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Biomarkers in the Prevention and Treatment of Atherosclerosis: Need, Validation, and Future

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***Abstract*——Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality in the developed world, and there is a clear need to develop novel therapeutic strategies to reduce cardiovascular risk further than is currently possible. Traditionally, the effectiveness of new cardiovascular drugs has been evaluated in clinical trials using cardiovascular outcomes as endpoints. However, such trials require large numbers of patients followed over long periods of time. Clinical trials using surrogate markers for CVD may be shorter in duration and involve fewer participants. Measurement of atherosclerotic progression is an ideal surrogate marker as it is predictive of future cardiovascular events. The "gold standard" for detecting and defining the severity, extent, and rate of atherosclerotic progression has been quantitative coronary angiography. However, this technique has fundamental limitations. More recently, measurement of carotid intima-media thickness using B-mode ultrasound and measurement of atheroma volume using intravascular ultrasound have emerged as more accurate techniques for detecting atherosclerotic progression. Both of these techniques have potential utility as surrogate endpoints in place of cardiovascular outcomes in clinical trials. Their use might facilitate the more rapid development of novel, safe, and effective therapies.**

I. Introduction

Cardiovascular disease (CVD[1]), including myocardial infarction (MI), heart failure, and stroke, represents the leading cause of mortality worldwide, accounting for half of the total number of deaths in the developed world (World Health Organization, 2002). Furthermore, the increasing prevalence of diabetes and obesity, combined with poor adherence to clinical guidelines that address both therapeutic lifestyle changes and pharmacologic interventions (National Cholesterol Education Program Adult Treatment Panel III, 2002; De Backer et al., 2003), bode poorly for the future prevalence of CVD. Thus, despite the many benefits that are obtainable under the current standard of care, based on current trends and in the absence of further therapeutic advances and their concerted application, it is expected that CVD will result in 20.5 million deaths annually by 2020 (World Health Organization, 2002) (Fig. 1). Clearly, the timely development of new pharmaceutical agents is necessary to moderate this health disaster, and to achieve this, new tools to evaluate clinical efficacy and

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[1] Abbreviations: CVD, cardiovascular disease; MI, myocardial infarction; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; QCA, quantitative coronary angiography; cIMT, carotid intima-media thickness; IVUS, intravascular ultrasound; CAD, coronary artery disease; ARIC, Atherosclerosis Risk in Communities; CI, confidence interval; CHS, Cardiovascular Health Study; CV, cardiovascular; CLAS, Cholesterol Lowering Atherosclerosis Study; PROCAM, Prospective Cardiovascular Münster; SCORE, Systematic Coronary Risk Estimation; ARBITER, Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol; REVERSAL, Reversal of Atherosclerosis with Aggressive Lipid Lowering; CRP, C-reactive protein; PROVE-IT, Pravastatin or Atorvastatin Evaluation and Infection Therapy; TIMI, Thrombolysis in Myocardial Infarction.

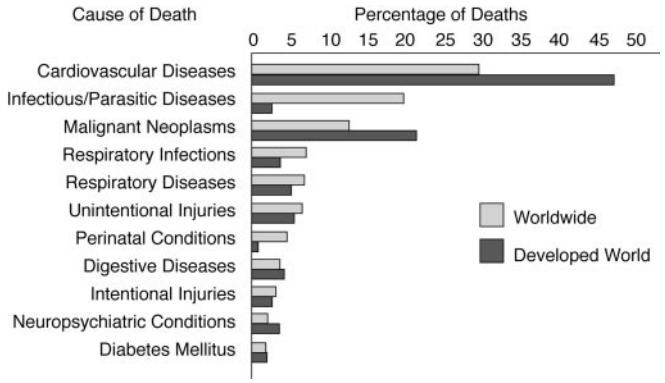

FIG. 1. Cardiovascular disease accounts for one third of deaths worldwide and half of deaths in the developed world. Data from World Health Organization (2002).

safety are required. More specifically, pharmaceutical companies and regulators must expedite the development and introduction of innovative pharmaceuticals that are directed toward new targets involved in the pathogenesis of CVD.

The "gold standard" for measuring clinical cardiovascular efficacy in drug development is the morbidity and mortality trial. However, such trials may require 10,000 to 15,000 subjects, followed for at least 5 years, to demonstrate a significant incremental benefit of a novel drug over and above that provided by currently available therapies. Moreover, the direct costs of conducting such trials and the costs resulting from the overall duration of the drug development and regulatory review process may well dampen enthusiasm for development of therapeutic agents in this area and, in some instances, may render advancement of novel treatments prohibitively expensive. On the other hand, if other, more efficient means of establishing the benefit of new drugs exist, the promise of timely access to new therapies remains. There is, therefore, potentially tremendous value to public health in accelerating the discovery and development processes for cardiovascular therapeutics through smaller, shorter studies, using validated endpoints other than mortality and irreversible morbidity. Of note, although the multiple reasons for differences in approach in cardiovascular therapeutics have been recognized, such concepts have long been applied in other disease areas, including infectious disease and oncology. The use, in part, of clinical trial evidence based on biomarker and surrogate endpoint effects (in lieu of morbidity and mortality endpoints) has the potential to revolutionize the drug development process and to thereby enhance the armamentarium of safe and effective cardiovascular therapeutics.

II. Definitions

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes or pathogenic processes or as a physiologic response to a therapeutic intervention. In clinical medicine, biomarkers are routinely used in disease diagnosis, prognostication, ongoing clinical decision-making, and follow-up to assess effects of therapy. Commonly used biomarkers include the electrocardiogram, isotopic and ultrasound imaging studies applied in multiple areas of disease management, bone densitometry in the assessment of osteoporotic fracture risk, dynamic pulmonary function testing, and angiography in the management of coronary and peripheral arterial disease. Commonly used soluble biomarkers include C-reactive protein, low- and high-density lipoprotein cholesterol (LDL-C and HDL-C), triglycerides, serum creatinine, and hepatocellular enzymes, as well as a host of other routine clinical laboratory measurements.

Surrogate endpoints are biomarkers that are predictors of clinical outcomes and that can therefore be used to assess the efficacy or safety of disease-modifying interventions. Typical surrogate endpoints used to assess the clinical efficacy of cardiovascular drugs include levels of LDL-C and blood pressure. Unlike simple biomarkers, measures of change in validated surrogate endpoints have sometimes served as a basis for the regulatory approval of pharmaceutical agents.

There have long been efforts to develop new biomarkers and to validate new surrogate endpoints in cardiovascular medicine. At this time, advances in cellular and molecular pathophysiology and in mechanism-driven pharmacology, the growing epidemic of CVD, and the plethora of opportunities to enhance the cardiovascular pharmacopoeia are together responsible for stimulating greater interest in accelerated drug development throughput in this clinical arena. The need for more rapid drug development highlights the role that surrogate markers may play in establishing the efficacy of drugs for managing CVD, and more specifically, atherosclerosis.

III. Validation of New Surrogate Markers

Presently, there is no agreed-upon standard with respect to the body of evidence that must be developed for a biomarker to be considered a surrogate marker of clinical efficacy. A framework for the validation of biomarkers was proposed by Boissel et al. (1992) and subsequently adapted by Espeland et al. (2005) in a discussion of the usefulness of carotid ultrasound to measure the clinical efficacy of lipid-lowering medications. Espeland et al., in modifying the terminology of Boissel et al., described clinical and statistical characteristics that a biomarker should have to be considered a surrogate marker of efficacy in atherosclerotic disease. The clinical criteria outlined for validating surrogate markers are efficiency, linkage, and congruence (Table 1).

The use of vascular imaging, combined with soluble molecular markers of disease activity, can provide valuable information to pharmaceutical companies and reg-

TABLE 1
Clinical criteria for surrogacy—Boissel et al. (1992) as modified by Espeland et al. (2005)

Efficiency	The surrogate marker should be relatively easy to evaluate, preferably by noninvasive means, and more readily available than the gold standard; the time course of changes in the surrogate marker should precede that of the endpoints so that disease and/or disease progression may be established more quickly via the surrogate; clinical trials based on surrogates should require fewer resources, less participant burden, and a shorter time frame
Linkage	The quantitative and qualitative relationship between the surrogate marker and the clinical endpoint should be established on the basis of epidemiologic and clinical studies; the nature of this relationship may be understood in terms of its pathophysiology or in terms of an expression of joint risk
Congruence	The surrogate should produce parallel estimates of risk and benefits that are related to the target disease process as endpoints; individuals with and without vascular disease should exhibit differences in surrogate marker measurements; in intervention studies, anticipated clinical benefits should be deducible from the observed changes in the surrogate marker

ulatory agencies during the development of novel treatments for atherosclerotic disease. This approach is not revolutionary. Indeed, in some countries, vascular imaging data have already been accepted in support of regulatory approval for supplemental indications for statins to slow the progression of atherosclerosis. An obvious means to expedite the availability of new cardiovascular therapies, therefore, is to base an initial regulatory approval on a combination of clinically validated vascular imaging endpoints and additional, suitably appropriate, clinical laboratory and safety data.

There are many vascular imaging technologies, both established and emerging, that permit investigators to collect information on vascular structure and on the development of atherosclerosis. Three of these vascular imaging technologies, quantitative coronary angiography (QCA), assessment of carotid intima-media thickness (cIMT) by ultrasound, and the determination of "plaque volume" using intravascular (or intracoronary) ultrasound (IVUS), meet or are at least close to meeting the established criteria for surrogacy (Boissel et al., 1992; Espeland et al., 2005). As discussed in further detail below, these methods are suitable for detecting atherosclerosis in specific vascular beds and for predicting clinical risk across populations. Furthermore, because atherosclerosis is a systemic arterial disorder, as documented in numerous post-mortem studies, and because a patient who has developed atherosclerosis in one vascular bed will also have it in other vascular beds, such methods can be used to support a clinical diagnosis of systemic atherosclerosis and overall cardiovascular risk (Mitchell and Schwartz, 1962; Wofford et al., 1991; Dormandy et al., 1999). Thus, with careful standardization in application and analysis, these techniques have been successfully adapted to clinical research to assess the safety and efficacy of new pharmaceutical therapies.

A. Quantitative Coronary Angiography

Coronary angiography was first used and is still used in the clinical setting to confirm the presence or absence of symptom-limiting atherosclerotic arterial narrowing. Typically, patients at risk of coronary artery disease (CAD) present with symptoms of angina pectoris or a positive stress test and, if clinical suspicion and concern are high, undergo diagnostic cardiac catheterization. Cardiac catheterization is an "invasive procedure" in which a catheter is advanced through a large vessel, typically a femoral artery, over the aortic arch, and selectively engaged into each of the major coronary arteries. During the catheterization, angiography is performed by injection of radio-opaque contrast material into the vessels. By use of X-rays, images are acquired either on film or on a digital detector. The images are then analyzed for the presence of atherosclerotic narrowing that might require further intervention and "revascularization," either with an intravascular device or by arterial bypass grafting (Fig. 2). Clinical cardiologists usually describe a coronary narrowing as a "percent stenosis," relative to a nearby "normal" reference segment of the vessel. Such determinations are typically subjective. After the introduction of QCA, the systematic measurement of coronary lumen diameter and calculated percent stenosis made the overall technique more applicable to clinical research. The initial studies using

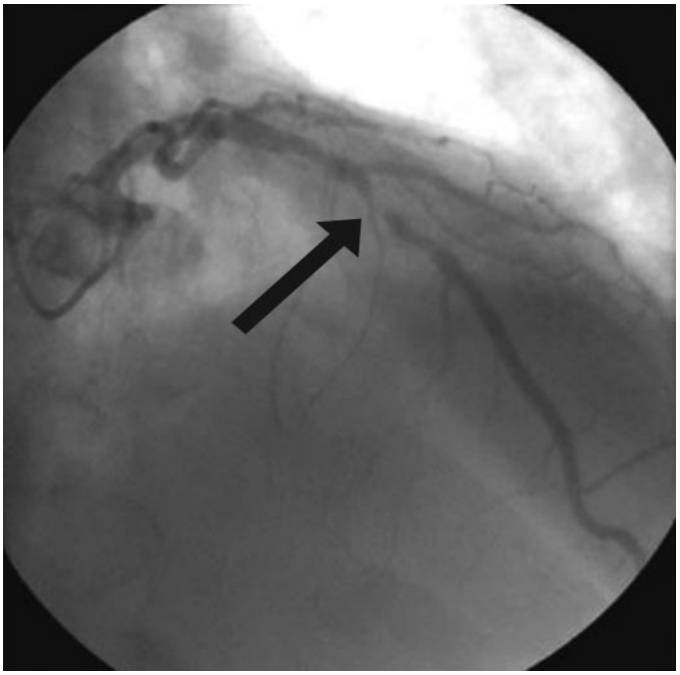

FIG. 2. Diagnostic coronary angiogram with subtotal occlusion of the left anterior descending coronary artery. Courtesy of Section of Cardiovascular Medicine, Yale University School of Medicine.

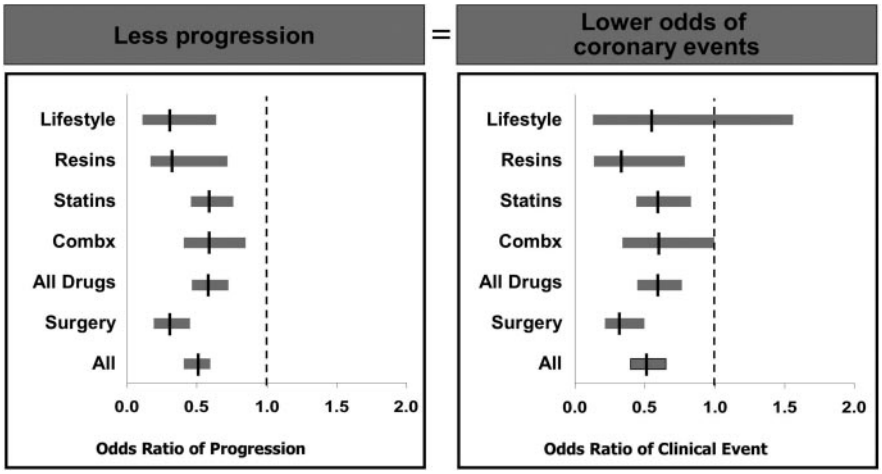

FIG. 3. QCA measures of atherosclerosis progression in cholesterol treatment trials using different therapeutic modalities. Adapted from a meta-analysis of Rossouw (1995).

QCA, conducted in the 1980s, were observational. Correlations between extent of disease by angiography and established risk factors such as hypercholesterolemia and hypertension were found. By conduction of sequential QCA studies, it became possible to measure rates of coronary atherosclerotic progression in human subjects. Subsequently QCA was used for the evaluation of antiatherosclerotic therapies, including lifestyle changes, mechanical revascularization with coronary angioplasty and stenting (including drug-eluting stents), and pharmacologic revascularization with thrombolytic therapies, lipid-lowering therapies, and anti-inflammatory agents. Specifically relating to the lipid-lowering arena, adequately powered, placebo-controlled studies of sufficient duration have demonstrated in parallel: 1) favorable changes in lipid levels, 2) favorable changes in angiographic atherosclerosis (measured as either a reduction in the percent coronary artery stenosis, change in minimal lumen diameter, change in percent progression or regression, or change in global coronary disease scoring), and 3) a reduction in the incidence of cardiovascular events (Brown et al., 1993; Vos et al., 1993; Rossouw, 1995) (Fig. 3).

Although QCA was once the gold standard for assessing the progression of atherosclerosis in clinical trials, its use has reverted to clinical diagnosis alone for two reasons: 1) the change from film to pixelated digital imagery created a loss of spatial resolution, making it more difficult to detect a treatment effect, particularly when the comparator is an active control, and 2) it is now understood that atherosclerosis is primarily a disease of the vessel wall and not the vessel lumen and that the latter is the only part of the vessel visualized using contrast angiography. It is now accepted that assessing the vessel lumen diameter using QCA provides a very limited look at atherosclerosis burden and only an inferential look at the disease itself. Indeed, a meta-analysis of angiographic studies in patients with CAD and MI revealed that in most instances, subjects who experienced an MI had coronary artery stenoses of <50% luminal narrowing, as measured with angiography. Those patients with coronary artery stenoses >70%, the type of lesions best detected with QCA, are underrepresented in populations with acute MI (Smith, 1996)

B. Carotid B-Mode Ultrasound

With the development of medical ultrasound, it became possible to evaluate vessel wall structure in both clinical practice and research. Ultrasound in the clinical setting is used to detect pathologic conditions such as aortic aneurysms, peripheral vascular disease of the lower extremities, and carotid artery stenoses in patients with cerebrovascular disease (stroke or transient ischemic attacks) and as echocardiography to evaluate cardiac structure and function. By using high-resolution ultrasound, measurements of vessel wall intima-media thickness and lumen diameter along the axis of the ultrasound beam may be made (Fig. 4). Another important advantage of ultrasound, compared with catheter-guided angiography, is its noninvasive nature, permitting serial measures of vessel structure, without exposing patients to risks of vascular injury or ionizing radiation. In recent years, measurement of cIMT by B-mode ultrasound has come to the fore as a quantitative research tool in the study of atherosclerosis (Simon et al., 2002).

Substantial epidemiologic evidence has demonstrated that quantitative measures of cIMT correlate with established cardiac risk factors and also with both cardiovascular and cerebrovascular events (Bonithon-Kopp et al., 1991; Heiss et al., 1991; Salonen and Salonen, 1991; Psaty et al., 1992; Wendelhag et al., 1992; Bots et al., 1993). In addition, the measurement of cIMT to quantify the risk of developing cardiovascular and cerebrovascular events has been used in prospective observational studies (Blankenhorn et al., 1993b; Bots et al., 1997; Chambless et al., 1997; Hodis et al., 1998; O'Leary et al., 1999; Chambless et al., 2000). The ARIC study included >12,000 asymptomatic men and women who were fol-

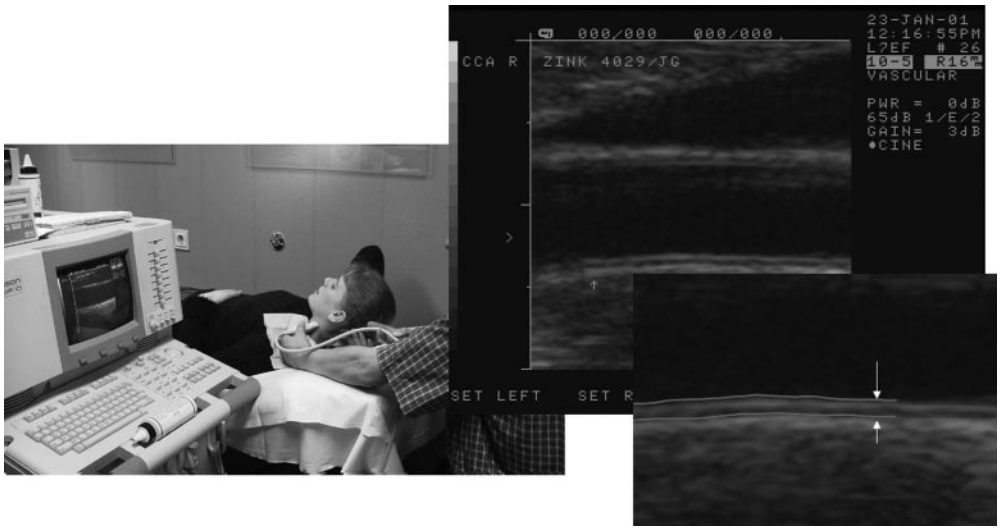

FIG. 4. Carotid ultrasonographer capturing images of the common carotid artery. The frame in the foreground shows the edges of far-wall carotid intima-media thickness. Courtesy of Academic Medical Center, Amsterdam, The Netherlands.

lowed for between 4 and 7 years to determine whether or not cIMT is associated with an increased risk of CHD (Chambless et al., 1997). For individuals whose mean baseline cIMT was >1 mm relative to those with a mean baseline cIMT ≤1 mm, the hazard ratio for CHD events was 5.07 for women [95% confidence interval (CI), 3.08–8.36] and 1.85 for men (95% CI, 1.28–2.69). The Rotterdam Study was a single-center, prospective, follow-up study (mean of 2.7 years) in which disease and disability in an elderly Dutch population was monitored (Bots et al., 1997). Carotid ultrasound data were available for 5965 subjects, 5130 of whom contributed clinical follow-up data. A case-control analysis revealed that for every S.D. increase in cIMT (0.163 mm), the odds ratio was 1.41 for stroke (95% CI, 1.25–1.82) and 1.43 for MI (95% CI, 1.16–1.78). In the multicenter CHS, 5858 individuals aged ≥65 years underwent carotid ultrasound, 4476 of whom had no evidence of clinical CVD (O'Leary et al., 1999). These individuals were followed up for CV events over a mean of 6.2 years. After adjustment for traditional CV risk factors, the relative risk for MI per 1 S.D. increase in average cIMT was 1.36 (95% CI, 1.23–1.52) and for stroke was 1.33 (95% CI, 1.20–1.47). The CLAS included 146 men who had undergone coronary artery bypass grafting and who subsequently underwent B-mode ultrasonography of the common carotid artery every 6 months during a 2-year treatment period with either usual care and placebo or niacin plus colestipol (Hodis et al., 1998). Study participants were followed for clinical outcomes over an average of 8.8 years after study completion. For each 0.03-mm increase in cIMT per year during the treatment period, the relative risk for nonfatal MI or coronary death was 2.2 (95% CI, 1.4–3.6) and the relative risk for any coronary event was 3.1 (95% CI, 2.1–4.5; $p < 0.001$).

In addition to these studies linking cIMT to atherosclerosis disease risk, a number of important clinical treatment studies have been conducted using cIMT as an endpoint to assess the efficacy of antiatherosclerotic therapies, some of which also included measures of cardiovascular outcome. Espeland et al. (2005), in a review of cIMT as a surrogate of CVD, conducted a meta-analysis that included seven statin trials (Furberg et al., 1994; Crouse et al., 1995; Salonen et al., 1995; Mercuri et al., 1996; de Groot et al., 1998; Hedblad et al., 2001; Sawayama et al., 2002). In this analysis, a −0.012 mm/year change in cIMT (95% CI, −0.015 to −0.007) was associated with an odds ratio of 0.48 (95% CI, 0.30–0.78) for cardiovascular events (Table 2).

C. Coronary Intravascular Ultrasound

Coronary IVUS represents an emerging vascular imaging modality that is conceptually similar to extravascular ultrasound of the arterial wall. During IVUS, a miniaturized transducer is attached to the tip of a catheter, permitting the acquisition of intravascular images of the vessel wall. The transducer rotates at ~1800 rpm, while the catheter is mechanically withdrawn at a fixed rate of 0.5 mm/s, acquiring serial images of vessel wall thickness throughout its 360° circumference. Approximately 30 images/s can be acquired. The data obtained are analyzed by trained readers who, either manually or using semiautomated systems, outline the intimal lining of the vessel lumen and the external elastic membrane that separates the media from the adventitia. The difference between the cross-sectional area (CSA) bordered by the external elastic membrane and the CSA of the vessel lumen represents the vessel wall or atheroma cross-sectional area (Fig. 5). When the multiple vessel wall CSA slices are summed along a vessel segment, the atheroma volume may be calculated. The primary disadvantage of coronary IVUS is that it is an invasive procedure performed at the time of a cardiac catheterization. As such and as with any invasive procedure,

TABLE 2
Clinical trials involving 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) using both cIMT and cardiovascular event outcomes

Clinical Trials	n^a	Statin	Relative Impact on IMT Progression of Primary Outcome[b]	Relative Impact on Reported Cardiovascular Endpoints	
				Abstracted CVD Events	Odds Ratio (95% CI)
			mm/yr		
ACAPS	919	Lovastatin	−0.015 (−0.023 to −0.007) (p = 0.001)	CVD death, MI, stroke	0.34 (0.12–0.69)
KAPS	447	Pravastatin	−0.014 (−0.022 to −0.006) (p = 0.005)	CVD death, MI, stroke	0.57 (0.22–1.47)
PLAC-II	151	Pravastatin	−0.009 (−0.031 to −0.013) (p = 0.44)	Clinical coronary events	0.37 (0.11–1.24)
CAIUS	305	Pravastatin	−0.014 (−0.021 to −0.005) (p = 0.0007)	CVD death, MI, stroke	1.02 (0.14–7.33)
REGRESS	255	Pravastatin	−0.030 (−0.056 to −0.004) (p = 0.002)	Clinical events	0.51 (0.24–1.07)
BCAPS	793	Fluvastatin	−0.008 (−0.013 to −0.003) (p = 0.002)	CVD death, MI, stroke	0.64 (−0.24 to 1.66)
FAST	164	Pravastatin	Significant benefit ($p < 0.001$)	CVD death, MI	0.32 (0.10–1.06)
Pooled estimate			−0.012 (−0.016 to −0.007)[c]		0.48 (0.30–0.78)

ACAPS, Asymptomatic Carotid Artery Progression Study; KAPS, Kuopio Atherosclerosis Prevention Study; PLAC-II, Pravastatin, Lipids, and Atherosclerosis in the Carotid Arteries; CAIUS, Carotid Atherosclerosis Italian Ultrasound Study; REGRESS, Regression Growth Evaluation Statin Study; BCAPS, Beta-Blocker Cholesterol-Lowering Asymptomatic Plaque Study; FAST, Fukuoka Atherosclerosis Trial.
[a] Arms used in meta-analyses.
[b] Data are means (95% CI) (reported p value).
[c] Excludes FAST.

there is a small risk of complications including, but not limited to, cardiac rhythm disturbances, vascular injury (such as spasm), thrombosis, dissection, or infection.

The clinical relevance of IVUS imaging data are supported by in vitro histopathologic (Siegel et al., 1991; Prati et al., 2001), epidemiologic (Tuzcu et al., 2001), observational, and clinical trial data. Although not as abundant as the cIMT data, accumulating evidence supports the use of coronary IVUS in the assessment of compounds intended to slow the progression of atherosclerosis. Similar to the observational cIMT data showing that intima-media thickness increases with age, there are observational data suggesting a relationship between coronary IVUS measures of atheroma burden and age. For example, studies in patients who have undergone heart transplantation show that the IVUS-determined coronary artery atheroma burden at the time of transplant increases with the age of the heart donor (Fig. 6). In addition, in a prospective follow-up study of 56 individuals with known CVD in whom serial coronary IVUS studies were performed, a relationship between IVUS-documented progression of atherosclerosis in the left main coronary artery and coronary risk factors was demonstrated (von Birgelen et al., 2004) (Fig. 7). In this study, the investigators also applied three different, commonly used cardiovascular risk scores to subjects [i.e., Framingham (Anderson et al., 1991), PROCAM (Assmann et al., 2002), and SCORE (Conroy et al., 2003)] and demonstrated a positive linear relationships between the calculated risk of CVD and plaque progression (r = 0.41–0.60; $p < 0.002$–0.0001) (Fig. 8). Finally, during 18 ± 9 months of follow-up, 18 subjects had adverse cardiovascular events. Those subjects had an average annual plaque progression greater than that of the other subjects (25.2 ± 19.5% versus 5.9 ± 15.6%, $p < 0.001$).

As with carotid ultrasound, a number of studies have used coronary IVUS to assess the efficacy of lipid-altering therapies directed at slowing the progression of atherosclerosis (Takagi et al., 1997; Schartl et al., 2001; Nissen et al., 2003, 2004, 2006a,b; Okazaki et al., 2004; Tardif et al., 2004) (Table 3). These studies ranged from 5 weeks to 2 years in duration and enrolled up to 500 participants/study. The most recent studies have used the nominal change in the percent atheroma volume as the primary endpoint. These studies have consistently shown a relationship between the on-treatment level of LDL-C, an accepted surrogate marker of cardiovascular risk, and the nominal change in percent atheroma volume as measured by IVUS (Fig. 9).

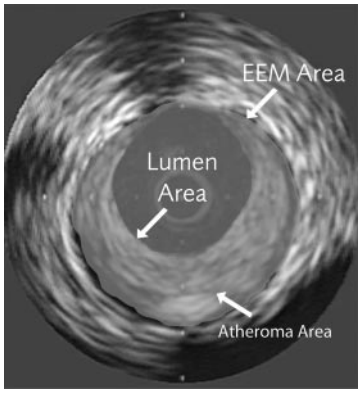

FIG. 5. External elastic membrane (EEM), lumen, and atheroma cross-sectional areas following the imaging core laboratory outlining of the lumen intimal and EEM edges of an IVUS "slice." Courtesy of the Cleveland Clinic IVUS Core Laboratory.

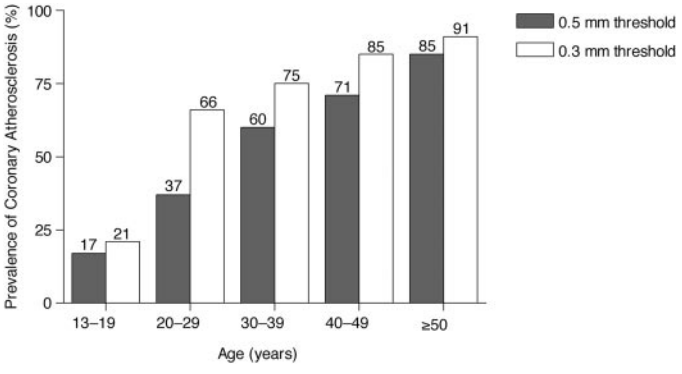

FIG. 6. Prevalence of coronary atherosclerosis as measured by coronary IVUS in transplant recipient hearts. Data from Tuzcu et al. (2001). Reprinted from *Circulation* **103:**2705–2710 with permission from Lippincott Williams & Wilkins (http://lww.com).

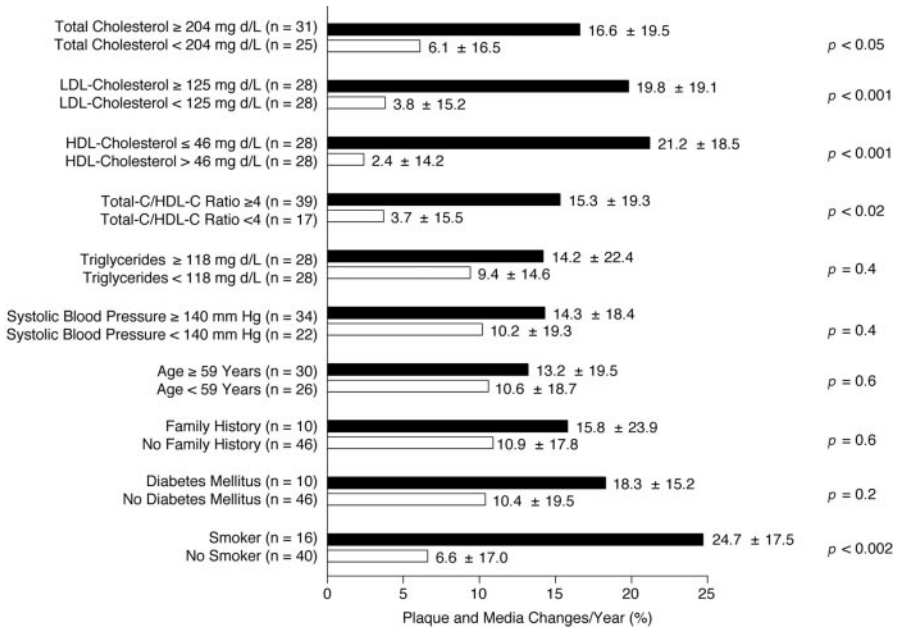

FIG. 7. Relationships between cardiovascular risk factors and IVUS percent atherosclerosis progression in the left main coronary artery. C, cholesterol. Data from von Birgelen et al. (2004). Reprinted from *Circulation* **110:**1579–1585 with permission from Lippincott Williams & Wilkins (http://lww.com).

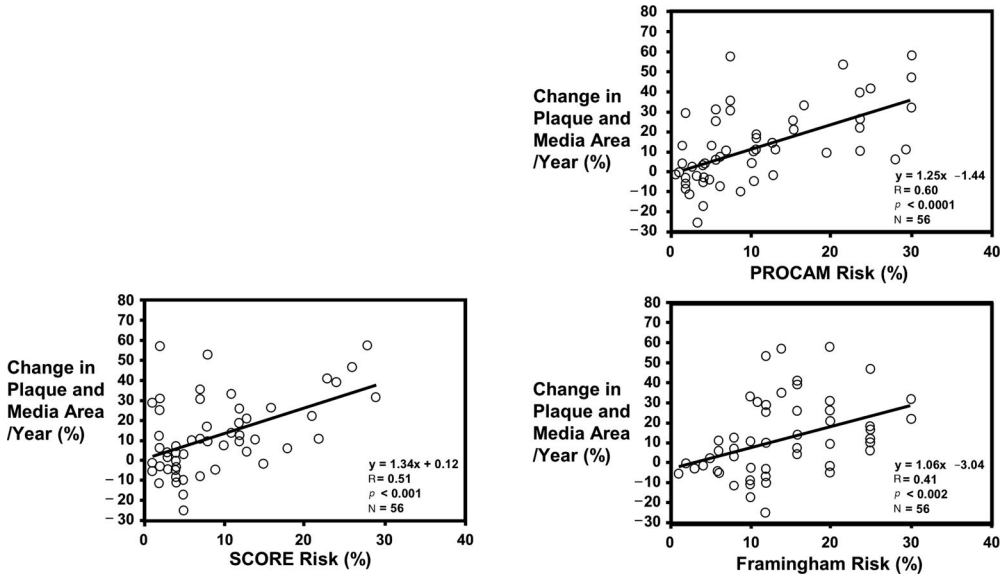

FIG. 8. Relationship between coronary risk scores and changes in left main coronary artery atherosclerosis progression by IVUS. Data from von Birgelen et al. (2004). Reprinted from *Circulation* **110:**1579–1585 with permission from Lippincott Williams & Wilkins (http://lww.com).

IV. Correlation between Carotid Intima-Media Thickness, Intravascular Ultrasound, and Clinical Events

The current standard of care for patients with CAD is statin therapy titrated to an LDL-C target of <100 mg/dl (National Cholesterol Education Program Adult Treatment Panel III, 2002; De Backer et al., 2003). Accordingly, most of the IVUS studies conducted to date have used active controls, meaning that they have had sufficient power to detect treatment differences in the progression of atherosclerosis but not in the frequency of cardiovascular endpoints. Even so, there is evidence that IVUS-measured differences in atherosclerosis are clinically relevant. Specifically, there have been three clinical trials in subjects with known CAD comparing the same therapies: pravastatin (40 mg/day) and atorvastatin (80 mg/day). Two of these studies used ultrasound to assess the progression of coronary atherosclerosis, one (ARBITER) with carotid ultrasound (Taylor et al., 2002) and one (REVERSAL) with coronary IVUS (Nissen et al., 2004). Both studies showed significant differences in the progression of atherosclerosis, favoring atorvastatin (80 mg/day). The third study (PROVE-IT TIMI-22) was a morbidity and mortality

TABLE 3

Representative coronary IVUS studies assessing therapies directed at slowing the progression of atherosclerosis

The table shows the baseline LDL-C and HDL-C levels and end of study treatment levels, as well as changes in primary imaging endpoints. Statin treatments: rosuvastatin, pravastatin, and atorvastatin; acyl coenzyme A:cholesterol acyltransferase inhibitors: avasimibe and pactimibe. Biologic: Apo A-I $_{Milano}$.

Trial Acronym	LDL/HDL	*n*	Years	Control (C)	Treatment (T)	%Δ LDL-C (T)	%Δ HDL-C (T)	IVUS Endpoint	Δ IVUS C/T (*p*)	Events C/T
	mg/dl									
ASTEROID	130/43	507	2	Baseline	Rosuvastatin (40 mg)	−53	+15	Δ PAV	Baseline/−6.8	N.A.
REVERSAL	150/42	502	1.5	Pravastatin (40 mg)	Atorvastatin (80 mg)	−46	+2.9	%Δ TAV	+2.7/−0.4 ($p = 0.02$)	9/6
GAIN	161/46	131	1	Usual care	Atorvastatin to target	−42	+9	%Δ PAV	+11.8/+2.5 ($p = 0.138$)	2/2
ESTABLISH	124/∼45	70	0.5	Usual care	Atorvastatin (20 mg)	−42	∼+2.4	%Δ TAV	+8.7/−13.1 ($p < 0.0001$)	0/0
PTCA-Pravastatin	170/35	25	3	Diet	Pravastatin (10 mg)	−26	+29	%Δ PI	+27/−7 ($p < 0.0005$)	N.A.
A-Plus[a]	<95/≤45	432	<2	Usual care	Avasimibe (50 mg)	+7.8	+0.8	Δ PAV	+0.4/+0.7 (N.S.)	7/4
					Avasimibe (250 mg	+9.1	+1.2		+0.4/+0.8 (N.S.)	7/9
					Avasimibe (750 mg)	+10.9	+2.3		+0.4/+1.0 (N.S.)	7/2
ACTIVATE[a]	N.A.	408	1.5	Usual care	Pactimibe (100 mg)	−4.9	−1.8	Δ PAV	+0.59/+0.69 (N.S.)	11/7
Apo A-I$_{Milano}$[a]	N.A.	47	0.1	Usual care	Apo A-I$_{Milano}$ (15 and 45 mg/kg)	N.A.	N.A.	Δ PAV	+0.14/−1.06	N.A.

PAV, percent atheroma volume; TAV, total atheroma volume; PI, plaque index; N.A., not applicable; N.S., not significant; PTCA, percutaneous transluminal coronary angioplasty; Apo A, apolipoprotein A; ASTEROID, A Study to Evaluate the Effect on Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden; GAIN, German Atorvastatin Intravascular Investigators; ESTABLISH, Early Statin Treatment in Patients with Acute Coronary Syndrome: Demonstration of Beneficial Effect on Atherosclerotic Lesions by Serial Volumetric IVUS Analysis during Half a Year after Coronary Event; ACTIVATE, Acyl-coenzyme A:cholesterol acyltransferase Intravascular Atherosclerosis Treatment Evaluation; A-Plus, Avasimibe and Progression of Lesions on Ultrasound.

[a] Nonstatin studies.

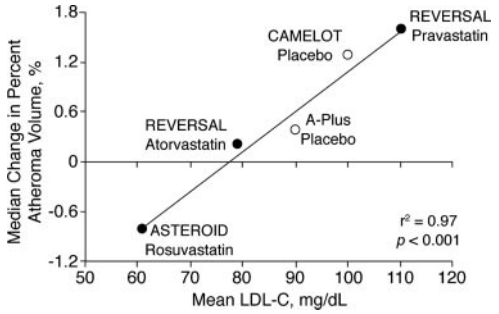

FIG. 9. Relationship of nominal mean change in percent atheroma volume, measured by IVUS, and end-of-study, on-treatment levels of LDL-C. Data from Nissen et al. (2006a). Reprinted from *J Am Med Assoc* **295:**1556–1565 with permission from the American Medical Association.

study in patients with acute coronary syndrome (Cannon et al., 2004), which showed a significant reduction in coronary events, again, in the patients treated with the more potent dose of statin (Table 4; Fig. 10). This cross-study comparison, although clearly not definitive, provides additional and substantial support for the notion that ultrasound measures of atherosclerosis, based on imaging of the arterial wall (with quantification of either vessel wall thickness or cross-sectional vessel wall area), have meaningful clinical relevance.

V. Circulating Biomarkers

As understanding of the relationship between metabolism, atherosclerosis, and inflammation has expanded, so has appreciation of the biochemical complexity of vascular disease (Fig. 11) (Hansson, 2005). It is now recognized that 20 to 50% of patients with CVD lack conventional risk factors (Khot et al., 2003). Indeed, a number of new biomarkers have been identified that have been posited as independent markers of cardiovascular risk. This ever-growing list of biomarkers includes cellular adhesion molecules, cytokines, proatherogenic enzymes, and CRP (Blake and Ridker, 2002; Tsimikas et al., 2006). Of these, CRP has received the most attention as a potential biomarker in ascertaining cardiovascular risk, independent of accepted surrogates, such as LDL-C and blood pressure. CRP was identified >50 years ago as an acute-phase reactant that was capable of activating the complement system (Tillet and Francis, 1930; Abernathy and Avery, 1941). It was subsequently noted to be one of a number of acute-phase biomarkers, along with the erythrocyte sedimentation rate and complement, which was elevated in acute MI (Boltax and Fischel, 1956). After the development of more sensitive, reliable, and readily available assays for CRP, a number of epidemiologic studies were conducted to assess the value of CRP in predicting CV risk. For example, levels of CRP were found to be more predictive of cardiovascular events than LDL-C in patients participating in the Women's Health Study (Fig. 12) (Ridker et al., 2000). Moreover, using the same Women's Health Study cohort, it was shown that modifying the Framingham Risk Score model to include CRP levels added predictive value (Cook et al., 2006). Whereas a number of large-scale prospective studies have demonstrated that CRP levels predict incident MI and stroke, the level of predictive power has varied (Ridker et al., 1997, 2002; Danesh et al., 2004; Koenig et al., 2004; Cushman et al., 2005). The U.S. Centers for Disease Control and Prevention and the American Heart Association have both advocated the use of CRP as an adjunct to global risk prediction among those at intermediate risk for CVD (Pearson et al., 2003). An analysis of CRP in the cohort of The Third National Health and Nutrition Examination Survey suggests that elevated levels of CRP in a general population are attributable largely to conventional risk factors (Miller et al., 2005).

Although CRP is recognized as an inflammatory marker associated with CHD risk, it is not yet known whether CRP is a promoter or a direct mediator of vascular disease. In secondary analyses of clinical trials with statins, which possess pleiotropic, anti-inflammatory properties, reductions in levels of LDL-C, atherosclerosis progression, clinical events, and levels of high-sensitivity CRP have all been observed in parallel (Nissen et al., 2005; Ridker et al., 2005). To date, however, there is no hard evidence demonstrating that CRP reduction per se lowers the risk of CVD. In fact, several therapies, such as glucocorticoids and COX-2 inhibitors, are known to lower CRP but without beneficial effects on cardiovascular risk. Furthermore, in a recent study in patients with stage 2 hypertension, which evaluated the effects of an angiotensin receptor blocker, valsartan, with and without hydrochlorothiazide, on blood pressure and CRP, CRP levels increased in the group with the

TABLE 4
Clinical trials comparing moderate to intensive statin therapy
ARBITER, carotid ultrasound; REVERSAL, coronary IVUS; PROVE-IT, morbidity and mortality.

Trial Name	LDL-C/HDL-C at Baseline	*n*	Years	Control (C)	Treatment (T)	%Δ LDL-C C/T	Δ Imaging Endpoint C/T (*p* Value)	Events C/T
ARBITER	~152/49	138	1	Pravastatin (40 mg)	Atorvastatin (80 mg)	−27/−49	cIMT + 0.025/−0.034 (mean mm/year) ($p < 0.03$)	1/0
REVERSAL	150/42	502	1.5	Pravastatin (40 mg)	Atorvastatin (80 mg)	−25/−46	Δ TAV + 2.7%/−0.4% ($p = 0.02$)	9/6
PROVE-IT	~110/~39	4162	≤3	Pravastatin (40 mg)	Atorvastatin (80 mg)	−21/−49 at 30 days	N.A.	10%/8.3% death/MI

TAV, total atheroma volume; N.A., not available.

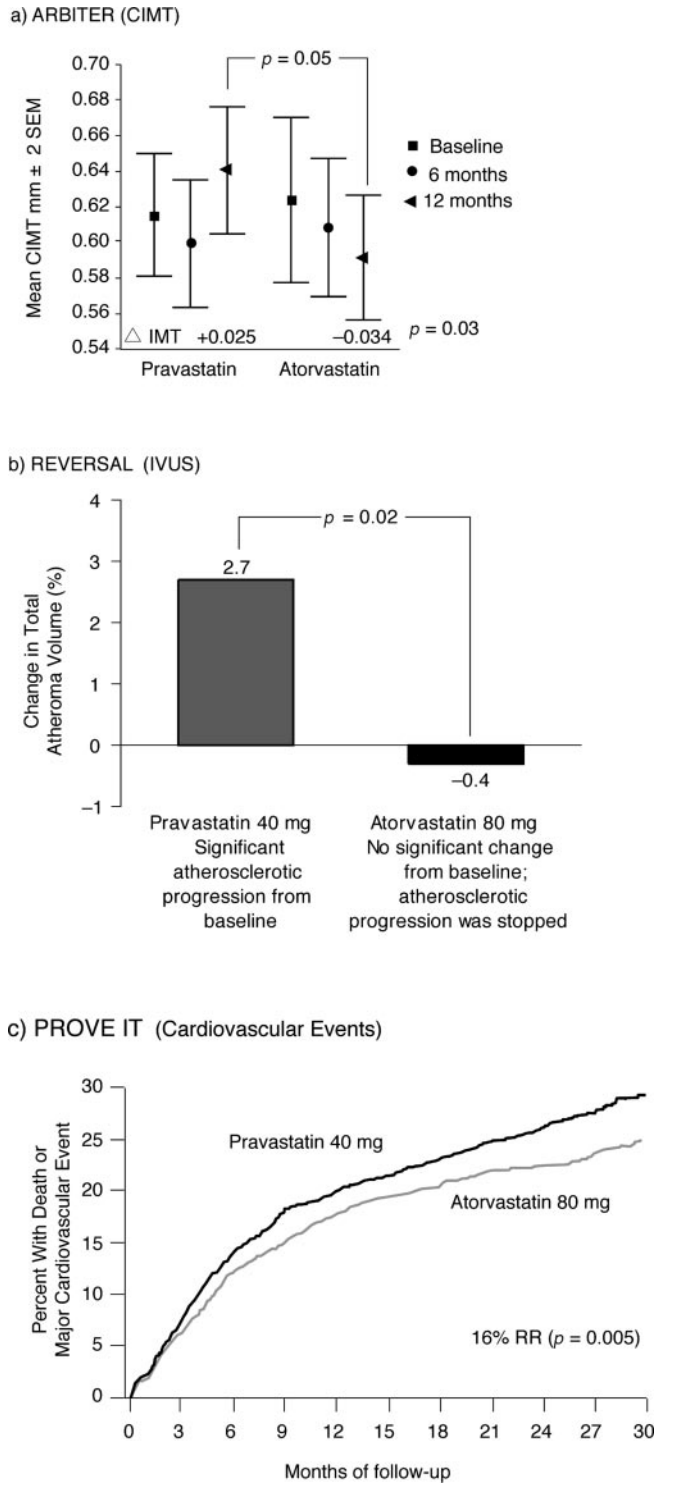

FIG. 10. Treatment differences in cIMT in the ARBITER trial (a), coronary total atheroma volume in the REVERSAL trial (b), and clinical events in the PROVE-IT study (c). Indirect evidence of clinical meaningfulness of ultrasound measures of vessel wall thickness. Data from (a) Cannon et al. (2004), (b) Nissen et al. (2004), and (c) Taylor et al. (2002). Panel a: Copyright © 2004 Massachusetts Medical Society; panel c is reprinted from *Circulation* **106:**2055–2060 with permission from Lippincott Williams & Wilkins (http://lww.com).

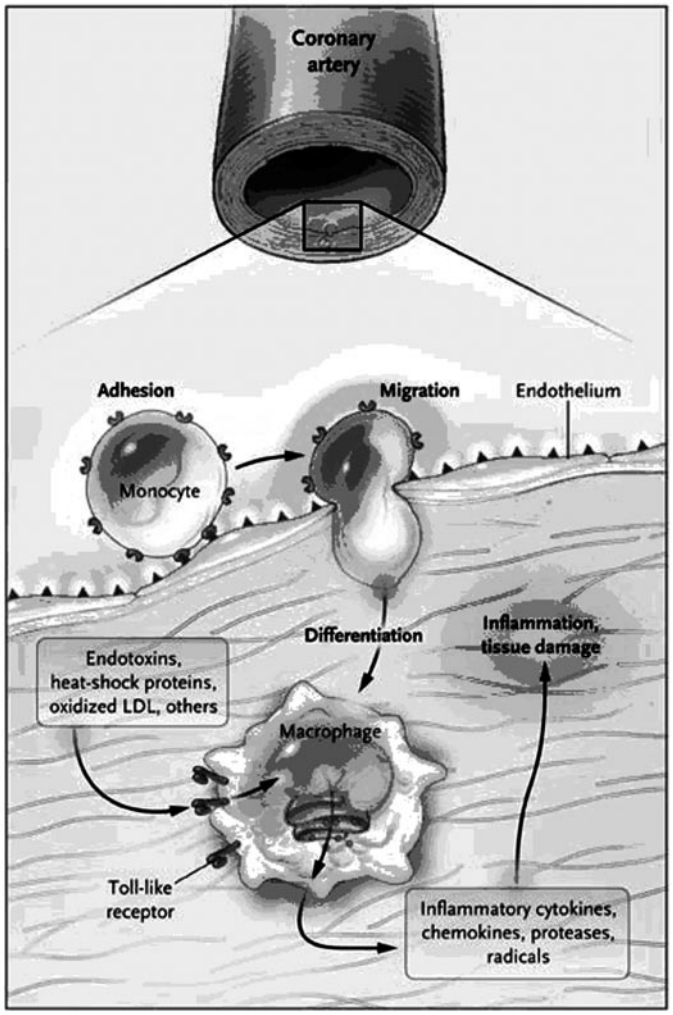

FIG. 11. Illustration of the dynamic pathophysiology of atherosclerosis showing relationships between potential metabolic, cellular, and inflammatory biomarkers. Data from Hansson (2005). Copyright © 2005 Massachusetts Medical Society.

greatest blood pressure reduction (valsartan-hydrochlorothiazide group) whereas CRP levels decreased in the group treated with valsartan alone, a less effective blood pressure-reducing regimen (Ridker et al., 2006). Thus, until more definitive studies are completed, it is unclear whether CRP will become a validated surrogate marker for CVD (Mora and Ridker, 2006).

VI. Surrogate Endpoints and Regulatory Approval

Applying the criteria of Boissel et al. (1992) and Espeland et al. (2005) for surrogacy to the imaging and CRP data described above, it is evident that only the imaging technologies of QCA and carotid ultrasound are supported by sufficient clinical evidence to demonstrate a direct relationship between slowing of the progression of atherosclerosis and a reduction in clinical events (Table 5). Nevertheless, the expanding database of coronary IVUS study results seems likely to lead to its acceptance as a reliable, clinically relevant measure of atherosclerosis progression in due course. At this time, data from a number of QCA and cIMT studies have been relied upon to support regulatory filings in the United States

TABLE 5
Matrix to evaluate biomarkers using the criteria for surrogacy of Boissel et al. (1992) as modified by Espeland et al. (2005)

Criteria	QCA	cIMT	IVUS	CRP
B1: Efficiency				
Easy to measure	?	√	√	√
Precedes the standard	√	√	√	√
Quicker, easier (noninvasive)	—	√	—	√
B2: Linkage				
Quantitative and qualitative relationship based on epidemiologic and clinical trials	√	√	√	?
Relationship understood via pathophysiology	√	√	√	?
B3: Congruency				
Parallel estimates of risk	√	√	√	?
Different with/without disease	√	√	√	√
Intervention trials show clinical benefit from Δ surrogate	√	√	?	?

√, yes; —, no; ?, questionable.

and Canada for supplemental indications of slowing the progression or promoting the regression of atherosclerosis (Blankenhorn et al., 1987, 1993a; Brown et al., 1990; Multicentre Anti-Atheroma Study, 1994; Waters et al., 1994; Jukema et al., 1995; Pitt et al., 1995; Salonen et al., 1995; Bots et al., 1997; Herd et al., 1997; Hodis et al., 1998; O'Leary et al., 1999) (Table 6). It is reasonable to expect that, in time, IVUS data will likewise be relied upon to support claims of slowing the progression of atherosclerosis and indeed may supplant QCA because of the latter's technical limitations, particularly with respect to spatial resolution and the failure to image the disease itself.

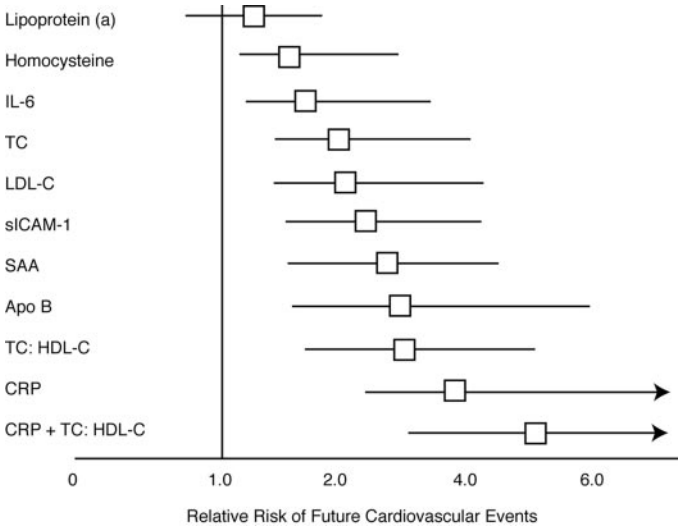

FIG. 12. Inflammatory biomarkers and cardiovascular risk prediction in women: relative risk of future cardiovascular events associated with biomarker levels in the Women's Health Study. Relative risks and 95% confidence intervals are shown for women in the top versus the bottom quartile. IL-6, interleukin-6; TC, total cholesterol; sICAM-1, soluble intercellular adhesion molecule-1; SAA, serum amyloid A; Apo B, apolipoprotein B-100. Data from Ridker et al. (2000).

VII. Future Prospects: Biomarkers and Surrogate Endpoints

Statistics suggest that there is a decline in the number of new molecular entities being submitted to global regulatory authorities for approval (http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html) (Fig. 13). There is evidence too that the stakeholders in the pharmaceutical enterprise (health care providers, regulatory authorities, industry, and payers) recognize the need for a shift in the approach to drug development. Indeed, the U.S. Food and Drug Administration has put forward a Critical Path Initiative that identifies a choice between the status quo, "stagnation," and a new path, "innovation" and describes critical path research as being

TABLE 6
Vascular imaging studies supporting regulatory filings for supplemental indications of slowing the progression of atherosclerosis using QCA or carotid ultrasound

Compound	Imaging Type: Endpoints	Supporting Studies	Imaging Indication
Lovastatin (Mevacor, 1995)	QCA: MLD, percentage of stenosis; cIMT, mean change in maximum IMT	QCA: CCAIT, MARS, FATS; cIMT, ACAPS	Slow progression of CAD
Pravastatin (Pravachol, 1996)	QCA: MLD; cIMT, mean change in maximum IMT, change in mean IMT	QCA: PLAC I, REGRESS; cIMT, PLAC II, REGRESS, KAPS	Slow progression of CAD
Simvastatin (Zocor, 1996)	QCA: MLD	QCA: MAAS	Slow progression of coronary atherosclerosis; reduce new lesion and total occlusions (Canada)
Fluvastatin (Lescol, 1997)	QCA: MLD	QCA: LCAS	Slow progression of CAD
Niacin (Niaspan, 1997) + resin	QCA: global change score, percentage of stenosis	QCA: CLAS, FATS	Slow progression or promote regression of CAD

MLD, minimum lumen diameter; CCAIT, Canadian Coronary Atherosclerosis Intervention Trial; MARS, Monitored Atherosclerosis Regression Study; FATS, Familial Atherosclerosis Treatment Study; ACAPS, Asymptomatic Carotid Artery Progression Study; PLAC, Pravastatin, Lipids, and Atherosclerosis in the Carotid Arteries; REGRESS, Regression Growth Evaluation Statin Study; KAPS, Kuopio Atherosclerosis Prevention Study; MAAS, Multicenre Anti-Atheroma Study; LCAS, Lipoprotein and Coronary Atherosclerosis Study.

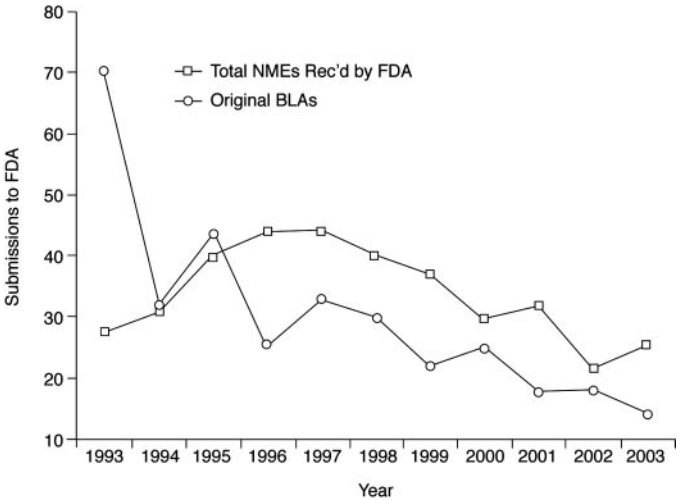

FIG. 13. The number of submissions of new molecular entities (NMEs) and the number of biologic license application (BLA) submissions to the U.S. Food and Drug Administration over a 10-year period. Data from U.S. Food and Drug Administration (2004).

"directed toward improving the product development process itself by establishing new evaluation tools" (http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html) (Fig. 14). The European Agency for the Evaluation of Medicinal Products, in collaboration with the European Society of Cardiology, has also recognized this opportunity to redesign the process of drug development and has developed a biomarker task force to address these issues (Hertog, 2006).

It is evident that new investigational paradigms in drug development must be advanced to facilitate both discovery and clinical development, without sacrificing basic regulatory standards of safety and efficacy. There are, however, obstacles to be overcome. Although biomarkers and surrogate endpoints have the potential to bring promising science to the clinic more expeditiously, there is as yet little agreement on the criteria for validating these new entities. The biomarker validation process itself is time-consuming and expensive. Intellectual property issues may also hamper validation. Perhaps the biggest hurdle is the need for stakeholders to agree that clinical investigation is not a perfect science, that

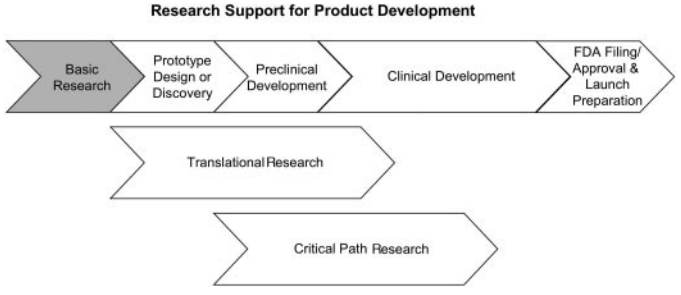

FIG. 14. U.S. Food and Drug Administration (FDA) Critical Path Initiative. The Critical Path Initiative is the FDA's effort to stimulate and facilitate a national effort to modernize the scientific process through which a potential human drug, biologic product, or medical device is transformed from a discovery or "proof of concept" into a medical product.

uncertainty always has and always will remain at the end of the development process (particularly regarding safety), and that the use of biomarkers and surrogates of efficacy need not necessarily amplify that uncertainty.

In summary, improved knowledge of the pathogenesis of atherosclerosis and of its molecular and anatomic pathology and the wealth of information correlating measures of atherosclerotic burden (obtained either invasively or noninvasively) with clinical disease risk arguably permit better means for assessment of the effects of new cardiovascular drugs than existed previously. It is evident from the discussions now ongoing between industry, government regulators, and academia that there is a shared recognition of the need for the application of new tools in drug development. This general philosophy, applied to atherosclerosis treatment, is critical to addressing the epidemic of CVD.

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