardiovascular outcomes. Because this link often is not entirely certain, atherosclerosis imaging can be used in phase 2 to identify signs of biological efficacy of a novel medication but not to replace a clinical outcomes trial, the latter also being essential to establish a sufficiently large safety database to warrant regulatory approval. In contrast, a neutral or negative well-designed phase 2 imaging study decreases the potential benefit on clinical outcomes of a new medication targeting atherosclerosis, and 2 such studies with agents from the same class demonstrating lack of beneficial effects most often should lead to the termination of the development of the entire class.

Conclusions

New antiatherosclerotic drugs must demonstrate benefits when added to the current standard of care that includes intensive use of statins, which results in the need for very large and long-term clinical outcomes trials before drug approval by regulatory agencies. Imaging biomarkers of atherosclerosis can contribute significantly to clinical drug development by allowing in phase 2 the identification of new chemical entities that have favorable vascular effects and should be evaluated in a large outcomes trial and of those that show no benefits on the arterial wall and should be rapidly abandoned. Imaging also can be used in phase 3b of drug development to target an indication of slowing atherosclerosis progression, particularly if benefits are observed in 2 vascular beds. Molecular imaging also holds great potential for the early stage of clinical development of drugs against atherosclerosis.

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