Company Profile

Aterovax

Aterovax is developing innovative products for patients with and at risk of cardiovascular disease. Based on its pioneering research in atherosclerosis, the company's first product is a diagnostic blood test to measure the activity of secreted phospholipase A2 enzymes, a powerful biomarker that provides physicians with clinically relevant information regarding the presence of destabilized atherosclerotic plaque. Detecting unstable plaque and its potential to rupture, is critical for monitoring the pathology of atherosclerosis and assessing the risk for events, such as myocardial infarction and stroke. Data have been collated from more than 23,000 tests to support the role of secreted phospholipase A2 activity in determining the risk of adverse cardiovascular events.

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Cardiovascular diseases & biomarkers

The global market for *in vitro* diagnostics reached US\$5.6 billion in 2007. By 2012, it is expected to have more than doubled to nearly \$13 billion. Cardiovascular biomarkers already play an important role in this market, as the second largest application after cancer, representing approximately 30% of the market share. By 2012, cardiovascular biomarkers will surpass cancer and represent the largest segment, with an estimated market potential of \$4.5 billion [101].

Cardiovascular disease (CVD) accounts for nearly 50% of all deaths in developed countries. By 2020, heart disease and stroke are projected to become the leading cause of death and disability worldwide, with the number of fatalities set to increase to more than 20 million a year. By 2030, this figure is set to increase to 23.6 million a year [102].

Atherosclerosis is a common and complex disease of the arteries and is responsible for many causes of CVD, including myocardial infarction, angina, stroke and leg ischemia. Our understanding of atherosclerosis has shifted dramatically in recent years. Rather than being a cholesterol-fuelled 'plumbing problem', atherosclerosis is now believed to result from chronic inflammation and buildup of plaque under the inner lining (intima) of arteries that swell into the lumen of the arteries. This gradual

accumulation of plaque occurs over many years making atherosclerosis difficult to diagnose accurately.

Although clinical complications of atherosclerosis can arise from plaque, causing flow-limiting stenoses (narrowing of the arteries), the most severe clinical events follow the rupture of plaque. Inflammation, which is implicated in the formation of early fatty streaks as well as at the onset of adverse cardiovascular events, causes cells within the plaque to secrete enzymes that degrade and weaken the fibrous cap, leading to rupture and thrombus (clot) formation. Ruptured plaques are the cause of acute coronary events and sudden cardiac death. However, thrombotic complications that arise from rupture or fissure (small rupture) of a vulnerable plaque may be clinically silent, yet contribute significantly to plaque progression and, ultimately, stenosis. It is, therefore, important to identify those patients in whom disruption of a vulnerable plaque is likely to result in a clinical event. This clearly points to the need for new diagnostic and therapeutic approaches.

Currently, the only way to determine the risk for atherosclerotic pathological events is to perform annual estimates of the overall risk of CVD over 10 years. This is accomplished using traditional risk factors, such as age, sex, cholesterol level, diabetes or hypertension, in predetermined scoring models, such as the Framingham's model or the European Systematic Coronary Risk

Evaluation (SCORE) model. Typically, this screening is routine from the age of 40 years in men and 50 years in women.

However, up to 50% of patients with CVD do not have high cholesterol or other traditional risk factors and, thus, are not recognized as being at risk [1]. This means that approximately 30% of heart attacks are never predicted. Among these, many patients had previously been considered as being at medium risk, so were never prescribed treatment. Diagnostic tools that are able to help distinguish between high and medium risk are greatly needed so that patients can be made more aware and, if appropriate, treatment such as statins given.

The ability to better predict the risk of cardiovascular events is one of the most important steps in reducing CVD and mortality. Most crucial to this is the ability to assess the risk of healthy individuals and detect atherosclerosis early – the largest target population for new biomarkers.

In addition, according to international guidelines on how to deal with CVD, all patients at high risk should be treated with statins and, in some cases, with blood pressure-lowering drugs. However, these guidelines are not always followed and it is not always economically possible to treat everyone. In addition, the doses of the medicines will vary depending on the gravity of the risk.

Therefore, there is a strong need in the cardiovascular field for novel diagnostic tools that could help physicians to determine who is the most crucial to treat and to adapt their treatment strategy accordingly.

Prognostic biomarkers & therapy response

Biomarkers for prognostic (predictive) use help to raise awareness in already sick individuals and support the necessary lifestyle changes or other preventive measures that a patient needs to undertake. Biomarkers can also be used to determine how an individual will respond to a particular therapy. This is an emerging and rapidly expanding market where the need for new potent biomarkers is enormous.

The pharmaceutical market, directed by regulatory agencies, has a growing need for these so-called theragnostic solutions. By using a specific diagnostic, those patients that would benefit most from a particular

treatment can be identified. This replaces the universal practice of prescribing the same drug to every patient.

Theragnostic solutions may enhance therapeutic differentiation and market penetration for new medicines by allowing selective recruitment of patients for trials and therapy, and as such, will become increasingly desirable to the industry.

A huge effort has been made by the pharmaceutical industry in the cardiovascular sector, with multiple new emerging therapeutic classes, in particular those targeting inflammation, such as secreted phospholipase (sPL)A2 inhibitors, lipoprotein-associated phospholipase A2 inhibitors or 5-lipoxygenase inhibitors. There will be a great interest in biomarkers linked to these new drugs, permitting a theragnostic approach.

Finally, biomarkers are of particular interest in the last phase of disease development for the monitoring and follow-up of a patient after treatment, and to assess whether the therapy will be efficient or not.

sPLA2 activity, a promising atherosclerotic biomarker

The sPLA2 constitutes a family of nine enzymes in humans that generate fatty acids and lysophospholipids, and trigger a variety of proinflammatory actions [2]. It is now well accepted that sPLA2 plays a crucial role in the formation and destabilization of atherosclerotic plaque and contributes to lipoprotein retention, foam cell formation and inflammation in a developing lesion (FIGURE 1). Furthermore, the potential clinical benefit of sPLA2 inhibitors in the treatment of CVDs has already been explored in several Phase II trials, and these molecules will enter Phase III in the coming months [3].

Last, but not least, sPLA2 activity recently emerged as a very promising biomarker for cardiovascular risk.

Pioneering work from Aterovax scientific founders, Alain Tedgui and Ziad Mallat, demonstrated that the activity of sPLA2 correlated strongly with the risk of experiencing a cardiovascular event, both in clinical and nonclinical populations [4,5]. In the Global Registry of Acute Coronary Events (GRACE) study, the unadjusted rate of death and recurrent myocardial infarction in patients with acute coronary syndrome increased in a stepwise fashion

with increasing tertiles of sPLA2 activity at baseline, and the association was significant across all subgroups of patients with myocardial infarction or acute coronary syndromes [4]. In the European Prospective Investigation of Cancer (EPIC)-Norfolk study, sPLA2 activity was determined at baseline in 2797 apparently healthy subjects (991 subjects with incident coronary artery disease and 1806 event-free controls) and the risk of incident coronary artery disease was associated with increasing quartiles of sPLA2 activity [5].

These initial results have now been further validated in more than 10,000 patients enrolled in six international clinical studies (FIGURE 2) [6–10]. Published clinical data obtained to date demonstrate that testing for sPLA2 activity is a powerful biomarker for the presence of destabilized plaque; hence, both a predictor of risk for coronary heart disease in the asymptomatic population as well as a predictor of cardiac events in patients with coronary syndromes.

By sharp contrast with other biomarkers currently under development in the cardiovascular field, sPLA2 is emerging as a risk factor and not a simple risk marker of CVD.

Aterovax sPLA2 activity test

Aterovax was incorporated in 2006 and initiated its operations mid-2007 with the objective of valorizing a patent portfolio filed by Institut National de la Santé Et de la Recherche Médicale (INSERM) and other academic institutions related to innovative products for patients with and at risk of CVD. In particular, Aterovax is the exclusive worldwide licensee of several patents covering the use of sPLA2 activity in the prediction of cardiovascular risk.

The company's first product was an enzymatic assay for the determination of sPLA2 enzymatic activity in human plasma and serum. This enzymatic activity is the result of the individual enzymatic activity of each sPLA2 protein isoforms.

Figure 1. Secreted phospholipase A2 enzymes play a crucial role in the development of atherosclerotic plaque. LDL: Low-density lipoprotein; HDL: High-density lipoprotein; sPLA2: Secreted phospholipase A2.

Figure 2. Secreted phospholipase A2 activity is emerging as a potent biomarker of cardiovascular risk assessment.

[†]Adjusted for traditional risk factors, treatments and biomarkers when available.

CAD: Coronary artery disease; CHD: Coronary heart disease; DIMU: Diagnostic In Medical Urgency; EPIC: European Prospective Investigation of Cancer; FAST-MI: French Registry of Acute ST-elevation or non-ST-elevation Myocardial Infarction; GRACE: Global Registry of Acute Coronary Events; PEACE: Prevention of Events with ACE inhibition. Data taken from [4–7,10,11].

Detecting sPLA2 activity in complex biological samples, such as serum and plasma, is a challenging task. This is mainly owing to issues related to nonspecific interactions of the tested biological samples and phospholipid substrate. Thanks to the expertise on both sPLA2 and *in vitro* diagnostics of its team and partners, Aterovax developed and industrialized, in record time, an innovative process for stabilizing phospholipid bilayers onto latex beads, leading to the development of a very robust and highly sensitive sPLA2 assay. The company filed several new patent applications protecting this method and its applications for detection of phospholipase enzymatic activity.

In the last 2 years, Aterovax has built a world-class academic network with leading cardiovascular clinical groups. This highly effective strategy enabled the company to have access to biological samples from large and very high-quality clinical trials and, thus, to rapidly expand the initial clinical demonstration of the interest of sPLA2 activity as a cardiovascular risk biomarker. To date, the clinical utility of Aterovax's sPLA2 Activity Test has already been validated and published in several large clinical trials [4–10]. In addition, two different hospital biochemistry laboratories in Europe independently validated the analytical performances and clinical utility of

the assay [GEORGES POMPIDOU EUROPEAN HOSPITAL, PARIS, FRANCE; LAPEYRONIE HOSPITAL, MONTPELLIER, FRANCE: UNPUBLISHED DATA]. The Aterovax sPLA2 Activity Test is then one of the best available tests for predicting cardiovascular risk, independently and over and above traditional risk markers, such as cholesterol, risk scores (Framingham's risk score), and well-established risk markers, such as high-sensitivity C-reactive protein [4–10].

At present, Aterovax's sPLA2 Activity Test is available for research use only and the company is in the process of submitting the test for regulatory approval both in Europe and the USA.

In clinical research and cohort trials, the sPLA2 Activity Test provides developers with a potent new biomarker to identify the best responders among patients enrolled in clinical trials. This is particularly true for anti-inflammatory drugs, such as sPLA2 inhibitors (varespladib; Anthera Pharma), Lp-PLA2 inhibitors (darapladib; GlaxoSmithKline) or 5-lipoxygenase inhibitors (VIA-2291; Via Pharmaceuticals) but is also of strong interest in other therapeutic classes, such as lipid-modifying or antithrombotic drugs. Monitoring sPLA2 activity in patients enrolled in trials is also a potent way of stratifying those who are at low-to-medium risk of cardiovascular events and,

thus, independently of other biomarkers or high-sensitivity C-reactive protein. Finally, sPLA2 activity is emerging as a strong biomarker of cardiovascular safety.

In secondary prevention, the need for robust biomarkers to adapt or personalize the different medication strategies available is high and still growing. Leveraging on initial results that demonstrated that sPLA2 activity is downregulated by effective cardiovascular therapies, such as hypolipidemic treatments, an ambitious clinical development plan is currently being conducted by Aterovax and its academic and medical network to further demonstrate the interest of sPLA2 activity as a treatment monitoring tool.

Finally, the primary prevention of atherosclerosis is still an area with crucial medical needs. Identifying healthy individuals with predisposition for the disease, independently or in addition to existing risk factors, is of critical importance and would have strong medical and economical benefits in the future. Thanks to this strong involvement in the pathophysiological pathways of atherosclerosis, sPLA2 activity, alone or combined with other biomarkers, established or currently under development, has the potential to become one of the leading biomarkers for cardiovascular primary prevention. Aterovax will have access to biological samples from the largest primary prevention retrospective studies in the cardiovascular field and will actively participate in the development of these new, highly demanded tools.

Future perspective

Thanks to its expertise in sPLA2 and its world-class international academic network, Aterovax has developed, industrialized and validated an innovative, highly sensitive and robust biomarker for the detection of cardiovascular risk in less than 2 years. The company is currently

conducting discussions with European, North American and Asian distributors and specialty laboratories to establish licensing and purchase agreements for its sPLA2 activity biomarker. Aterovax's first product would then be available for the clinical community in the coming months and will generate the first revenues of the company.

Atherosclerosis is a chronic, complex and multifactorial disease. The consensus view among clinicians tends to be that a multi-marker test could bring better detection of the vulnerable plaque than a single marker alone. Therefore, combining measurement of sPLA2 activity with other biomarkers could provide a better indicator of the vulnerable plaque. Specifically, the combined measure of sPLA2 activity with the oxPL/apoB biomarker holds great promise [9]. In that aspect, it should be noted that oxidized phospholipids are actually a substrate of sPLA2. Aterovax is developing next-generation products, combining the detection of sPLA2 activity and/or mass with other biomarkers. In particular, Aterovax is engaged in several collaborative programs focused on identifying new diagnostic products based on the detection of sPLA2 activity and sPLA2 isoform concentrations. Finally, and in parallel with its sPLA2 program, the company is also pursuing efforts to discover and develop its own pipeline of novel *in vitro* diagnostic product candidates.

Financial & competing interests disclosure

The author is an employee of Aterovax SA. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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