APPLICATION OF PREDICTIVE BIOSIMULATION TO THE STUDY OF ATHEROSCLEROSIS: DEVELOPMENT OF THE CARDIOVASCULAR PHYSIOLAB® PLATFORM AND EVALUATION OF CETP INHIBITOR THERAPY

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Abstract
An unexpected increase in the number of cardiovascular events reported in recent clinical trials has highlighted the difficulty of translating epidemiological and preclinical data into safe and effective treatments for cardiovascular disease (CVD). New approaches are needed to better predict the response of diverse patient populations to novel therapies. The Cardiovascular PhysioLab® platform, a large-scale computer model of the biology and pathophysiology of CVD, was developed to explore the progression of atherosclerosis, a prerequisite for the majority of cardiovascular events. Virtual patients have been created in the platform to reproduce the dynamics of plaque progression and response to therapies. Virtual patients have been used to investigate mechanisms of therapeutic response, predict the efficacy of potential therapeutics, and identify pathways with the greatest impact on efficacy. We present a case study in which virtual patient responses to torcetrapib/atorvastatin therapy suggest that “pure” CETP inhibition, in the absence of additional off-target effects or vascular toxicities, is sufficient to reproduce the lipid and plaque endpoints of the ILLUSTRATE trial. Cohorts of virtual patients can be prevalence weighted to establish a virtual population, a powerful tool for identifying responder and non-responder patient subtypes and optimizing clinical trials.

Keywords
Biosimulation, Atherosclerosis, Virtual population

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INTRODUCTION
Cardiovascular disease (CVD) is the single largest cause of death in the United States. Despite widespread use of medications targeting two major risk factors, hypertension and high blood cholesterol levels, the nearly 80 million U.S. adults with CVD accounted for 36.3% of all deaths in 2004 (American Heart Association, 2007). Despite intense research, the last significant improvement in CVD-related mortality was the introduction of statins (4S, 1994).

The complex biology underlying the pathophysiology of CVD, combined with the long disease developmental time course, present challenges to drug development. Much is known about risk factors identified from epidemiological studies (e.g., (Splansky et al., 2007)) which form the basis of current detection and treatment guidelines (ATP III, 2001). However, the lack of predictive animal models and unambiguous biomarkers for human disease progression has led to recent clinical failures (Gulseth, 2005; Nissen et al., 2006b; Tall et al., 2007; Tardif et al., 2004). In addition, several therapies targeting known CVD risk factors have demonstrated unexpected increases in events despite
achieving the desired effect on risk factors (e.g., diabetes (Nissen & Wolski, 2007), thrombin activity (Gulseth, 2005), and estrogen status (Vickers et al., 2007)).

Technological advances (genomics, lipidomics, proteomics) have identified multiple genes and pathways involved in CVD (Samani et al., 2007). These data demonstrate the extensive diversity of the patient population and disease etiology. However, to identify promising therapeutic targets and determine key biological pathways responsible for efficacy and safety, a comprehensive understanding of the dynamic mechanisms that lead to CVD is required.

To this end, Entelos has developed the Cardiovascular PhysioLab® platform (CV platform), a large-scale computer model of the biology and pathophysiology of human CVD. The platform mathematically represents the relative contribution of circulating lipids, systemic inflammation and local vessel inflammation to the progression of atherosclerosis, a necessary precursor for the bulk of cardiovascular events. A top-down modeling approach (Bangs, 2005) was used, incorporating only biological functions necessary to characterize the disease state and reproduce key clinical outcome measures, consistent with the available literature. The CV platform not only enables identification of key molecules and pathways involved in CVD, but it can also be used to evaluate the potential efficacy of novel therapeutic interventions which affect these processes, either directly or indirectly.

To investigate the impact of environmental and genetic diversity on plaque progression and therapeutic efficacy, virtual patients (Friedrich & Paterson, 2004) and virtual populations are developed to represent specific hypotheses or assumptions about the dynamics and relative contribution of disease pathways to atherosclerosis. The simulated responses of virtual patients to published treatment protocols can be compared to reported clinical data to develop virtual populations that reproduce the demographics, mean responses and variances of real clinical populations. Given that the successful design of a clinical trial depends on identifying a significant mean response, the predicted individual responses of virtual patients in a virtual population can be used to identify the unique characteristics and prevalences of responder and non-responder patient sub-populations. This allows patient inclusion/exclusion criteria or drug compound characteristics to be optimized, increasing the probability of clinical success.

**BIOLOGY OF THE CV PLATFORM**

The CV platform was developed in a modular fashion to represent the key subsystems controlling the development of atherosclerotic plaques, plaque instability, and the risk of potential rupture (Figure 1). Each module of the platform was constructed to reflect current literature on the underlying biology of CVD.

![Figure 1. The four modules of the CV platform. Each arrow represents output data that informs subsequent modules](image)

The cholesterol metabolism module (Figure 1a) represents the synthesis, metabolism and clearance of lipoprotein particles. High levels of circulating cholesterol, particularly in the low density lipoprotein (LDL) fraction, has long been recognized as a major contributor to atherosclerotic disease and CV risk (Steinberg, 2004). Conversely, high density lipoprotein (HDL) cholesterol levels are inversely correlated with risk (Berchtold & Berger, 1978; Miller, 1978).

Apolipoprotein B-100 (apoB-100) is an essential component of pre-LDL and LDL particles produced by the liver. ApoB-100 particles transport cholesterol and other lipids to peripheral body tissues, where they are used in a variety of cellular processes. HDL particles transport cholesterol from the peripheral tissues back to the liver. Intensive research has focused on therapeutic approaches to maximize the HDL/LDL ratio and, hopefully, reduce the number of CV events.

Serum lipoprotein levels are determined by genetic, dietary and other lifestyle factors. Sequence variations in the genes that code for molecules involved in the metabolism of lipoproteins, e.g., HMG-CoA reductase (HMGCR) (Hubacek et al., 1999), cholesterol ester transfer protein (CETP) (de Grooth et al., 2004), the LDL receptor (LDLR) (Russell et al., 1989) and the intracellular transporter ABCA1 (Brunham et al., 2006) have been linked to increased atherosclerosis. These and other potential therapeutic targets are represented within the cholesterol module. The most successful class of drugs to exploit these targets are the statins, which bind to and inhibit HMGCR, reducing LDL...
cholesterol and apoB-100 (Baigent et al., 2005). Despite their wide use and efficacy, statins have only achieved about a 30% reduction in cardiovascular events (Steinberg, 2006). Additional therapeutic approaches are needed to further reduce risk, either by modulating lipoprotein metabolism or altering the composition and stability of the plaque itself.

The life cycle of atheromatous plaque formation and growth is represented in the atherogenesis module of the CV platform (Figure 1b). Plaque progression is dependent on the accumulation of cholesterol in the vessel wall. Virtual plaques respond dynamically to the inputs from the cholesterol metabolism module and the level of local and systemic inflammation according to the range of variation reported in the literature. Each virtual plaque for each virtual patient in the CV platform develops uniquely in response to a balance in the contributions of lipid and inflammatory drivers.

The accumulation of cholesterol in the atherogenesis module is driven by the levels of circulating lipoprotein particles and by metabolic or inflammatory modification of these particles. Influx of cholesterol is dependent on apoB-100 particles and their level of modification. Similarly, efflux of cholesterol is dependent on the level and functionality of HDL. Retention of cholesterol in the arterial wall leads to monocyte infiltration and activation. Activated macrophages secrete cytokines and chemokines, recruiting additional pro-inflammatory cells to the tissue. Endothelial cells, fibrous, and smooth muscle cells also contribute to this inflammatory milieu. Inflammation increases lipoprotein modification and function, leading to further lipid retention (Stocker & Keaney, Jr., 2004).

Macrophages accumulate retained lipid, forming foam cells. Buildup of free cholesterol in these cells leads to reduced fluidity of the cell membranes, eventually leading to toxicity (Tabas, 2000). Dying cells contribute their contents to the necrotic acellular core of the plaque (Stary, 2003). Unstable plaques have a large lipid core and increased inflammatory cells (Felton et al., 1997). The fibrous cap overlying this thrombogenic core thickens in response to growth factors secreted by various plaque cells. However, the cap thins when the level of inflammatory mediators reaches a level where cell death predominates. Thin caps are prone to break under the pressure of systolic/diastolic movement, exposing the lipid core and increasing the risk of a cardiovascular event (Finet et al., 2004).

Virtual plaques in the CV platform grow or regress at different rates and exhibit highly variable composition. For example, a rapidly-growing virtual plaque may be quite stable if the inflammatory cell and lipid content is modest. A second virtual plaque growing at an equal rate with a higher inflammatory cell content may be more unstable, increasing the likelihood of rupture.

Model parameters in the CV platform are calibrated such that virtual plaques respond to therapies known to affect plaque size in a manner consistent with clinical data (detailed in the case study that follows). Selected data is used to validate the predictions of the CV platform as a whole, i.e., virtual plaques are calibrated to regress at a specific rate based on clinical patient responses to 40 mg/day atorvastatin therapy, and subsequent model predictions of plaque regression rate are validated against patient responses to 20 mg/day rosuvastatin therapy.

Measurements of virtual plaque geometry, lipid core size, cellular content, and the thickest and thinnest dimensions of the protective cap are provided as inputs to the plaque stability module of the CV platform (Figure 1c). A finite element model is used to calculate the magnitude and location of the peak mechanical stress within the plaque. The threshold stress for rupture is a function of the inflammatory and fibrous cell densities in the virtual plaque. These two factors determine the Plaque Instability Index (PII). The PII can be used to monitor how much a virtual plaque is changing due to disease progression or in response to therapy over a fixed time period.

Finally, the CV risk module of the CV platform (Fig. 1d) can be calibrated to relate the output of the previous modules to the relative risk of a cardiovascular event in virtual patients, consistent with clinical data from real patients.

TORCETRAPIB, A CASE STUDY

Entelos used the CV platform to predict the effects of CETP inhibition in CVD patients. Several observations combine to make CETP an attractive therapeutic target. CETP activity has a large impact on lipoprotein profiles (Huesca-Gomez et al., 2004) and potentially on cardiovascular risk (Blankenberg et al., 2003). CETP transfers cholesteryl esters and triglycerides between HDL and apoB-100 lipoproteins, including LDL. Simulations in the CV platform predict that increased CETP activity reduces HDL cholesterol. Low levels of HDL cholesterol are found in patients with an increased atherosclerotic burden (Wilt et al., 1997). Increased CETP activity has also been observed in patients with insulin resistance (Julius et al., 2007) who are known to have accelerated atherosclerosis (Bansilal et al., 2007). In contrast, a polymorphism of the
CETP gene that results in lower serum CETP levels has been linked to increased longevity (Barzilai et al., 2003). Studies in transgenic models have supported both proatherogenic (Marotti et al., 1993) and antiatherogenic (Foger et al., 1999) effects for CETP. Roche/Japan Tobacco’s JTT-705 (de Grooth et al., 2002) and Pfizer’s torcetrapib (Clark et al., 2004) are two CETP inhibitor (CETPi) compounds that have increased levels of HDL cholesterol in clinical studies. Patients treated with torcetrapib alone or in combination with atorvastatin (T/A therapy) exhibited increased HDL and decreased LDL levels (Millar et al., 2006).

Despite the promising effects of CETP inhibition on lipoprotein profiles, Pfizer terminated the 15,000-patient T/A ILLUMINATE trial in December 2006 due to an increase in mortality and the incidence of cardiovascular events (O'Riordan, 2006). Recently-released data from the 1,200-patient T/A ILLUSTRATE trial in patients with coronary artery disease demonstrated that although T/A therapy increased HDL levels by 60% and decreased LDL levels by 20% relative to atorvastatin therapy alone, there was no statistically-significant change in plaque atheroma volume (PAV) as measured by intravascular ultrasonography (IVUS) (Nissen et al., 2007). Furthermore, torcetrapib was associated with an average increase in systolic blood pressure of 4.6 mm Hg (McKenney et al., 2006).

Although torcetrapib was shown to have effects on blood pressure, an additional hypothesis suggests that the observed increase in levels of cholesterol-rich HDL may represent dysfunctional HDL with proatherogenic effects (Ishigami et al., 1994; Norata et al., 2006). These observations may ultimately provide an explanation for the increased mortality and morbidity observed in the ILLUMINATE trial, but a central question remains: are these effects unique to torcetrapib? A “compound effect” hypothesis leaves the door open for continued development of CETPi compounds. Alternatively, the unexpected clinical behavior of torcetrapib may be the first indication of a target-specific or “class effect” that results from CETP inhibition. The CV platform provides a unique tool in which to evaluate these competing hypotheses.

Simulations in the platform assessed the effect of “pure” CETP inhibition (inhibition of the enzymatic transfer of cholesteryl esters and triglycerides between HDL and apoB-100 lipoproteins), distinct from any additional off-target effects or vascular toxicities that might be associated with torcetrapib. To conduct these simulations, Entelos created a single virtual patient to represent patients that exhibit a “moderate cardiovascular risk” phenotype (moderate risk virtual patient). This virtual patient was validated by ensuring that its simulated responses to atherosclerosis trial protocols (i.e., changes in circulating lipoproteins and PAV) were consistent with published data. Specifically, the virtual patient responded appropriately to simulated protocols for three HMGCR inhibitors (REVERSAL (Nissen, 2005), CAIUS (Baldassarre et al., 2000; Mercuri et al., 1996) and ASTEROID (Nissen et al., 2006a)) and two ACAT inhibitors (ACTIVATE (Nicholls et al., 2006) and A-PLUS (Nissen et al., 2006b)). The lipid profile represented in the moderate risk virtual patient was consistent with the ILLUSTRATE trial patient inclusion criteria (Nissen et al., 2007).

### Table 1. Comparison of ILLUSTRATE trial data to simulated moderate risk virtual patient responses

<table>
<thead>
<tr>
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<th>ILLUSTRATE Trial Data</th>
<th>Moderate Risk Virtual Patient (VP)</th>
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<tbody>
<tr>
<td></td>
<td>atorvastatin only</td>
<td>T/A therapy</td>
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<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
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<tr>
<td><strong>Baseline</strong></td>
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<tr>
<td>(on statin therapy)</td>
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<tr>
<td>LDL-C (mg/dL)</td>
<td>84.3</td>
<td>83.1</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>45.2</td>
<td>46</td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>1.9</td>
<td>1.88</td>
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<tr>
<td>Plaque burden (PAV)</td>
<td>37.1</td>
<td>37</td>
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<tr>
<td><strong>After 24 months of</strong></td>
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<td></td>
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<tr>
<td>additional therapy</td>
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<tr>
<td>LDL-C (mg/dL)</td>
<td>87.2</td>
<td>70.1</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>43.9</td>
<td>72.1</td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>Plaque burden (PAV)</td>
<td>37.3</td>
<td>37.1</td>
</tr>
</tbody>
</table>

Simulations predicted a relative increase in HDL-C and a decrease in LDL-C in the moderate risk virtual patient in response to CETPi/statin combination therapy, with no difference in the plaque burden endpoints (Table 1). These predictions are consistent with the IVUS data, and suggest that the lack of incremental improvement in PAV despite an additional 20% reduction in LDL with combination therapy can be supported by a class effect hypothesis for CETP inhibition. Analysis of the simulation results revealed that although HDL particle levels increased, their
capacity to remove additional cholesterol from the virtual plaque decreased. This change results in a slowing of cholesterol catabolism, consistent with an observed lack of increase in biliary cholesterol secretion (Brousseau et al., 2005).

This initial study did not investigate the increase in CV events seen in the ILLUMINATE trial (O'Riordan, 2006). It is important to note that this increase is observed in the absence of increased plaque burden, as demonstrated in CV platform simulations and the ILLUSTRATE data. Several hypotheses have been proposed to explain the increase in cardiovascular events, including variability in expression and function of cholesterol transporters in plaque, dysfunctional HDL particle effects, and changes in lipoprotein particle number and composition.

Entelos plans future studies in the CV platform to evaluate these hypotheses. One advantage of the CV platform is the ability to monitor changes within virtual plaques which are difficult to observe clinically. Not only can these measures be rapidly evaluated in virtual patients, but the CV platform is capable of addressing the relative contribution of elevated blood pressure on changes in plaque biology and mechanical stress that lead to rupture. Entelos also plans to develop a virtual patient population that represents the full variability in lipid profile and plaque baseline characteristics observed in the general CVD population. This virtual population will support retrospective and prospective trial simulations that can be used to evaluate the impact of patient heterogeneity on clinical outcomes and explore the biological mechanisms responsible for plaque formation, stability and rupture.

VIRTUAL POPULATIONS

A virtual population is a prevalence-weighted cohort of virtual patients calibrated to reproduce the means and variances of lipid and plaque measures from clinical trials. It is designed to incorporate the same correlations as the clinical population of interest.

Variation in clinical lipid measures is represented in the CV virtual population by using the cholesterol metabolism module to generate thousands of virtual patients that explore the impact of genetic, environmental, and lifestyle factors that affect circulating lipoprotein levels (Figure 2). For example, variations in at least 13 metabolic pathways represented in the CV platform were identified, based on a literature search, to have a potential effect on the diversity of lipoprotein profiles in the human population.

Figure 2. Creating a virtual population from all potential virtual patients
Although the activity of the CETP enzyme (Huesca-Gomez et al., 2004) and the level of hepatic LDLR expression (Pisciotta et al., 2006; Russell et al., 1989) have a significant effect on circulating lipoproteins, lipoprotein lipase and hepatic lipase activities and the circulating levels of triglyceride and free fatty acids also play a role. By systematically varying all of these pathways within the reported range of biological activity, tens of thousands of combinations can be generated to represent ‘potential’ virtual patients (Figure 2a).

However, not all of these combinations are biologically ‘feasible’. Clinical data are used to select only those ‘potential’ virtual patients whose lipoprotein profiles match baseline data from real clinical patients (Figure 2b) and respond appropriately to standard therapies (Figure 2c). In the example shown, over 2000 ‘feasible’ virtual patients (Figure 2d) were obtained, representing valid clinical characteristics within the range of reported variability for a given clinical population.

Cohorts of virtual patients created in this manner represent the theoretical variability that could comprise a patient population. The actual clinical population, however, is likely composed of a subset of these individuals. Therefore, Entelos developed methodologies to represent population variability in a cohort of virtual patients. These methodologies combine the specific virtual patients in a cohort using statistical approaches (e.g., principle component analysis and observed phenotypic correlations (Figure 2e) to bridge the gap between the existence of a phenotype, i.e., the virtual patient, and its prevalence in an epidemiological profile of the clinical population. The resulting virtual population (Figure 2f) is calibrated to the actual means and variances of clinical endpoints observed in a given CV patient population. A similar process is repeated in the atherogenesis module to represent the variability in clinical plaque measures.

Utilizing a virtual population, simulations can be used to predict population-level outcomes. By systematically altering trial design criteria in the platform, optimal protocols can be developed to meet the requirements of clinical, marketing, safety, and regulatory groups.

CONCLUSION

The Cardiovascular PhysioLab platform was constructed to reproduce the underlying biology of lipoprotein metabolism, atherosclerotic disease development and plaque stability in humans. Normal and disease physiology are represented to ensure that the platform reproduces the stability and dynamics of a homeostatic system. The set of model parameters that define each virtual patient represent a hypothesis for a pathophysiological state, subject to validation against external standards such as the reported response of a clinical patient to a particular therapeutic protocol.

Virtual patients respond differently to treatment, just as subpopulations of actual patients representing a specific disease phenotype would respond in the clinic. The platform simulates the effect of specific therapies on virtual patients by computing changes in plaque geometry and composition that can be used to predict the long-term risk of a major cardiovascular event for each patient type.

Virtual populations encompass the diversity of variations that may result from genetic factors as well as environment and lifestyle factors. Clinically relevant virtual populations are calibrated by constraining the inclusion of virtual patients to those that reproduce responses to therapeutics seen in the clinic, and by using clinical patient data to determine the prevalence of individual phenotypes.

Clinical patients with the same lipoprotein profile and similar plaque burden do not necessarily have the same risk of a cardiovascular event. The relationship between plaque size and cardiovascular risk can be uniquely examined using the CV platform by exploring the variability in plaque composition due to known and hypothesized differences in component cell life-cycles and inflammatory mediators. The predictive capabilities of the platform have been validated by demonstrating consistency between simulation results and published clinical data for a range of therapeutic interventions, as exemplified by preliminary simulation experiments to demonstrate that “pure” CETP inhibition is sufficient to reproduce reported data from the ILLUSTRATE trial.

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REFERENCES


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