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To:
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The European Heart House
Councils Relations
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Topic: Report on the Outcome of the ESC First Contact Initiative Grant

Frankfurt, 14.11.2011

Dear Sir/Madam

I would like to thank very much the Council on Basic Cardiovascular Science of the European Society of Cardiology for providing me with the First Contact Initiative grant which has enabled me to pay a visit at Prof. Klaus Ley Lab (La Jolla Institute for Allergy and Immunology, Division of Inflammation Biology, CA, USA) and start up a fruitful cooperation.

Regarding my scientific background:
I graduated from the Democritus University of Thrace Medical School, Greece, in July 2005. Since September 2005 I have been working on the field of vascular biology first at the University of Tübingen and later at the Goethe University Frankfurt, Germany. My medical dissertation revealed the role of platelet-derived stromal cell-derived factor-1 (SDF-1) in progenitor cell recruitment on vascular wall, which was awarded the top score "summa cum laude" by the Medical Faculty of the University of Tübingen, Germany *(Stellos K, Circulation 2008)*. Since 2007 I have been leading a junior research team on "Vascular and Regenerative Cardiology" parallel to my full-time clinical duties at the Department of Internal Medicine III of the University of Tübingen, supervising medical and veterinarian doctorate students. My research work was mainly focused on the mechanisms of platelet and endothelial interaction with human $CD34^+$ progenitor cells *(Stellos K, Arterioscler Thromb Vasc Biol, 2010, Stellos K, Semin Thromb Haemost, 2010)*, as well as in the role of platelet activation in patients with coronary artery disease *(Stellos K, Eur Heart J, 2009; Stellos K, J Thromb Haemost 2010)*, atrial fibrillation *(Stellos K, J Thromb Haemost 2011)* or Alzheimer's disease *(Stellos K, J Cereb Blood Flow Metab, 2010)*. Since first of July 2011, I have joined the Goethe University Frankfurt establishing an independent junior research team on "Vascular Homeostasis" at the Institute of Cardiovascular Regeneration (Director: Prof. Stefanie Dimmeler) parallel to my clinical duties at the Coronary Care Unit of the Cardiology Department of Prof. Andreas Zeiher. My current research activities are mainly funded by the independent young investigator program 2011 of the Goethe University Frankfurt.

Regarding the host lab:
Prof Klaus Ley has been one of the early discoverers of the leukocyte adhesion cascade

providing new evidence in leukocyte interplay with vascular wall in development of arteriosclerosis. Through his pioneering work Prof Klaus Ley established and further developed high-tech lab methods including stroboscopic epifluorescence intravital microscopy of transgenic and knockout mice, total internal reflection fluorescence microscopy (TIRF), 2-photon and calcium imaging microscopy and dynamic footprinting, literally visualising the microcirculation in mice and the adhesion and transmigration process of blood cells and accumulation at sites of tissue injury. More Info about his lab is available at www.liai.org/pages/ley-home

Regarding the project:
First of all, we discussed with Prof Klaus Ley the role of platelet factor 4 in atherosclerosis: Platelet factor 4 (PF4, also known as CXCL4) is a chemokine-like molecule exclusively expressed in megakaryocytes and platelets. PF4 is stored in platelet granules and secreted upon platelet activation. Platelets are known to be activated in patients and mice with atherosclerosis *(Huo Y, Nat med, 2003; Massberg S, J Exp Med, 2002; Stellos K, Eur Heart J, 2009; Stellos K, J Thromb haemost, 2010)*. They form heteroaggregates with circulating monocytes and promote monocyte recruitment to atherosclerotic lesions *(Huo Y, Nat med, 2003;Furman MI, JACC, 1998)*. PF4 can form heterodimers with CCL5 (RANTES) and promote monocyte arrest from rolling *(Koenen RR, Nat Med, 2009)*. Platelet antigens like CD41 and PF4 are found in atherosclerotic lesions, suggesting that platelet material persists in lesions. Therefore, it is reasonable to expect that PF4 may have effects on macrophages and dendritic cells (DCs) in atherosclerotic lesions.

Klaus Ley team has recently found that PF4 induces a unique phenotype in primary human monocyte-derived macrophages *(Gleissner CA, J Immunol, 2010)*. In the differentiation process, PF4 completely suppresses expression of CD163 at the mRNA and protein levels *(Gleissner CA, Circ Res, 2010)*. CD163 is the receptor for hemoglobin (Hb)-haptoglobin (Hp) complexes *(Kristianden M, Nature, 2001)*. Normally, perivascular macrophages express CD163 *(Kim W, Am J Pathol, 2006)*, which enables them to take up Hb-Hp complexes formed after minor bleeding and hemolysis of extravasated erythrocytes, which could derive from bleeding out of plaque neovessels. Upon uptake in lysosomes, Hb dissociates from Hp and is processed to biliverdin, bilirubin and carbon monoxide (CO) by heme oxygenase. These products have anti-inflammatory effects. Consistent with this, mice lacking *Hmox1*, the gene encoding heme oxygenase, show accelerated and exacerbated atherosclerosis *(YET SF, Faseb J, 2003)*. CD163 engagement also leads to production and secretion of the anti-inflammatory cytokine IL-10 *(*Graversen, JH, *Int J Biochem Cell Biol, 2002)*. Glycated hemoglobin Hb1Ac, which is elevated in diabetes, remains an oxidant even when bound to Hp *(Asleh R, Circ Res, 20003)*, and its uptake by CD163 may be impaired *(Sachais BS, Thromb Haemost, 2003).*

Atherosclerotic arteries contain macrophages and dendritic cells. Several subsets have been described, including $CD11b^+F4/80^+$ macrophages, $CD11b^+CD11c^+F4/80^+$ macrophages and $CD11b^-CD11c^+F4/80^-$ dendritic cells. Among the $CD11c^+$ cells, our preliminary data define at least three phenotypes: $CD103^+$, $CD8\alpha^+$ and $CD8\alpha^-$. CD163 expression has not been studied in atherosclerosis. Based on preliminary findings of Klaus Ley Lab, PF4 downregulates CD163 expression, which disables the generation of anti-inflammatory CO, bilirubin and IL-10. Consistent with the expected pro-inflammatory effect of PF4, $Pf4^{-/-}Apoe^{-/-}$ mice have greatly reduced atherosclerotic lesion sizes in the aortic root and along the aorta.

Moreover, we discussed with Prof Klaus Ley the role of other macrophage modulators and we concluded that the clinical significance of the observed *in vitro* and *in vivo* novel findings shall also be investigated in patients cohorts with documented atherosclerosis. This could be a field of cooperation since as a clinical fellow at the Department of Cardiology of the Goethe University Frankfurt I treat everyday patients with cardiovascular diseases. We therefore agreed to start up a joint clinical study evaluating the diagnostic and prognostic value of macrophage differentiation modulators in patients with heart failure and coronary artery disease.

Other scientific activities in La Jolla/San Diego:
During my short staying at Ley Lab I had the opportunity to further discuss the ongoing projects and the methods applied with Prof. Klaus Ley's experienced postdocs and to learn the basics of intravital microscopy in mice. Morover, I was invited by Prof. Joe Witztum and Prof. Yury Miller (Department of Endocrinology and Metabolism, UCSD) to give a lecture on the UCSD Atherosclerosis and Vascular Biology Series about the role of platelets and platelet interaction with progenitor cells in atherosclerosis. Furthermore, during my staying in San Diego I had the opportunity to meet and extensively discuss current research projects as well as collaboration opportunities with Prof. Zaverio M. Ruggeri (Scripps Institute), Prof Antony N. DeMaria (Editor of the Journal of American College of Cardiology, Director of the Cardiovascular Center San Diego), Prof. Sotirios Tsimikas (UCSD), Prof. Neil C. Chi (UCSD), Prof Joel Linden and Prof Cathrine Hedrick (La Jolla Institute), and Prof Eric Topol (Director of the Scripps Transnational Science Institute and Genomic Medicine Program). I would like at this point to thank all of them for their time devoted to me and the vivid discussions we had.

Acknowledgements:
Last but not least, I would like at this point to extend my gratitude to Prof Klaus Ley for his scientific advice and mentorship, to his great team for the many hours of scientific discussions and presentation of methods applied and to his research administrative assistant, Daisy Varbanova, for the great hospitality and all the help provided to me to get around in the lab, in the research institute, at the University of California San Diego and the city.

Yours faithfully

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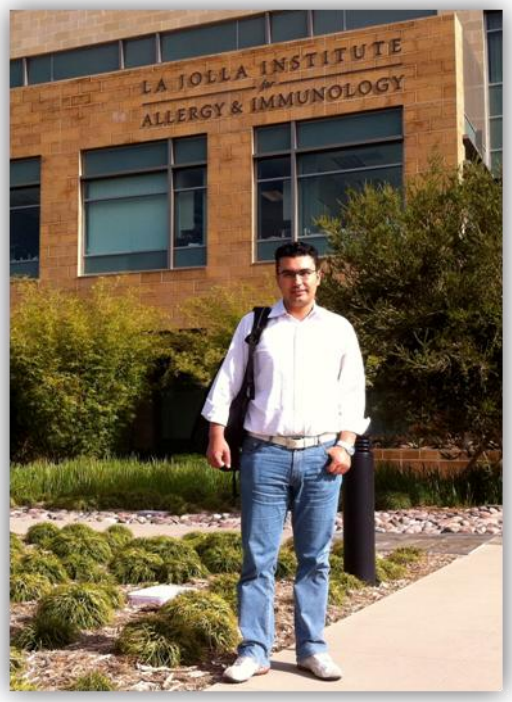

Prof. Klaus Ley and Dr. Konstantinos Stellos