The Pursuit of Atheroma Regression:
The Proposed Role of ApoA-I Production & Reverse Cholesterol Transport in Reducing Percent Atheroma Volume and CVD Event
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Abbreviations

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<tr>
<td>ACS</td>
<td>Acute Coronary Syndromes</td>
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<tr>
<td>ApoA-I</td>
<td>Apolipoprotein A-I, main protein in HDL</td>
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<td>ApoA-IMilano</td>
<td>Variant of ApoA-I where arginine in position 173 has been substituted with a cysteine</td>
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<td>ABCA1</td>
<td>ATP Binding Cassette A1 Transporter</td>
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<td>CETP</td>
<td>Cholesterol Ester Transfer Protein</td>
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<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>EBT</td>
<td>Electron Beam Tomography</td>
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<td>HDL</td>
<td>High Density Lipoprotein, density 1.063-1.21mg/mL</td>
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<tr>
<td>Hypo-alpha</td>
<td>Low ApoA-I concentration</td>
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<td>IVUS</td>
<td>Intravascular Ultrasound</td>
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<td>Lp-PLA2</td>
<td>Lipoprotein Phospholipase A2, also known as platelet activating factor acetylhydrolase (PAF-AH)</td>
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<td>MDCT</td>
<td>Multi Detector Computer Tomography</td>
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<td>PAV</td>
<td>Percent Atheroma Volume</td>
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<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>Preβ-HDL</td>
<td>Small discoidal HDL particle with negative charge, same as nascent HDL</td>
</tr>
<tr>
<td>RCT</td>
<td>Reverse Cholesterol Transport</td>
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Executive Summary

This white paper describes an ongoing paradigm shift in cardiovascular disease (CVD) research and drug development. The paradigm shift is the pursuit of atheroma regression as the goal of lipid and atherosclerosis management. The landmark Esperion trial (1) clearly illustrated for the first time that atherosclerosis, the leading cause of premature death in coronary artery disease, is a dynamic process and can be reversed. In this important trial, patients with atherosclerotic coronary disease were infused with ApoA-I formulated with phospholipids to create preß-HDL like particles. ApoA-I is the dominant protein of HDL (so called “good cholesterol”). The stunning results of this trial showed highly significant regression of atherosclerosis in only 5 weeks. This outcome sparked several other trials including ERASE, PERISCOPE, CHICAGO and ASTEROID (2-6) to further support the pursuit of atheroma regression. In comparison, targeting non-ApoA-I parameters to reduce atheroma regression have faired poorly. For example, none of LDL lowering, LpPLA2 and PPAR targets have shown potential for atherosclerosis regression anywhere near ApoA-I enhancing technologies, as illustrated in both the Esperion (1) and ERASE (2) ApoA-I infusion trials.

The above information summarizes studies that demonstrate the importance of raising ApoA-I. That such therapies regress atherosclerosis stemmed from the use of IVUS (intravascular ultrasound) to study patients with CVD enrolled in the regression trials (1-6). Data collected using IVUS to measure atherosclerosis, show a clear relationship between atheroma burden and CVD events. IVUS measurements of atheroma size provided numbers to calculate percent atheroma volume (PAV) (7). This parameter is a sensitive reflection of atheroma burden because the baseline PAV value is predictive of CVD events. Moreover, the change in PAV over 12-24 months is also predictive of CVD events. Thus patients that accrue more atheroma burden leading to increased PAV over baseline have a greater likelihood of future CVD events versus those who have no change in their PAV. IVUS has therefore become the ‘gold standard’ method for assessing treatment effects on atherosclerosis. Data derived from the use of IVUS in Phase 2 trials will be essential for designing pivotal Phase 3 studies that will define the beneficial actions of ApoA-I enhancing treatments on major adverse coronary and cerebral events.

The objective of this white paper is to define the rationale that supports the use of RVX-208, or any other ApoA-I production therapies, that leads to the beneficial effects on atherosclerosis and CVD events. This paper is comprised of our review of pertinent literature dealing with ApoA-I biology, atherosclerosis regression studies and discussions with key scientists in the field. Based on our understanding of this information we propose the following hypothesis:

A rise in ApoA-I production of 4%-10% along with corresponding elevations of functional HDL particles preß-HDL and α1-HDL over a 9-12 month period, would supersedes standard of care for atherosclerosis regression and CVD event prevention, i.e. rosuvastatin 40mg OD. This outcome would fill a large unmet medical need particularly in the population with low plasma ApoA-I who are known to be at high risk for CVD.

The intent of this white paper is to establish the following understanding:

1.) atherosclerosis is a reversible and dynamic process affecting the walls of blood vessels;
2.) production of ApoA-I and HDL functionality as exemplified by preß-HDL and α1-HDL elevations is the key pathway for achieving atherosclerosis regression;
3.) utilizing the average 8% elevation over 5 weeks in the two ApoA-I infusion studies as a benchmark, a 4%-10% permanent increase in ApoA-I production via a novel orally available small molecule would be compatible with unprecedented potential for atherosclerosis regression;
4.) IVUS is the ‘gold standard’ for assessing impact of treatment on atherosclerosis, and;
5.) Percent Atheroma Volume (PAV) derived from IVUS is predictive of CVD events.
The Importance of ApoA-I and Functional HDL

Numerous epidemiologic studies have shown that the incidence of cardiovascular events correlate inversely with the plasma level of HDL (8, 9) and its main protein ApoA-I (10-12). With the wider use of ApoA-I analysis we find that ApoA-I, better than HDL cholesterol, reflects CVD-protective effects. A case in point is found in the recent analysis of EPIC-Norfolk trials data showing that subjects with very high levels of HDL may not be protected from CVD (13). On the contrary, ApoA-I’s inverse relationship to CVD was consistent across all levels suggesting that ApoA-I is a better biomarker than HDL cholesterol. This finding is not surprising in view of ApoA-I’s known role in protein-protein interactions with ABCA1 and LCAT (see below). ApoA-I is not only the dominant (70%) protein component of HDL, it is the substrate that initiates the synthesis of nascent or preβ-HDL particles (14, 15). These young particles of HDL are known to be nature’s strongest ligands to the ABCA1 transporter and the rate limiting step in RCT, the natural defense system against atherosclerosis. In brief, newly formed and non-lipidated preβ-HDL particles may interact with the foam cell surface ABCA1 transporter in a high affinity-low capacity interaction that drives the first step in RCT (16, 17). This interaction leads to the removal of excess cholesterol from the foam cell, thus triggering the shrinkage of the atheromatous plaque. The reduction in cellular cholesterol content underlies changes in the macrophage phenotype that results in stabilizing the atheromatous plaque, making it less prone to rupture followed by clot formation and thus reduce CVD events (18). This process of effluxing cholesterol from the foam cells by preβ-HDL is now described as one of the functional roles of HDL or HDL-functionality. Additional cholesterol efflux at the cell level is achieved by ABCG1 transporter. The action by LCAT with ApoA-I as co-factor then occurs in plasma where the esterification of free cholesterol facilitates the growth in HDL particle size (i.e. maturation). Other beneficial effects of ApoA-I as the anti-inflammatory, anti-thrombotic and immuno-modulating effects may or may not be related to its RCT action (19, 20). Below is a pictorial illustration highlighting the critical role of the ApoA-I protein, HDL particles and ABCA1 transporter to enhance RCT leading to PAV regression and a reduction in CVD events.

Figure 1. Source: Nissen et al, New England Journal of Medicine 2006
The development of CETP inhibition molecules and their clinical testing have provided additional understanding of reverse cholesterol transport (RCT). As shown in Figure 1, CETP facilitates the transport of cholesterol ester molecules from HDL to ApoB containing particles (VLDL or LDL) in exchange for either a cholesterol ester (homo-exchange) or a triglyceride molecule (hetero-exchange). CETP inhibition causes an increase in HDL cholesterol and decrease in LDL cholesterol (21, 22). The decrease in LDL cholesterol is a reflection of decreased RCT to ApoB particles and may thus paradoxically negatively impact atheroprotection, as a substantial portion of RCT is estimated to be mediated via the CETP-ApoB-LDL receptor route. It has been suggested that a CETP inhibition in the case of low LDL receptor activity would be favorable since the RCT route via ApoB particles may be negligible. This would for example be the case in coronary artery disease, familial hypercholesterolemia or ApoB mutations where LDL receptor uptake is severely inhibited.

Regarding RVX-208 treatment, we postulate that the increased ApoA-I production and increased RCT from peripheral tissue to HDL may in turn shuttle cholesterol ester to LDL VLDL via CETP. In this situation a LDL increase would be part of RCT from peripheral tissue. In fact this appears to be the case for Nevirapine, a non-nucleoside reverse transcriptase inhibitor used for HIV treatment that has been shown to increase ApoA-I production, increase LDL cholesterol (23) and decrease relative risk of CVD in the HIV population (24). Such a LDL cholesterol increasing effect by enhanced RCT from peripheral tissue would be less pronounced in situations of LDL receptor upregulation, for example in animals on chow diet (for example non-human primates) or humans on statin treatment (25). The ideal combination statin with RVX-208 to achieve complete RCT from peripheral tissue to the liver would thus be a statin with maximal LDL receptor upregulation.
The Independent Effects of Raising ApoA-I and Lowering LDL

The preceding section details the rationale for developing therapies that raise ApoA-I production to treat atherosclerotic disease. Now we will examine how statin treatment resulting in lower LDL cholesterol is the current standard of care for CVD. Statins prevent about 1 out of 3 events, both in primary (26) and secondary intervention trials (27, 28). This beneficial action of a statin arises from its ability to slow or lessen progression of the atherosclerotic process but these drugs do not cause regression of the disease. The inability of a statin to regress atherosclerotic disease is clearly evident in intervention studies revealing that lowering LDL-cholesterol via use of statins (regardless of dose) for 18-24 months have minimal effects – if any - on coronary atherosclerotic plaque regression (6, 29), Figure 2, right side panel (vide infra). Thus the general belief is that the best outcome, given optimal LDL lowering, is slowed or lessens the progression of atherosclerosis. Only the rosuvastatin trial (ASTEROID, see Fig 2 below) showed a regression in atheroma burden, i.e. 0.8% PAV regression (6). The modest effect on atherosclerosis was achieved by high dose treatment in selected patients allowing very low on-treatment LDL concentrations rarely experienced in medical practice, i.e. 61 mg/dL. In striking contrast to the poor statin effects on atherosclerosis reduction, ApoA-I infusions for only 5-6 weeks regress atheroma burden substantially (1, 2), see Figure 2 left side panel. Furthermore, it is important to point out that the stark difference between LDL lowering and ApoA-I raising therapies are independent actions. Therefore, either lowering LDL cholesterol or raising ApoA-I separately should benefit patients with atherosclerosis. But highly attractive is the option of combining the two therapies, the use of which is expected to exert a very potent effect on atheroma plaque and CVD events.

![Graph showing the effects of ApoA-I and LDL reduction](image)

*Figure 2. Source: Compilation Select IVUS Trials, adopted from Nissen and Nicholls.*
The hypothesis that raising ApoA-I production will reduce CVD risk requires proof from intervention trials. One critical step in gathering such data is assessing the effects of therapy on atheroma burden. The use of imaging technology to visualize atheromatous plaques for assessing medical therapies is rooted in the fact that such lesions cause CVD events. Data from pathology (30), early imaging studies using coronary angiography and carotid ultrasound (31-34), forge a strong link between atheroma burden, its progression and CVD outcome. Modalities that slow the progression of atheroma burden are known to reduce clinical events. This finding has been shown repeatedly in randomized clinical trials. Thus drug regulatory agencies have accepted the idea of stabilizing or slowing atheroma burden progression as a goal of medical therapy. Many methods are available for assessing the atherosclerosis burden in the coronary arteries. Non-invasive methods include electron beam tomography (EBT) assessing coronary calcium score (35) and multi-detector computer tomography (MDCT) assessing coronary plaque volume (36, 37). Both methods have been used to examine CVD subjects to show that patients with higher HDL have less degree of atherosclerosis, albeit correlations have been weak.

Compared to these non-invasive techniques higher resolution, precision and less variability are achieved by using the Intravascular Ultrasound (IVUS) methodology (7). The connection between atherosclerosis and CVD events was established long before the use of IVUS. Given the precision by which IVUS technology quantifies the extent of coronary atherosclerosis, it is expected that data gathered using IVUS will provide further support to this relationship. There are two key observations from clinical trials using IVUS to gather data that supports this expectation. The first is that a reflection of atheroma burden called PAV (percent atheroma volume) at baseline and subsequent serial increases are both greater in patients who experience a cardiovascular event. For example, analysis of combined data from the treatment groups in the ILLUSTRATE trial showed (38) that patients who had an event during the trial had a higher PAV at baseline followed by greater progression, see Figure 4 left panel. The difference in change in PAV between those who had an event and those who were event-free ranged from 0.5%-0.6%. This difference in the PAV was subsequently confirmed in pooled analysis of 7 clinical trials performed at the Cleveland Clinic involving more than 4500 patients (oral communication Dr. S. Nicholls). Therefore, the use of IVUS to detect a 0.5% to 0.6% difference between baseline and subsequent PAV is expected to translate into a clinically meaningful outcome. This expectation is supported by results of many previous studies, i.e. Esperion, ASTEROID, PERISCOPE (1, 6, 5).

**ILLUSTRATE = 1180**

Incidence of cardiovascular death, myocardial infarction, hospitalization for unstable angina, stroke and coronary revascularization

![Graph: Baseline Percent Atheroma Volume vs. Change Percent Atheroma Volume](image)

*Figure 3. Source: Dr. S. Nicholls, AHA 2007*
The second key observation is that therapies which slow atheromatous plaque progression as detected by IVUS translate into a reduction in clinical events. Three months following report of the REVERSAL study (29), the results of the PROVE-IT study (28) also confirmed the superiority of high-dose atorvastatin compared with pravastatin on both disease progression and cardiovascular events see Figure 4, right panel. Similar data was demonstrated in the CAMELOT study (39) that had an imaging component embedded within the larger clinical trial. Data from this aspect of CAMELOT showed that amlodipine resulted in the slowest plaque progression rate was associated with the lowest event rate. The finding that pioglitazone arrests arterial disease progression in diabetes is consistent with its benefit on ischemic end points in the PROactive study (40). More recently, it has been demonstrated that raising HDL cholesterol with torcetrapib was associated with a lower hazard ratio for events and slower disease progression. Similar findings have been demonstrated beyond native atherosclerosis in the heart transplant literature, in which the immuno-modulatory agent everolimus slowed progression of vasculopathy and reduced rejection rates. Together the preceding data is very consistent and convincing to support therapies that cause changes in atheromatous plaques which may be detected using IVUS correlate with their effects on clinical outcome. These findings provide strong support for the use of IVUS as a tool for the clinical assessment of novel anti-atherosclerotic therapies.

**Figure 4. Source: Nicholls et al. Effects on PAV regression are consistent with event reduction.**

IVUS can be used to assess atherosclerosis plaque volume along a coronary segment of approximately 40-60 mm with high precision and a standard deviation for the change over time in the order of 2.5%-3.0% (6, and oral communication Dr. S. Nissen). Below is a series of images that illustrates the use of IVUS to measure an atherosclerotic lesion within a coronary artery to define atheroma burden.
Based on concordance between PAV from IVUS and clinical events at follow up, it is generally accepted that IVUS assessment of pharmacological intervention is the ‘gold standard’ method for assessing efficacy of a treatment modalities. Such data is helpful for optimizing treatment regimen and for deciding on implementation or not of Phase 3 trials (7) in drug development. More recently, the ability of radiofrequency echo detection added to IVUS enables it to detail plaque composition. This feature will most likely increase the clinical utility of IVUS (41). Together the above summary and the documented capabilities of IVUS build a strong case for the use of this ‘gold standard’ technique in course of developing drugs to benefit CVD.
Valuable Lessons from ApoA-I Infusion Studies

Results from the proApoA-I infusion study in familial hypercholesterolemia (FH) patients (42) lay the foundation for dosing regimen and duration of the Esperion and ERASE trials. In the proApoA-I infusion study in FH patients, subjects received a single 2g dose leading to a transient increase in plasma ApoA-I and HDL. More importantly, the ApoA-I infusions lead to a >33% increase in sterol excretion sustained over 12 days. Since cholesterol production and cholesterol uptake were unchanged the net effect was estimated to be 5%-7% removal of the body’s total cholesterol pool. In view of cholesterol’s fundamental role in biology this effect was astonishing. The landmark Esperion trial that used ApoA-IMilano infusions (1) was thus designed to increase the preβ-HDL pool significantly over 5-6 week duration of the study (personal communication Dr. J. Johansson) and test the hypothesis that the expected RCT would result in regression of coronary atherosclerosis, as assessed by IVUS.

In humans, the Esperion and ERASE trials infused ApoA-IMilano dimer (1) and wild type ApoA-I (2), respectively, formulated with phospholipids to mimic preβ-HDL like particles. Both studies used the identical treatment regimen of once weekly ApoA-1 infusions for 5 weeks in acute coronary syndrome (ACS) patients. Assessment of coronary vasculature using IVUS was performed before starting and 1 week following completion of the last infusion. The Esperion trial had a low (15mg/kg) and high (45 mg/kg) dose arm, but the IVUS data surprisingly showed no statistical difference between doses to regress atheroma burden. This meant that the low and high doses were equally efficacious in regressing plaques. This unexpected finding may be explained by a potentially rate limiting capacity or a saturation for ApoA-IMilano to induce RCT, i.e. the high affinity-low capacity nature of the preβ-HDL and ABCA1 transporter interaction at the macrophage surface (16, 17). The plasma ApoA-I concentration is approximately 1mg/mL. It is estimated that the ApoA-I total mass in interstitial fluid/extravascular space is equal to that in plasma (43, 44). In vitro the concentration of ApoA-I yielding half maximal efflux (EC50) from macrophage cells is about 1µg/mL (45). In other words, already at a concentration of about 2mg/L the maximal efflux is achieved and saturation of the system accomplished (45). Most people have a plasma ApoA-I concentration of 1 mg/mL and a lymph concentration 1/10 of that, thus about 1000 to 100-fold higher than the EC50 value.

The active preβ1-HDL fraction comprise about 5% of total ApoA-I (46), still about a concentration 5-fold higher than the EC50 value. Even though the extrapolation from in vitro to in vivo has limitations, it is clear that the ApoA-I to ABCA1 interaction functions at low preβ1-HDL concentrations, close to a saturation kinetics. Thus the characteristics of the high affinity and low capacity interaction may limit the usefulness of raising ApoA-I and preβ-HDL beyond a certain point. This feature may explain why in the ApoA-IMilano infusion 15 mg/kg was equally efficient to 45 mg/kg in reducing plaque volume. Because of cholesterol efflux saturation related to the ABCA1 transporter’s high affinity-low capacity nature, it is likely that a lower and more sustained production of ApoA-I over an extended period would constitute the preferred regimen for treating atherosclerosis.
In support of the above finding in the Esperion trial, a dose-response atherosclerosis study in hyperlipidemic rabbits (47) infusions of ApoA-IM/PL-complex (synthetic HDL) showed effects with a 5mg/kg dose. The atherosclerosis reduction reached a maximum between 20 and 40 mg/kg with no additional effect by 150 mg/kg. Applying allometric scaling from rabbit to human (approx. 3:1), the 40 and 150 mg/kg dose in the rabbit corresponds roughly to 15 and 45 mg/kg in humans.

In the ERASE trial patients with ACS were given either a low or high dose of the ApoA-IWT/PL complexes at 40 and 80mg/kg, respectively. The higher dose was terminated due to side effects likely attributed to cholic acid (ingredient for complex formation) so no dose-response could be established for ApoA-IWT. As there is no convincing data supporting one of the two ApoA-I forms, Milano or WT, to be more efficient (48-51) we assume that a 15mg/kg dose or lower would be optimal for once weekly infusion treatment regiments.

What increase in ApoA-I and what duration is required to reduce coronary atheroma burden? The reported half-life of ApoA-I is 3-4 days (43, 52, 53). According to this half-life, a 5 week average increase in ApoA-I arising from a 15mg/kg weekly injection would be about half of that, i.e. 7.5 mg/kg which is a 7.5% plasma ApoA-I increase, ceteris paribus. No accumulation or tolerance effects have been shown and the ~8% average ApoA-I increase estimate would be assumed to remain constant throughout the 5 week treatment period. A more permanent increase arising from newly synthesized ApoA-I, to trigger creation of nascent HDL particles, induced by an orally active small molecule that stimulates endogenous ApoA-I production from the liver and small intestines, would be a very attractive regimen for treating atherosclerosis.

The infusions studies achieved unprecedented atheromatous burden regression by infusing 15 mg/kg ApoA-I or plasma ApoA-I increase of approximately 8% during the 5 week study. We hypothesize that a chronic ApoA-I production increase in the order of 4% (half of 8%) over a 3 month or longer period of time would have the potential to achieve similar effect on atheroma regression compared to the ApoA-I infusion studies. However, a more appropriate benchmark for a small molecule for oral administration like RVX-208 would be best standard of care, i.e. rosuvastatin.
The enhancement of ApoA-I production using an orally active small molecule added to current LDL-lowering therapies as such as rosuvastatin, would potentially provide needed additional PAV regression and CVD risk reduction for many CVD patients. ApoA-I infusion studies have changed the paradigm of atherosclerosis from one that is static to a biologically dynamic disease. The ApoA-I/RCT pathway is the only described pathway with the ability to substantially regress atherosclerosis. The known strong relationship between atherosclerosis and CVD events points to ApoA-I therapies utility in CVD prevention. ApoA-I production via oral administration of small molecules is ideal for chronic treatment in contrast to infusion therapies. To estimate the potential of ApoA-I production treatment we utilize the infusion studies which increased the ApoA-I levels by only 8% for 5 weeks.

A small molecule that raises levels of ApoA-I from 4%-10% by increasing endogenous production of the protein would, particularly in the low HDL patient population, be hypothesized to have treatment effects far exceeding best standard of care for small molecules for oral administration. Best standard of care effect is achieved by 40mg rosuvastatin over 2 years causing 0.8% reduction in PAV.
Product Differentiation and ApoA-I Production Rate

Based on non-clinical and clinical data there are good reasons to believe that the mechanism for raising ApoA-I and HDL is the preferred path for atherosclerosis. We postulate that therapies which increase the ApoA-I production rate provides the most logical approach for treatment efficacy. Increased ApoA-I production triggers the synthesis of nascent ApoA-I particles or preβ-HDL that are most efficient in picking up cholesterol from atherosclerotic lesions and channeling it via the RCT pathway. The use of this pathway to get rid of cholesterol should protect against atherosclerosis, prevent CVD events and possibly regress atheroma burden. The newly synthesized nascent ApoA-I particles or preβ-HDL and their interaction with the high affinity-low capacity ABCA1 transporter (16, 17) facilitates removal of excess cholesterol from the macrophage foam cells in the arterial wall, thereby promoting plaque stability and reducing plaque volume. Furthermore, data from the infusion studies paired with knowledge of ApoA-I physiology leads to the hypothesis that a 4%-10% permanent increase in ApoA-I production rate would have pronounced effects on atherosclerosis. The removal of excess cholesterol from the macrophage foam cells within atherosclerotic lesions would change the plaque phenotype from being rupture-prone to being stable (18).

Therapies which raise HDL and ApoA-I by slowing the catabolic rate have had neutral and possibly negative effect on atherosclerosis. For example, the valuable lessons learned from the use of Torcetrapib, a potent CETP inhibitor is in keeping with this hypothesis. Also, therapies that affect HDL levels indirectly (by increasing it aside from production) would not be able to take advantage of the powerful and beneficial actions of the ApoA-I RCT pathway. This scenario would apply to the lowering of VLDL triglycerides or LDL cholesterol as a way to increase HDL. Such an approach requires the transfer of lipids from the surface of VLDL and LDL to HDL particles. Drugs that act via the preceding mechanism include; niacin or fibrates which lower triglycerides and statins which lower LDL cholesterol to raise HDL.

In the ASTEROID trial rosuvastatin caused LDL cholesterol to be lowered by 53%, the HDL cholesterol increased by 14.7% and ApoA-I by 8.9%. New diagnostic techniques have illustrated that these increases are not via a direct production mechanism of ApoA-I. Turn-over studies with rosuvastatin showed that it does not affect HDL production but rather increased catabolic rate (54, 55). It is believed that the statin induced reductions in VLDL and LDL caused a substrate dependent CETP inhibition, resulting in a slower catabolic rate of HDL particles. This appears to be the case also for simvastatin where the approximate 5%-10% increase in HDL is strongly and inversely correlated to the decrease in LDL-cholesterol (56). Atorvastatin has neutral effects on HDL and in turnover studies it did not change the production or catabolic rates (57). In keeping with our prediction, the ability of a statin to lower LDL enables it to stabilize or slow progression of atherosclerosis. The added ability of rosuvastatin to raise HDL, in a fashion that does not seem to raise the production rate, might account for its limited effect on regression of atherosclerosis as seen in the ASTEROID study.

Fenofibrate is a PPARα agonist that increases ApoA-I synthesis in hepatic cells. Kinetics studies prior to and 5 weeks following treatment with micronized fenofibrate (200mg/day) in metabolic syndrome subjects showed increased ApoA-I production rate and increased catabolic rate leading to a rise in plasma ApoA-I of about 16% (57). Data from the VA-HIT study showed that gemfibrozil increased HDL and at the same time decreased triglycerides associated with a 21% reduction in primary end points. But in similar studies in subjects with elevated cholesterol, the increased HDL production and catabolic rate were reproduced but instead, the plasma ApoA-I levels decreased (58). A decrease in plasma ApoA-I by fenofibrate was also seen by long term fenofibrate treatment in the FIELD study paired with an increase in ApoA-II resulting in change to smaller HDL particle size (59). Also Tramblay et al. (58) showed equally large production and catabolic increases by fenofibrate with no increase in plasma ApoA-I. Fenofibrate along with other PPARα agonists exert their most prominent effect on HDL via an ApoA-II increase that is larger than the rise in ApoA-I (61, 62). In view of the above and recent studies on the strong interaction between VLDL triglycerides and HDL (62) it is believed that most of the HDL effects...
by fenofibrate are secondary to its effects on triglyceride metabolism. These findings may help explain the poor effects by fenofibrate on CVD risk, as predicted from our hypothesis.

Vitamin B3 or niacin at gram doses has many effects (63). Clinically effective doses of niacin causes flushing, initially misinterpreted as generated by increased metabolism, and the drug was introduced by Altschul in the mid 1900s for weight reduction purposes. Albeit weight lowering was never achieved, subsequent studies showed that niacin lowers triglycerides and increases HDL cholesterol, and also to a lesser extent ApoA-I. Niacin also increases blood glucose in subjects with glucose intolerance. Several studies have suggested that niacin has favorable effects on CVD events in the non-diabetic population. (64, 65). Compliance is an issue and remains a problem despite use of slow- or extended-release formulations. A recent attempt to overcome the compliance problem led to creation of anti-flushing agents (laropiprant). Original turnover studies of niacin in young normolipidemic subjects showed no change in ApoA-I production or catabolic rate (43, 66). Schaefer et al. (67) recently performed a study in 5 non-diabetic men with combined hyperlipidemia. In a double blind cross over design he compared niacin alone to niacin + lovastatin and placebo. Niacin increased ApoA-I production rate by 24% with no change in fractional catabolic rate. These changes led to increased HDL-c of 35%, plasma ApoA-I rose 15% while plasma ApoA-II was unchanged. These data support the important role of niacin in treating lipids and its use in the prevention of CVD in the in non-diabetic/ non-glucose intolerant population (64). Its main draw backs include significant side effects and patient compliance. The uncertain actions of niacin on HDL production makes it premature to predict its effects on CVD based on our hypothesis at this time.

In this section, we have outlined the current therapies used to raise HDL, briefly summarized their modes of action. It is noted that none of them raised ApoA-I production exclusively. As current understanding of lipoprotein biology emerges it is become clear that there remains significant gap in our therapeutic options for atherosclerosis. Emerging data strongly suggests that small molecules that raise ApoA-I production will potentially fill the critical unmet need that remains with the key underlying cause of CAD and CVD, namely atherosclerosis.
Conclusion

This white paper summarizes current knowledge detailing the ability of ApoA-I production and emerging RCT biomarkers preβ-HDL and α1-HDL to regress atherosclerosis. ApoA-I infusion studies form the basis for postulating that increasing ApoA-I production to raise the body’s HDL levels is essential in harnessing the beneficial actions of this class of lipoprotein particles. This benefit is likely due to enhanced channeling of cholesterol through the reverse cholesterol transport pathway. Furthermore, therapies that raise plasma HDL by impacting the degradation of this lipoprotein class will not regress atherosclerosis. Our hypothesis is based on data from coronary atherosclerosis studies that compare LDL lowering statin therapy with ApoA-I/preβ-HDL infusion studies. The LDL-lowering studies show that optimal statin treatment for at least 2 years, at best, leads to stabilization of disease. In contrast the ApoA-I/preβ-HDL infusion treatments for less than 2 months show striking regression of atherosclerosis. These powerful data were gathered using IVUS to calculate the PAV, a reflection of atheroma burden. PAV has been shown to be predictive of CVD events in more than 4500 subjects, thus providing an attractive biomarker to gauge the impact of ApoA-I producing therapy on hard endpoints. Thus atherosclerosis regression is feasible given a therapy that raises ApoA-I production to remove cholesterol from atherosclerotic lesions and get rid of it via the RCT pathway.

To understand how to best regress atherosclerosis we compiled data from ApoA-I infusion studies in humans and rabbits. These data suggested that maximal effect would be achieved by an 8% increase in the ApoA-I pool leading by preβ-HDL addition. This number is based on the rate limiting/saturation features imposed by the high affinity-low capacity interaction between the ApoA-I (i.e. preβ-HDL) protein and ABCA1 transporter. Thus a permanent increase in ApoA-I production within a range of 4% to 10%, with a similar increase in plasma ApoA-I would have an effect on atherosclerosis far beyond current best standard of care, i.e. rosuvastatin 40 mg/day. None of the existing marketed drugs in atherosclerosis and CVD has effectively had effects on atheroma regression. The future for reducing the morbidity and mortality associated with CVD is the development of novel therapeutic agents that can safely and effectively regress the grievous burden of atherosclerosis.

Establishing modalities which increase ApoA-I production and subsequent the key RCT particles preβ-HDL and α1-HDL are important in treating, preventing and possibly regressing CVD. RVX-208 is well positioned to meet this critical unmet medical need in CVD, the largest therapeutic market in the world.
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