

## Endothelial Progenitor Cell (EPC) capture to aid vascular repair following coronary stenting: a new frontier in stent technology?

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### Introduction

Drug eluting stents (DES), that locally release antiproliferative, immunosuppressive or anti-inflammatory drugs to retard intimal hyperplasia and restenosis, have significantly improved the outcome of percutaneous coronary interventions. In-stent restenosis was reduced by approximately 70% by the introduction of DES, both in paclitaxel or sirolimus eluting stents<sup>1-3</sup>. However, the local toxicity of the drug on the vessel wall and endothelium have also been shown to concomitantly impair endothelial regeneration and induce local hypersensitivity reactions leading to long-term endothelial dysfunction<sup>4-10</sup>. As endothelial stent coverage was found to be the most powerful histological predictor of late stent thrombosis (LST), delayed re-endothelialisation remains a big concern<sup>5</sup>. Even though the incidence of LST is low, the sheer volume of coronary interventions nowadays leads to significant numbers with high morbidity and mortality. LST is associated with non-fatal MI and cardiac death in as many as 75% and 45% of the cases respectively<sup>11-13</sup>. An accelerated re-endothelialisation response of the bare stent struts and facilitated arterial repair therefore is desirable. This may not only prevent stent thrombosis by inhibition of platelet adhesion, but it also might impede smooth muscle cell proliferation and migration<sup>14</sup>. This eventually might result in reduced neo-intimal hyperplasia with preserved arterial integrity and endothelial vasomotor function.

### Endothelial progenitor cells

The arterial repair response after stent implantation is a multi-step process that involves de-differentiation, migration and proliferation of neighbouring endothelial cells<sup>14</sup>. Equally important is the incorporation of systemically circulating endothelial progenitor cells (EPCs) originating from the bone marrow. These cells are estimated to contribute to re-endothelialisation of the neo-intima for up to 25%<sup>15,16</sup>. The beneficial role of these endothelial progenitor cells in neoangiogenesis and arterial repair was initially suggested in 1997 by Asahara et al<sup>17</sup>. EPCs are immature cells that are capable of migrating, proliferating and differentiating into endothelial cells under influence of angiogenic growth factors, including vascular endothelial growth factor, cell-cell interactions and interactions with the extra cellular matrix. Several studies have suggested, that these EPCs play an essential role in postnatal vasculogenesis as well as in the vascular repair response<sup>4,15,18,19</sup>.

Ever since their discovery, there has been controversy about the actual flow cytometric phenotype of EPCs. Whereas the classical phenotype comprises CD45+/ CD34+/ VEGFR2+(KDR) cells, several alternate populations have been identified as potential endothelial cell (EC) progenitors<sup>20</sup>. Moreover, EPCs can be characterised by functional analysis in cell culture (migratory and colony forming potential, expression of EC specific proteins, uptake of acLDL), rather than by flow cytometry<sup>20,21</sup>. Recently, two functionally distinct

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subpopulations of EPCs were described: “early outgrowth EPCs” and “late outgrowth EPCs”. Early outgrowth EPCs proliferate slowly, express monocytic (CD14), leukocyte (CD45) and EC (VEGFR2, CD31) surface markers and die after a few weeks in cell culture<sup>22,23</sup>. These cells stimulate angiogenesis in a paracrine fashion by secreting angiogenic, anti-apoptotic and chemotactic factors and cytokines. Late outgrowth EPCs arise from adherent colonies amongst early outgrowth EPCs and have been shown to proliferate more rapidly and they can be expanded almost innumerably. These late outgrowth EPCs do not express CD14 and CD45 and are thought to promote neoangiogenesis by transdifferentiation into functional ECs of newly formed structural blood vessels<sup>22-25</sup>. Considering the expansion potential, these late outgrowth cells are speculated to be in fact stem cells. A recent paper by Sieveking et al confirms this hypothesis<sup>26</sup>. Despite the clear *in vitro* difference in phenotype and function of both early and late outgrowth EPCs, the *in vivo* (clinical) relevance remains uncertain. Since most of the clinical research and trials to date have been focussed on early outgrowth EPCs, the majority of the data that we will discuss, refer to these specific early outgrowth EPCs.

### Clinical implication of endothelial progenitor cells

The titer of EPC is known to increase upon ischaemic insults and induction of vascular damage, including myocardial infarction and stent implantation, indicative of ongoing arterial repair and compensatory vasculogenesis<sup>27-30</sup>. EPC number, function and migratory capacities however were found to be decreased in patients with stable coronary artery disease (CAD)<sup>31</sup> and in patients with several cardiovascular risk factors<sup>32</sup>. For instance, EPC function was impaired with age<sup>33</sup> or with smoking<sup>34</sup>, as well as in patients with type II diabetes mellitus<sup>35</sup>, dyslipidaemia<sup>36</sup> and hypertension<sup>31</sup>. Conversely, low EPC count or impaired EPC function *in vitro* was shown to be a strong predictor of in-stent restenosis, (progression of) atherosclerotic disease, cardiovascular events and death from cardiovascular causes<sup>37-40</sup>.

Several factors are associated with improved function and recruitment of EPCs from the bone-marrow including VEGF, estrogen, exercise and erythropoietin<sup>41-45</sup>. Also, statin therapy is known to augment EPC mobilisation from bone marrow and improvement of EPC function in both mice and patients<sup>46,47</sup>. It is believed that the increase of bio-availability of eNOS by statins and the activation of the PI3-kinase/Akt-pathway underlies these beneficial and pleiotropic effects<sup>41,48,49</sup>.

### Vascular healing and re-endothelialisation

To accelerate re-endothelialisation after coronary intervention several approaches have been explored to date. Direct seeding of mature endothelial cells on bare metal stents proved to be laborious, whereas the cultured endothelial monolayers covering the stent struts is severely damaged by balloon expansion of the stent. Blood flow along the stent surface after implantation washed away most of the remaining cells. Although this approach is feasible, it has not been pursued in a clinical setting<sup>50-52</sup>.

Alternatively, angiogenic growth factors such as VEGF have been explored to stimulate the endogenous ability to promote regrowth of

the endothelial layer. The mode of delivery of VEGF, that has been described, varied from direct intracoronary infusion of plasmids encoding VEGF prior to PCI<sup>53,54</sup> to recombinant VEGF protein-coated stents<sup>55</sup> and stents coated with VEGF plasmids (gene eluting stent)<sup>56</sup>. Although these techniques proved to be safe in animals and small scale clinical trials, they failed to show a reduction in neointima formation.

### The CD34 antibody-coated bioengineered coronary stent (Genous R stent)

The key role of EPCs in the arterial repair response after stent implantation prompted the concept that recruitment of the patient's own EPCs to the site of vascular injury could aid stent re-endothelialisation and initiate the endogenous arterial repair response. A few years ago, this “pro-healing” concept led to the development of the first bio-engineered stent by the Thoraxcenter Rotterdam in conjunction with OrbusNeich, The Netherlands (Genous R-stent). This stent was specifically designed to promote the arterial healing response by a coating of immobilised murine antibodies raised against human CD34. As a result, the Genous R-stent captures and sequesters circulating CD34-positive progenitor cells to the luminal stent surface and so theoretically initiates re-endothelialisation.

Several pre-clinical studies were conducted to prove safety and feasibility. In pigs, anti-CD34-coated stents exhibited accelerated coverage with an endothelial cell population and, equally important, showed no sign of mural thrombus formation in contrast to bare metal stent control pigs, 48 hours post implantation. Complete coverage of the Genous-stent by a functional endothelial monolayer was achieved after seven to 14 days, whereas DES controls showed eminent delayed vascular healing.

Re-establishment of a functional endothelial cell layer restored the cellular vascular integrity and homeostasis. The recovery of vascular function resulted in prevention of platelet aggregation and in-stent thrombus formation, inhibition of smooth muscle cell migration and proliferation, thereby prevention of neointimal hyperplasia, preserved vasomotor response, and angiogenesis<sup>14</sup>.

### The EPC capturing Genous stent in clinical practice

The encouraging pre-clinical studies in rabbits, pigs and primates led to the HEALING-FIM study in which safety and feasibility of the anti-CD34-coated stent was demonstrated in 16 patients with stable single vessel CAD. In this study the procedural success rate was 100%, whereas the composite major adverse cardiac and cerebrovascular event (MACCE) rate was 6.3% due to a target vessel revascularisation in one patient. Despite only one month of dual anti-platelet therapy, there was no acute stent thrombosis observed in the treated patient group. Mean late luminal loss after six months was 0.63±0.52 mm and stent volume obstruction was 27.2%±20.9%<sup>57</sup>. Where HEALING-FIM demonstrated the safety of the anti-CD34-coated stent, the HEALING II registry was designed to provide additional safety data and initial efficacy data as well by use of QCA and IVUS follow-up. HEALING II was a prospective, non-randomised, multicentred study, enrolling 63 patients with single vessel CAD. The coronary intervention was successful in 98.4% of

the cases. No acute or sub-acute stent thrombosis was found during the 18 month follow-up, despite only one month of dual antiplatelet therapy. An analysis of the six and eighteen month data disclosed a clinically-driven target lesion revascularisation of 17.4% and a target vessel revascularisation of 7.9%. The overall MACCE rate was 7.9%. Angiographic follow up revealed a late luminal loss of  $0.78 \pm 0.39$  mm at six months and a regression of late luminal loss to  $0.59 \pm 0.31$  mm at 18 months<sup>58,59</sup>. IVUS confirmed this neointimal regression, although less pronounced as compared to the QCA data. Interestingly, it was found that EPC titer correlated closely with angiographic outcome at six months, but also with the regression in late luminal loss after 18 months follow-up. This would suggest an ongoing vascular repair and remodelling process even after eighteen months of follow-up.

Since the anti-CD34-coated stent is capturing circulating EPCs that initiate and govern the vascular healing, the number and function of EPCs appear to be of paramount importance to the response to this bio-engineered stent. Indeed, in the HEALING II registry a clear correlation was found between the EPC titer and restenosis rates. All patients with significant restenosis or MACCE/revascularisation events had low EPC titers at six month follow-up, whereas patients with normal EPC titer did not show TLR or MACCE.

Recently, the use of the EPC capturing stent in the treatment of acute myocardial infarction was described by Co et al<sup>60</sup>. In this study 120 patients with acute ST elevation MI were treated with a primary PCI using a Genous R stent. Procedural success was achieved in 95% of the cases. At one year follow-up, MACE rate was 9.2% and TLR/TVR 5.8%. One patient suffered from acute stent thrombosis and one patient from a sub-acute stent thrombosis. No LST was reported, despite only one month of dual anti-platelet therapy.

## Upcoming results and trials

Data analysis of the HEALING II study showed that the majority of patients treated with concomitant statin therapy had normal EPC titers, while patients that were not on statin therapy had low EPC counts<sup>59</sup>. The latter observation led to the design of the HEALING IIb registry, in which all patients receiving the anti-CD34-coated stent also were initiated with statin pharmacotherapy (Atorvastatin 80 mg qd) at least two weeks prior to stent placement. HEALING IIb is a multicentre, prospective, non-randomised trial with angiographic follow-up at six and eighteen months, in which the effectiveness of the anti-CD34-coated stent in combination with optimal statin therapy will be assessed. By the beginning of 2008, inclusion of all 100 patients was finished and final outcome is expected in 2009. This study will render more insight into the correlation between EPC titer, accelerated vascular healing and angiographic outcome after placement of the OrbusNeich Genous R-stent.

Forthcoming studies with the anti-CD34-coated stent comprise the HEALING-AMI as a single centre study in which 60 patients with ST elevation myocardial infarction (AMI) receive a Genous stent in the culprit lesion and Atorvastatin 80 mg qd for six months. Enrolment of all 60 patients has recently been completed and the final study report will be due in September 2008.

The e-HEALING registry recently reached complete enrolment of their 5,000 patients. The e-HEALING registry is a multicentre

(100-120 centres), worldwide, prospective registry of an all-comer patients population treated with the Genous Bio-engineered R stent. The primary objective of this registry is to collect post-marketing surveillance data on patients that have received a Genous R-stent. The study protocol recommends that patients receive standard statin therapy for at least two weeks prior to the intervention and only one month of clopidogrel treatment after the procedure. The primary endpoint of the registry is target vessel failure at 12 months. Only recently, enrolment has started of the HEALING-Vasomotion study. This is a multicentre study of 36 patients, in which the vasomotor response of a stented coronary artery will be assessed six months after the stenting procedure. Patients will be randomised to receive either a Genous R stent or a Xience V stent after at least two weeks of optimal statin therapy. At baseline and six months after stent implantation, IVUS will be performed as well as an acetylcholine provocation test. Vasomotor response after acetylcholine provocation of the stented vessel will render more insight into the difference in vascular healing and endothelial function after placement of the bio-engineered stent or DES.

Finally, the TRIAS-HR and TRIAS-LR studies are multicentre, prospective, randomised trials of approximately 1,200 patients that only recently have been initiated. In the TRIAS-HR study, non-inferiority of the Genous R-stent versus TAXUS Liberté paclitaxel eluting stents in *de novo* lesions with high-risk for restenosis will be investigated. The TRIAS-LR study was designed to show non-inferiority of the Genous R stent versus bare metal stents in *de novo* lesions with a low risk of restenosis. Primary endpoints of both studies are target lesion failure within one year.

## Conclusion

In conclusion, acceleration of the endogenous vascular repair response by EPC capturing is an interesting novel approach to impede in-stent restenosis formation in percutaneous interventions. Safety and proof of principle have been established in several (pre-) clinical studies, although the outcome of larger randomised clinical trials has to be awaited.

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