To the ESC Council Members
European Society of Cardiology
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Milan, May 30, 2013

Dear Council members,

First, I would like to thank you for awarding me the ESC First Contact Initiative Grant in autumn 2012. This grant gave me the opportunity to visit the group of Prof. Massimiliano Mazzone at the Vesalius Research Center (VRC) of Leuven (Belgium) in April 2013.

The VRC is a renowned and leader institution mainly devoted to the study of vascular biology, which is the topic of my current studies. My willing to visit the laboratory of Prof. Mazzone has been driven by the excellence of research quality of this group. Prof. Mazzone, with a pioneering work published in *Cell*, has been the first to identify and characterize the reprogrammed "phalanx" endothelial cell (EC) phenotype in the context of $PHD2^{+/-}$ genetic background (Mazzone et al., Cell, 2010). As an independent researcher, he is already considered an expert in the field of vascular biology and indeed his findings have led to outstanding publications.

I benefited from this experience in many different ways. First, I had the chance to discuss the data of my current research with all the members of the group. This helped me to define my future experiments in a more fruitful and focused way. Second, I had the possibility to attend the internal lab meetings where the ongoing research projects were discussed. This experience broadened my knowledge in vascular biology and provided me with a more comprehensive view of the main research lines of the group, of their future goals and of the internal organization of the laboratory life. Furthermore, from the lab meetings I got a broad spectrum of technical insights that, together, with the methodologies that I learned hands-on during my stay, will be very helpful in my future studies. Finally, I improved my technical skills in mouse surgery and learnt different protocols for the analysis of the vasculature, as I will discuss in the following paragraph.

The goal of my project is to understand the role of an immunoglobulin-like glycoprotein called L1 in vascular biology. L1 has been extensively characterized in the nervous system, where it mediates intercellular recognition, neuronal migration, axon pathfinding and fasciculation. However, L1 expression has also been reported in certain non-neural tissues and in various cancer types where its function remains unclear. In particular, our group recently contributed to this field by reporting the

dual, cell-context-dependent role of L1 in ovarian carcinoma (Zecchini et al., Cancer Res., 2008). More importantly, we observed the expression of L1 in certain lineages of inflammatory cells and in the vessels associated to various human tumor types, while no expression has been detected in the normal vasculature (Maddaluno et al., J Exp Med., 2009). Tumor vascularization is stimulated by angiogenic factors secreted by tumor cells themselves and/or by recruited inflammatory cells. Along this line, our laboratory showed that the inflammatory cytokine TNF-α stimulates L1 expression in ECs both *in vitro* and *in vivo*. This raised the question of whether L1 plays a functional role in vascular biology and, in particular, in tumor angiogenesis. We decided to address these questions in pancreatic ductal adenocarcinoma (PDA), a very aggressive tumor type in which neovascularization plays a key pathogenic role.

The laboratory of Prof. Mazzone has recently reported that the orthotopic transplantation of the mouse cell line Panco02 into the head of the pancreas results in the formation of highly vascularized PDA (Mazzone et al., Cell, 2010). Hence, this animal model appeared particularly suitable to investigate the molecular mechanisms regulating PDA-associated neovascularization. Thus, during my visit, I learnt and practiced this mouse model of cancer.

Such a model requires both good technical skills and long practice, due to many aspects: first, the mouse is open via abdominal midline incision and the stomach exteriorized in order to expose the head of the pancreas for the injection, the capability to pinch the stomach without damaging all the other organs of the abdominal cavity requires long practice; second, the injection has to be performed in the head of the pancreas, which represents a small part of the entire organ; third, the pancreas is really soft, meaning that it can be easily damaged by an improper use of forceps and by the injection itself; fourth, the injection into a so soft organ is really difficult and requires long practice due to the high probability to hole the organ itself and, thus, to let the tumor cells spreading into the whole abdominal cavity. The result of a successful injection is shown in Figure 1.

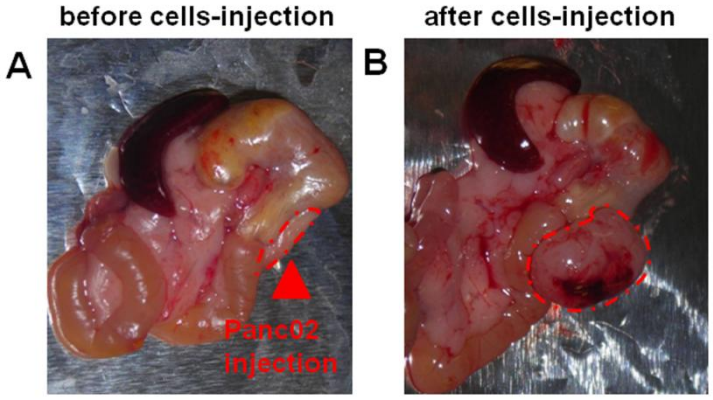

Figure 1. Images of pancreas before (A) and after (B) 14 days from Panc02 injection into the head of the organ (circled by a dotted line).

In the past, I tried to perform this surgical procedure in my lab, unfortunately with poor results; this experience made me learn a series of fundamental tricks to success in this procedure. Besides the improvement in mouse surgery, I became familiar with other protocols and technologies, among them embedding the pancreas and performing proper staining to assess the tumor vasculature.
I intend to explore the role of L1 in tumor angiogenesis by inducing Panc02 tumors in mice with the endothelial-specific ablation of L1. To this goal, I will capitalize on Tie2-Cre/L1^{floxed} mice, already available in our laboratory. Tie2-Cre transgenic mice are widely used to target gene expression in the endothelium (Kisanuki et al., Dev Biol, 2001), while L1^{floxed} mice carry two floxed alleles of the *L1cam* gene (Law et al., J. Neurosci., 2003). Tie2-Cre/L1^{floxed} mice are viable and fertile and the specific ablation of L1 in the endothelium has been already verified in my lab by the observation that the protein is induced by TNF-α in the vessels of control L1^{floxed}, but not Tie2-Cre/L1^{floxed} mice (Maddaluno et al., J Exp Med., 2009).

Thus, the ESC First Contact Initiative Grant gave me the opportunity of a useful experience in Prof. Mazzone's laboratory. During this visit, I realized that moving to this lab could be a good possibility for the development of my career. Besides his excellent reputation as a scientist, Prof. Mazzone is surely dedicating a big effort to training junior investigators, providing them with scientific and technical knowledge which are fundamental to develop an independent career. I was impressed by his sincere enthusiasm for science, his energy and open mind.
As a result of my ESC First Contact Initiative Grant, I have decided to apply for a fellowship to join this group upon completion of my current project in Milano.
Thus, I would like to thank you once again for providing me with the support for such a formative experience which may prove fundamental for my scientific career.

Best regards,
Elena Magrini