Overview of the main findings

This project has some important findings that can be translated into clinical practise. This project provides robust information supporting that statins exert a rapid, direct effect on:

a) human saphenous vein grafts (SVs) used for coronary artery bypass grafting (CABG) operations

b) internal mammary artery grafts (IMAs) used for CABG in these patients and

c) human myocardium

by reducing the activity of key enzymes involved into the regulation of vascular and myocardial oxidative stress (redox state). Since oxidative stress is involved in the development of atherogenesis (in the vascular wall) and myocardial complications post CABG (i.e. atrial fibrillation), it is suggested that statin treatment may have important clinical implications. However, this is a small mechanistic, pilot study and the clinical value of our results should be evaluated/confirmed by large clinical trials.

In more details, the potential impact of the study results can be summarised as:

A) The impact of statins on arterial and vein grafts redox state: Implications for cardiac surgery[1]

In this study we show that even 3 days treatment with atorvastatin (just 3 tablets or 40mg for 3 consecutive days), results into a rapid reduction of oxidative stress in both venous and arterial grafts used in CABG. This effect is due to direct changes of the biology of these grafts, and independent of LDL lowering. These findings are clinically important, since they suggest that the pleiotropic effects of statins on the vascular wall (well demonstrated by basic science studies in the past) is also present at a clinical level. They also provide the mechanistic basis that explains the findings of large clinical trials showing that statins improve clinical outcome post-CABG, and provides potential mechanisms for their effects. It is likely that the improvement of redox state in SV and IMA grafts used in CABG may result into better patency of these grafts, explaining the overall better clinical outcome of patients receiving statins, post CABG.

Importantly, our study demonstrates that the use of statins modifies grafts' redox state independently of LDL lowering therefore, it implies that LDL should not be the only biomarker to monitor the responsiveness to statin treatment. Importantly, the beneficial effect of statins on grafts' redox state does not seem to be mediated through the well known antiinflammatory effects of statins, since classic markers of inflammation (CRP, cytokines, soluble adhesion molecules etc) had not been changed by short-term statin treatment.

In conclusion, the results of the current project suggest that statin treatment, even for just 3 days results into striking improvement of redox state in venous and arterial grafts used in CABG, and this may lead to improve patency of these grafts, improving the clinical outcome post CABG.

The main effects of statin treatment on SV grafts' redox state as documented in the current study are summarised in **Figure 1**.

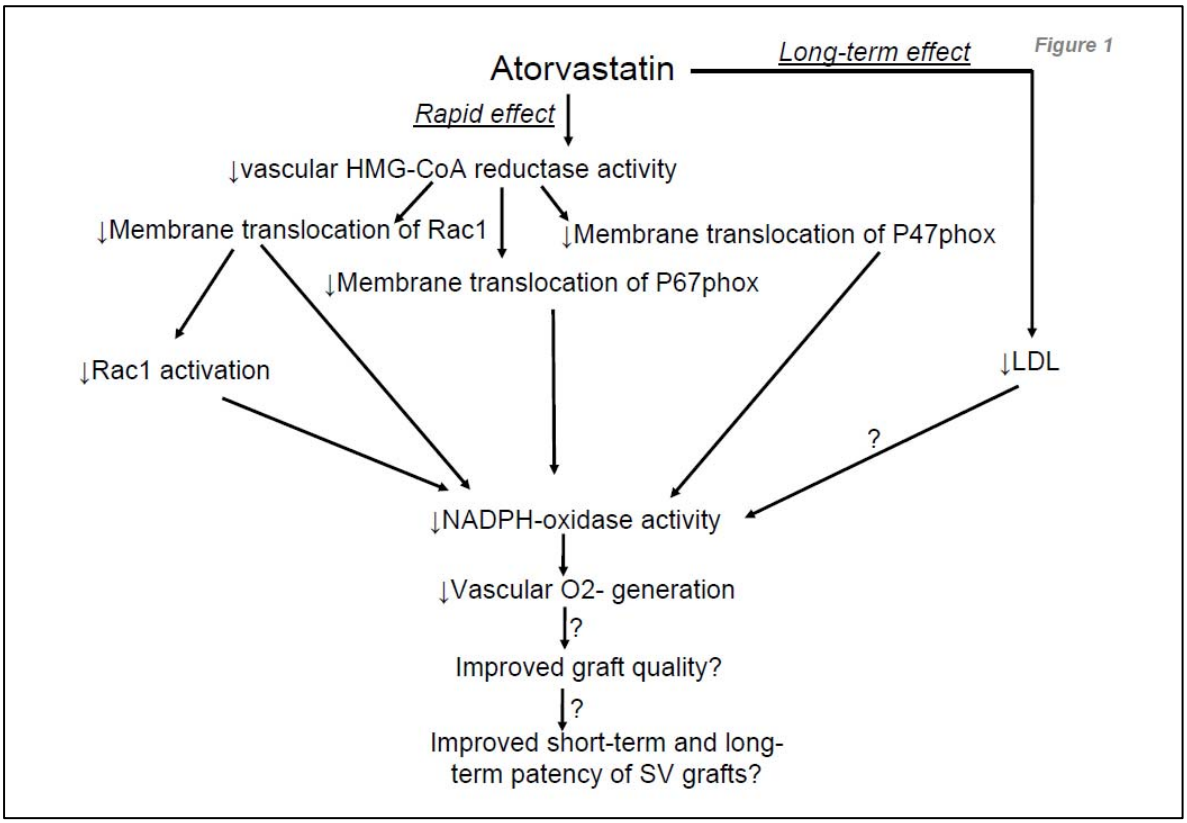

Figure 1: Atorvastatin, by inhibiting HMG-CoA reductase in the vascular wall, leads to a respective reduction in phosphorylation/membrane translocation of NADPH-oxidase subunits Rac1, p67phox and probably P47phox. This results into reduced NAPH-oxidase activity that leads to reduced O2- generation in saphenous vein (SV) grafts. Improved redox state in SV grafts may lead to improved short-term and long-term graft patency in patients undergoing coronary artery bypass grafting operation (CABG). A long term effect of statin treatment on these mechanisms through LDL reduction is also likely, although not documented in the present study.

B) The impact of statin treatment on arterial redox state: Implications for coronary atherosclerosis[2]

The use of statins in coronary atherosclerosis is already included into the clinical guidelines for the management of these patients.[3] However, the only documented mechanism by which statins exert their antiatherogenic effects at a clinical level is through LDL lowering. Although several basic science studies suggested that statins exert also pleiotropic (non LDL-mediated) effects on the vascular wall, this concept has not been well documented in humans.

In this project, we used IMAs obtained from patients undergoing CABG, as a model system to evaluate the effects of statin treatment on key mechanisms involved in atherogenesis, such as arterial superoxide generation (arterial redox state) and endothelial function. Importantly we demonstrate for the first time in humans, that statins exert a direct effect on these arteries, reducing oxidative stress, even after only 3 days of treatment with atorvastatin 40g/d. This pleiotropic effect of statins was also documented for the first time in humans by using the model of IMA, since atorvastatin rapidly improved redox state in IMA segments even in the absence of LDL, in an LDL-free environment ex vivo. In addition, we demonstrate that atorvastatin rapidly improved endothelial function (flow mediated dilation of the brachial artery) independently of LDL lowering. The mechanisms by which statins affect arterial redox state, as demonstrated in the present study, are demonstrated in **Figure 2**.

Figure 2

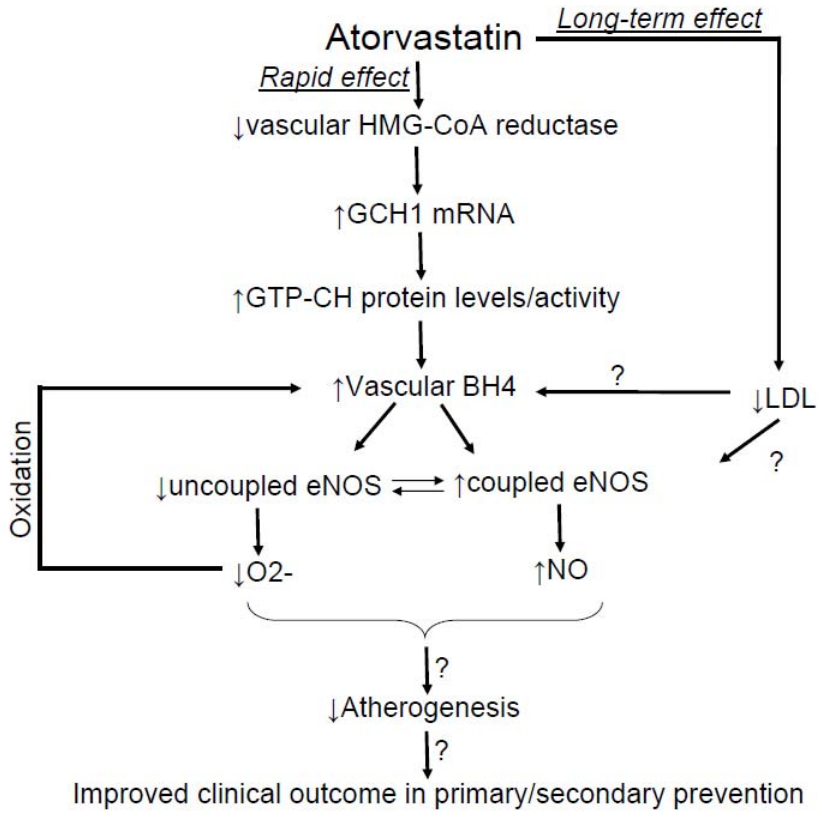

Figure 2: Atorvastatin, by inhibiting HMG-CoA reductase in the vascular wall, it leads to a respective reduction in mRNA levels of GCH1 gene that encodes GTP-cyclohydrolase 1 (GTP-CH). This is the rate limiting enzyme for the biosynthesis of tetrahydrobiopterin (BH4), a critical co-actor for the maintenance of endothelial nitric oxide synthase (eNOS) enzymatic coupling. As a result, the improvement of BH4 bioavailability improved eNOS coupling, resulting into improved NO synthesis and reduced O2- generation. This new balance partly explains the antiatherogenic effects of statins and the beneficial effect of statin treatment on clinical outcome in primary and secondary prevention. A long term effect of statin treatment on these mechanisms through LDL reduction is also likely, although not documented in the present study.

Our data suggests that statins rapidly modify key mechanisms involved in atherogenesis, and that these effects are independent of LDL lowering or changes in systemic inflammation. These mechanisms involve both endothelial function and arterial redox state, and we provide robust mechanistic data documenting the exact enzymatic systems involved in these responses.
Overall, this project confirms the importance of small mechanistic/translational studies to provide a robust mechanistic basis for the clinical observations of large cohorts, supporting the regular use of statin for the prevention and treatment of coronary atherosclerosis, that has been introduced into the clinical guidelines on the basis of the improvement of clinical outcome by statin treatment in these patients.

C) The impact of statin treatment on myocardial redox state: Implications for cardiac surgery[4]
In this study we demonstrate for the first time in humans, that measurement of myocardial redox state in right atrium appendages samples obtained from patients undergoing cardiac surgery, has a strong predictive value for the post-operative outcome of these patients. We demonstrate that myocardial redox state is strongly associated with the development of

post-operative complications such as atrial fibrillation or acute heart failure, and it predicts the length of hospital stay post cardiac surgery.

Therefore, by detecting the individual sources of reactive oxygen species that determine post-operative outcome, we identify potential therapeutic targets to improve clinical outcome post cardiac surgery. The key candidate therapeutic targets are myocardial O2- and ONOO-, and mainly myocardial NADPH-oxidase.

After identifying the main therapeutic target for myocardial redox regulation post-operatively, we then demonstrate that atorvastatin treatment for 3 days (40mg/d) results into a rapid, direct reduction of NADPH-oxidase activity in the human myocardium. This effect is independent of LDL lowering or the possible systemic antiinflammatory effects of statin treatment. We also provide further mechanistic insights into the exact molecular mechanisms by which statins modify NADPH-oxidase activity, which is observed even in an LDL-free environment ex vivo. These mechanisms are presented in **Figure 3**.

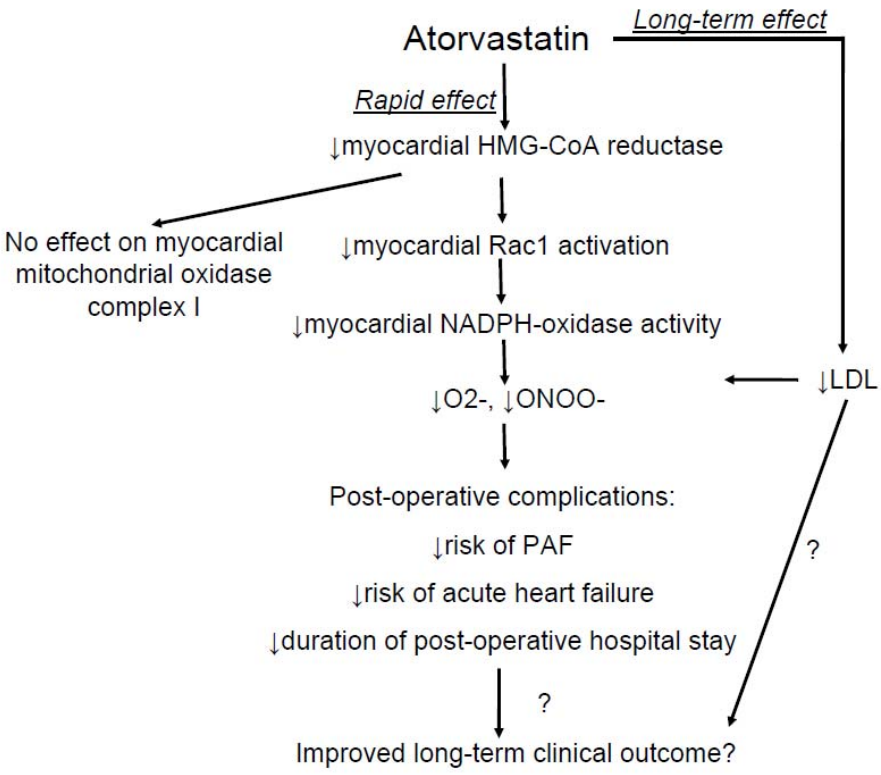

Figure 3: Atorvastatin, by rapidly inhibiting HMG-CoA reductase in the human myocardium, leads to a respective reduction of Rac1 activation, that results into reduced NADPH-oxidase activity. This results into reduced production of O2- and ONOO- radicals, both molecules that induce paroxysmal atrial fibrillation (PAF), acute heart failure and prolong hospital stay post cardiac surgery, as we have demonstrated in this study. A long term effect of statin treatment on these mechanisms through LDL reduction is also likely, although not documented in the present study.

In conclusion, we now clearly show that statins exert their beneficial effects on post-operative clinical outcome of patients undergoing CABG, by directly inhibiting NADPH-oxidase activity in the human myocardium. This me

Overall scientific conclusions and clinical implications

In this study we show that even 3 days treatment with atorvastatin, results into a rapid reduction of oxidative stress in both venous and arterial grafts used in CABG. This effect is due to direct changes of the biology of these grafts, and independent of LDL lowering. These findings are clinically important, since they suggest that the pleiotropic effects of statins on the vascular wall (well demonstrated by basic science studies in the past) are also present at a clinical level. They provide the mechanistic basis that explains the findings of large clinical trials showing that statins improve clinical outcome post-CABG, and propose potential mechanisms for their effects. It is likely that the improvement of redox state in SV and IMA grafts used in CABG may result into better patency of these grafts, explaining the overall better clinical outcome of patients receiving statins, post CABG.

Importantly, our study demonstrates that the use of statins modifies grafts' redox state independently of LDL lowering, therefore it implies that LDL should not be the only biomarker to monitor the responsiveness to statin treatment. Importantly, the beneficial effect of statins on grafts' redox state does not seem to be mediated through the well known antiinflammatory effects of statins, since classic markers of inflammation (CRP, cytokines, soluble adhesion molecules etc) had not been changed by short-term statin treatment in the present study.

In conclusion, the results of the current project suggests that statin treatment, even for just 3 days results into striking improvement of redox state in venous and arterial grafts used in CABG, and this may lead to better patency of these grafts, improving the clinical outcome post CABG.

Socio-economic impact and wider societal implications of the project so far

The findings of the project may have direct socioeconomic implications, since statin treatment before cardiac surgery may reduce post-operative complications, and may result into shorter hospital stay and lower overall hospitalisation cost for this type of surgery, having also a positive impact on patient's psychology.

By identifying new therapeutic targets in the vascular wall and the myocardium, the project may trigger new research for the development of new drugs able to modify these enzymatic systems, further to statin treatment. This may have a direct impact on both primary and secondary prevention, with benefits to the social finance and the patients' healthcare in general. However, this is a small mechanistic, pilot study and the clinical value of our results should be confirmed by large clinical trials before any clinical application.

Dissemination activities and exploitation of results

During the fellowship period, the results of the project were presented in multiple scientific meeting such as the scientific sessions of European Sociey of Cardiology, the American Heart Association or the American college of Cardiology.

An abstract derived from this project received the First Prize in the Best Poster award competition of the American College of Cardiology 2011 in New Orleans, and that received a lot of publicity since it was presented in the highlights of the congress.

Another abstract from the current project has been selected as finalist for the Young Investigators Award Competition of the European Society of Cardiology 2011 (to be presented next August in Paris). The Commission will be informed about the outcome of the competition next September.

The current project leaded to 7 full length publications in high impact journals such as Circulation (Impact factor ISI 2009: 14.8), J Am Coll Cardiol (Impact factor ISI 2009: 12.5), European Heart J (Impact factor ISI 2009: 9.8) and others.

During the fellowship, we continued the collaboration with the University of Oxford UK. This allowed us to combine data from all institutions, in order to improve the quality of the scientific manuscript, that has reached the highest level in the field of cardiovascular medicine.

References

1. Antoniades C, Bakogiannis C, Tousoulis D, Reilly S, Zhang MH, Paschalis A, Antonopoulos A, Demosthenous M, Miliou A, Psarros C, Marinou K, Sfyras N, Economopoulos G, Casadei B, Channon KM, Stefanadis C. Preoperative Atorvastatin Treatment in CABG Patients Rapidly Improves Vein Graft Redox State by Inhibition of Rac1 and NADPH-Oxidase Activity Preoperative Atorvastatin Treatment in CABG Patients Rapidly Improves Vein. *Circulation.* 2010;122:S66-73.

2. Antoniades C, Bakogiannis C, Leeson P, Guzik T, Zhang MH, Tousoulis D, Antonopoulos A, Demosthenous M, Marinou K, Hale A, Paschalis A, Psarros C, Triantafyllou C, Bendall J, Casadei B, Stefanadis C, Channon KM. Rapid, Direct Effects of Statin Treatment on Arterial Redox State and Nitric Oxide Bioavailability in Human Atherosclerosis via Tetrahydrobiopterin-Mediated eNOS Coupling. *Circulation.* 2011;(in Press).

3. Fraker TD, Jr., Fihn SD, Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS, Ferguson TB, Jr., Fihn SD, Fraker TD, Jr., Gardin JM, O'Rourke RA, Williams SV, Smith SC, Jr., Jacobs AK, Adams CD, Anderson JL, Buller CE, Creager MA, Ettinger SM, Halperin JL, Hunt SA, Krumholz HM, Kushner FG, Lytle BW, Nishimura R, Page RL, Riegel B, Tarkington LG, Yancy CW. 2007 chronic angina focused update of the ACC/AHA 2002 Guidelines for the management of patients with chronic stable angina: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines Writing Group to develop the focused update of the 2002 Guidelines for the management of patients with chronic stable angina. *Circulation.* 2007;116:2762-2772.

4. Demosthenous M, Antoniades C, Tousoulis D, Margaritis M, Antonopoulos A, Paschalis A, Ekonomopoulos G, Reilly S, Cassadei B, Stefanadis C. Abstract 17030: Atorvastatin Treatment for Three Days Before CABG Improves Myocardial Redox State, by Modifying NADPH-Oxidase Activity and NOS Coupling. *Circulation.*122:A17030-.