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Report on the outcome of the ESC First Contact Initiative Grant

Dear Sir or Madam,

first, I would like to extent my gratitude towards the European Society of Cardiology for providing me with the First Contact Initiative Grant which has enabled me to pay a visit at Prof Kevin Croce's Lab (Center of Excellence in Cardiovascular Biology, Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA) and to consolidate our cooperation.

Main research interest and scientific background
I entered the area of cardiovascular science when I started working on my MD thesis project in the laboratory of Prof Daniel Sedding and proceeded working with him following graduation as a cardiovascular physician-scientist. The group of Prof Sedding predominantly focuses on the molecular mechanisms of vascular remodeling and regeneration and the functional interplay of different cell types within these pathophysiological processes. My main research interests comprise the pathophysiology of neointimal lesion formation following vascular injury as well as the molecular course of atherosclerosis. Specifically, I am excited about the molecular and functional control mechanisms of smooth muscle cell (SMC) function, growth, and phenotypic modulation. Most recently, I started to investigate the impact of the adventitial layer on the development of a smooth muscle cell-prone neointimal lesion due to the observation of high adventitial proliferation rates following vascular injury *in vivo*. Therefore, we removed the whole adventitial layer following femoral artery dilation in *C57BL/6J* mice and found a significant reduction in neointima formation.

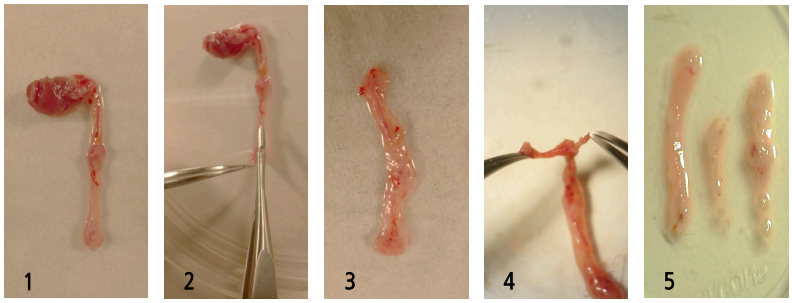
Aim of the visit
The aim of my visit was to establish appropriate *in vitro* and *in vivo* techniques to investigate the functional interplay of different vascular tissue layers and certain cell types in the course of neointimal lesion formation. In detail, I intended to investigate the effect of circulating inflammatory cells on adventitial cell proliferation as well as the specific impact of the adventitial layer on neointimal lesion formation.

Host lab

Prof Croce and his co-workers at the Cardiovascular Division of the Brigham and Women's Hospital are highly regarded for their expertise in a wide range of *in vitro* and *in vivo* models to investigate the pathophysiology of vasculo-proliferative disease with special reference to the inflammatory interplay of certain cell types in vascular remodeling processes.

Funded Project: Activities at the host lab

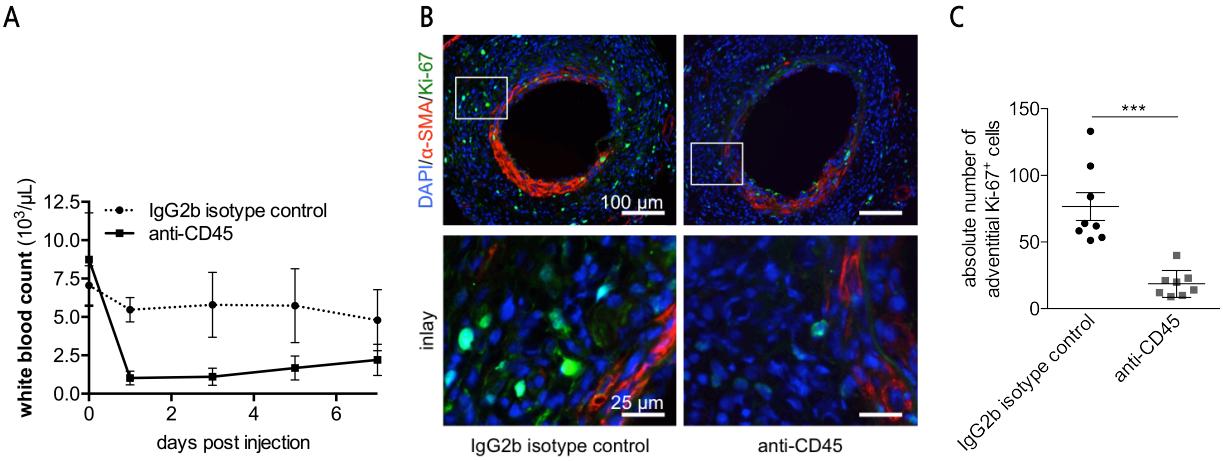
The ESC First Contact Initiative Grant enabled me to establish a model of specific leukocyte depletion in close cooperation with Prof Croce's group. In contrast to "classical" animal models of leukocyte depletion, i.e. whole body radiation, injection of an anti-CD45 antibody does not affect the viability and proliferation of non-leukocytes, which empowers us to investigate the impact of leukocytes on the proliferative response of other cells.
Furthermore, I got the opportunity to learn the detailed preparation of the murine aorta, especially the demanding detachment of the certain vascular layers (figure below). This set the stage for the development of a new model of adventitia transplantation.



1. Harvesting of the full aorta of donor mice.
2. Rinsing the sample in PBS. Longitudinal incision.
3. Opened aorta (luminal side en face).
4. Careful removal/pulling apart of the perivascular layer (left forceps: intimal and medial layer, right forceps: perivascular layer) and preparation of the adventitia.
5. Left: intimal and medial layer. Middle: adventitia. Right: perivascular fat.

Funded Project: Results

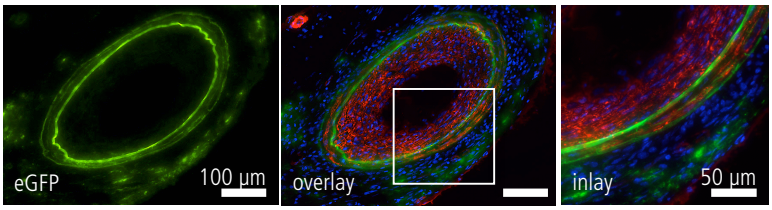
Leukocyte depletion by anti-CD45 injection (A) and subsequent wire-induced injury of the femoral artery revealed significantly impaired adventitial cell proliferation at 7 days following injury compared to injection of an IgG2b isotype control antibody and subsequent vessel dilation (***P<0.001, n=8, B and C).



As mentioned above, we found neointimal lesion formation in response to vascular injury markedly attenuated after complete removal of the adventitial layer in *C57BL/6J* mice. To determine the amount of adventitial cellular contribution to the neointimal lesion, we carefully dissected the abdominal aortic adventitial layer of ubiquitously enhanced green-fluorescent protein (eGFP) expressing *C57BL/6-Tg(CAG-EGFP)1Osb/J* mice (as learned in Prof Croce's lab). We then

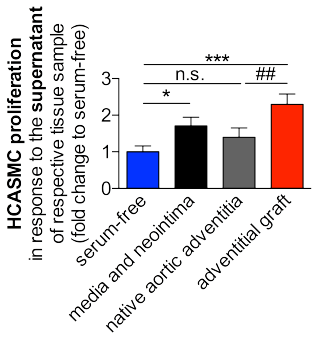
dilated the murine femoral artery, coated the medial vascular layer with the prepared adventitial sample and found a barely cellular contribution of eGFP^{+} cells to the neointimal lesion at 21 days following injury (D).

D



Genotype-identic transplantation of aortic adventitial samples of *C57BL/6J* mice, removal of the samples 14 days following wire-induced injury (peak of neointimal cell proliferation), and incubation in serum-free media enabled us to investigate paracrine effects of the adventitial layer. After a 24-hour-period of incubation, we used the supernatant to treat human coronary smooth muscle cells (HCASMC), determined their proliferative and migratory capacity, and found significantly higher proliferation and migration rates in cells treated with the supernatant of adventitial grafts compared to cells treated with serum-free media, the supernatant of the neointimal and medial layer, or the supernatant of a native (not-transplanted) aortic adventitia (*P<0.05, **P<0.01, ***P<0.001 compared to serum-free, $^{\#}$P<0.05, $^{\#\#}$P<0.01 compared to native aortic adventitia, n=6, E and F).

E



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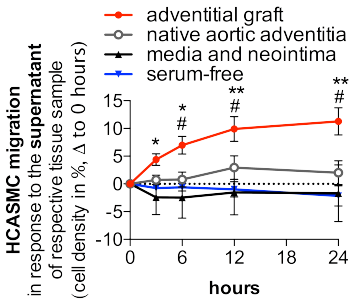

In close cooperation with Prof Croce's group, I was able to complete the experiments back in Hannover, Germany, to replenish these functional advances with transgenic and mechanistic/molecular approaches and submit the results for publication. In summary, the ESC First Contact Initiative Grant provided indispensible support to me to conduct the above mentioned experiments and thus to prove the substantial role of the adventitial layer in vascular remodeling processes.

Acknowledgment
Last but not least, I would like to thank very much Prof Kevin Croce for his scientific advice and mentorship and to his great team, especially Yevgenia Tesmenitsky, for the many hours of scientific discussions and presentation of methods applied. I would further like to extent my gratitude towards Prof Daniel Sedding for his willingness to support the application for this grant, to support the stay at the host laboratory, and – most important – to support the continuation of the collaboration with Kevin's group in his lab.

Yours faithfully

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