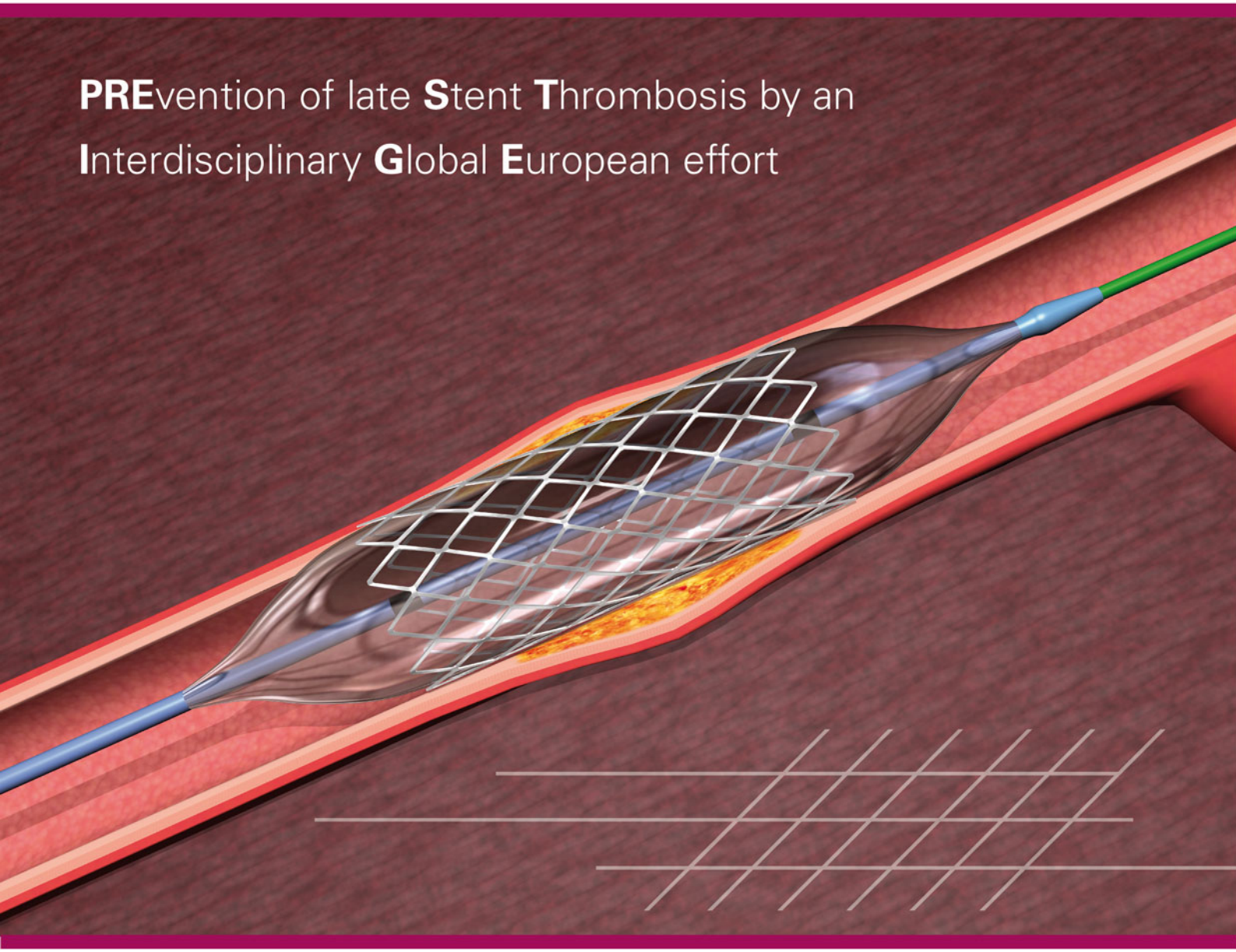
PRESTIGE

PREvention of late **S**tent **T**hrombosis by an **I**nterdisciplinary **G**lobal **E**uropean effort



SEVENTH FRAMEWORK PROGRAMME

PROJECT FINAL REPORT

Grant Agreement number:

260309

Project acronym:

PRESTIGE

Project title:

PREvention of late Stent Thrombosis

by an Interdisciplinary Global European effort

Period covered:

01 December 2010 – 30 November 2014

PRESTIGE: Executive Summary

Stent thrombosis after implantation of stents for coronary artery disease represents a major European health care challenge. Incident cases are associated with high and mortality and the impact of this condition is very significant. Against this background we formed a multi-disciplinary consortium – the PREvention of late Stent Thrombosis by an Interdisciplinary Global European effort (PRESTIGE) consortium – combining in a unique way the expertise of basic researchers (with focus on platelet and endothelial biology, or coagulation), histopathologists, bioimaging engineers and interventional cardiologists. The scientific work of PRESTIGE was divided into 4 work packages. These work packages were supplemented by 2 specific work packages dealing with project management (WP5) and dissemination of findings (WP6).

WP1 established the first successful mouse model of stent thrombosis. Moreover investigators elucidated key mechanisms by which CoCr – the most common material used in stents – activates platelets and thrombosis, elicits inflammatory cell recruitment and undergoes re-endothelialization. WP2 investigators developed novel stent coatings which promote more complete and/or rapid endothelial healing after stent thrombosis. The most successful approaches involved plasma treatment of stent surfaces, attachment of peptidomimetics to attract endothelial cells, chitosan polymer coatings and dextran-based co-polymers to favourably modulate platelet-stent interactions and coagulation pathways. In WP3 the consortium focused on novel imaging approaches to predict stent thrombosis in man. Key outputs included the development of a novel NIRF functional imaging catheter specifically designed for animal studies. Long-term it is expected that this catheter can be used in clinical practice. In addition clinically-important progress was made in the application of existing OCT imaging to determine mature and immature healing tissue after stenting using tissue characterization analysis and this analysis technique is already being investigated clinically. Finally in WP4, the largest databank of stent thrombosis cases with multimodal assessment worldwide was compiled. Early insights reveal an unexpected high prevalence of neoatherosclerosis in the aetiology of these events. Further exploitation of the data for risk factor algorithm construction is planned following presentation of the primary registry results in 2015.

The broad wealth of data acquired from bench work, pre-clinical testing and clinical investigation has already contributed unique and important knowledge and meaningfully advanced investigation of this clinically important problem. The key findings have already been broadly disseminated through published scientific papers, public information workshops and scientific presentations at high profile meetings. In addition, the work of the consortium has already stimulated pan European cooperation on a number of related research topics outside of the scope of PRESTIGE. We envisage that this will strengthen the European pre-eminence in cardiovascular research.

PRESTIGE: Project context and objectives

Stent thrombosis after implantation of stents for coronary artery disease (CAD) represents a major European health care challenge. Moreover it is expected to assume increasing importance in clinical practice in coming years for a number of reasons. Firstly, underlying coronary artery disease (CAD) is the leading cause of morbidity and mortality in the western world in general and in Europe in particular. Its incidence is strongly related to age. With the increasing age of the global population – in the coming years the number of adults over 65 worldwide is expected to outnumber the number of children under the age of 5 for the first time – the burden of the disease will increase significantly. Secondly, technological advancements mean that treatment of obstructive CAD by percutaneous coronary intervention (PCI) has now become the predominant form of revascularization for this condition. In particular the advent of drug-eluting stents (DES) has resulted in significant reduction in the incidence of stent failure after PCI – mainly due to in-stent restenosis – by an order of 35-70% in comparison with uncoated stents. This progress has enabled the expansion of PCI to treat patients with complex disease morphology who previously would have required cardiac surgery as well as to patients with complex co-morbidities who previously would have been ineligible for myocardial revascularization. For both of these reasons the number of patients treated with PCI continues to rise in absolute terms. This means that although acute stent failure due to stent thrombosis remains a relatively infrequent event – 0.5-3% at 3 years – it has become an increasingly important health care issue. Moreover as incident cases are associated with high morbidity – around 90% suffer a myocardial infarction – and mortality (around 20-40% will die) the impact of this pathophysiological condition is very significant.

The exact mechanisms leading to stent thrombosis are incompletely understood. However, delayed healing of the DES-stented coronary artery segment characterized by persistent late platelet accumulation and activation of coagulation appear to play an important role. The occurrence of stent thrombosis has led guideline writing authorities both in Europe and the United States to revise their recommendations for postprocedural therapy after DES stenting: Due to a lack of reliable risk predictors and particularly of sensitive imaging technologies allowing for appropriate risk stratification, a potent dual antiplatelet therapy (DAPT) – consisting of aspirin in combination with a thienopyridine – is currently recommended for at least 6-12 months across the spectrum of patients receiving DES therapy. However, DAP not only targets thrombotic events within DES, but also inhibits normal haemostasis. Consequently, at present mitigation of the risk of stent thrombosis occurs at the expense of a significant burden both in terms of economic cost of DAPT, consequences of bleeding events due to DAPT and deferral of necessary surgeries due to requirement for extended DAPT after stenting.

Although DES have indisputably improved patient outcomes, the excess of stent thrombosis events late after implantation and the consequential need for protracted DAPT along with its

attendant bleeding risks makes dedicated research in this field an urgent priority. Indeed, it was a major task of the current project to not only develop therapies that further reduce the overall risk of stent thrombosis after DES, but in particular to identify and advance strategies and technologies that prevent stent thrombosis and at the same time induce a minimum of collateral damage. The low frequency occurrence of stent thrombosis means that assessing the influence of any intervention on its incidence in a clinical setting is problematic – very large cohorts of treated patients need to be recruited in order to demonstrate the impact of a given intervention. Therefore, this objective can only be achieved by a collaborative interdisciplinary approach synergizing the expertise of multiple large clinical centres.

Against this background we formed a multi-disciplinary consortium, the PREvention of late Stent Thrombosis by an Interdisciplinary Global European effort (PRESTIGE), combining in a unique way (a) basic researchers (with focus on platelet and endothelial biology, or coagulation), (b) histopathologists, (c) bioimaging engineers and (d) interventional cardiologists. The general objective of PRESTIGE was be to identify and validate more selective strategies that reduce the risk of stent thrombosis and at the same time minimize the bleeding complications associated with the current anti-thrombotic regimens. PRESTIGE focused on strategies to promote DES-related arterial healing and to prevent local thrombotic events, thereby reducing the requirement for prolonged DAPT and to better identify, by means of surrogate markers of delayed healing, those patients who remain at-risk and in whom the risks of prolonged DAPT therapy may be more justified. Using this twin-track approach it is anticipated that substantial inroads into the burden of late stent thrombosis can be made.

The focus of PRESTIGE was on four general objectives:

1.) to perform basic research to define the molecular and cellular mechanisms underlying stent thrombosis

2.) to use bioengineering in order to advance novel DES technologies fostering vascular healing

3.) to develop and validate novel imaging technologies for early diagnosis of events contributing to late ST

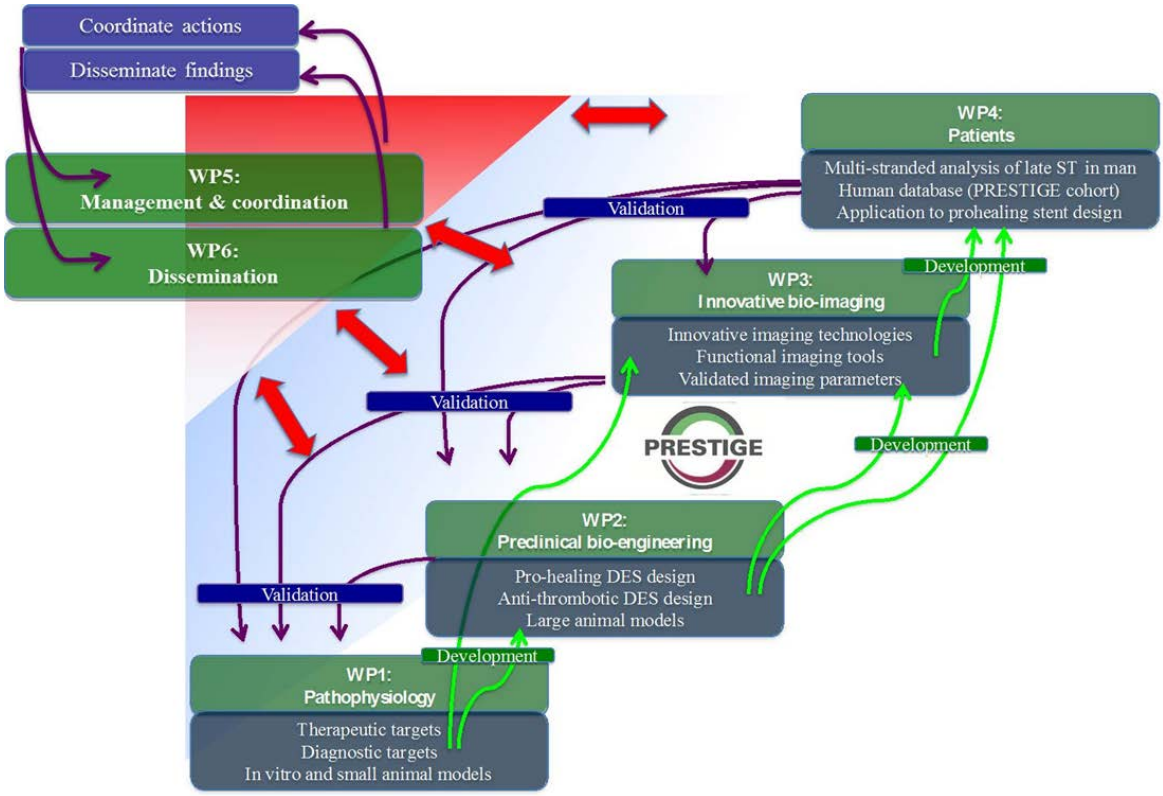
4.) to establish a clinical database in order to evaluate clinical surrogate parameters for risk stratification

These aims were planned to be achieved through the interactive work of four scientific and technical work packages (WP1-4), which aimed to translate focused basic experimental work through preclinical models into clinical practice. In terms of scientific knowledge, the goal of PRESTIGE was to deliver enhanced understanding of platelet-stent, coagulation-stent, and endothelium-stent interactions (WP1) and a detailed characterisation of the processes of arterial regeneration after DES implantation (WP2-3). In terms of new technology, the goal

was: (i) to investigate and develop novel "pro-healing" stent platforms (WP2) including assessment of novel platforms in clinical use (WP4); and (ii) to develop a comprehensive functional and morphological dual imaging approach including novel technology that would identify surrogate markers of ongoing delayed arterial healing (WP3). Finally the aim of our broad-reaching multinational clinical registry of incident stent thrombosis cases (WP4) was to better characterize patients with stent thrombosis and to more accurately risk stratify patients at potential risk of developing this condition. These scientific work packages were supplemented by 2 specific work packages dealing with project management (WP5) and dissemination of findings (WP6). The organization of the work of the consortium is shown in **Figure 1**.

The principle *medical objective* of PRESTIGE was to develop new strategies to prevent stent thrombosis at a cost of minimum bleeding risk. The main *scientific objective* of PRESTIGE was to dissect the mechanisms contributing to the occurrence of stent thrombosis. This would be achieved through mechanistic research in coagulation as well as platelet and endothelial biology using relevant *in vitro* and *in vivo* model systems in combination with novel imaging technologies. A better mechanistic understanding of late ST is a *conditio sine qua non* for the development of more specific anti-thrombotic regimens that have minimal effects on normal haemostasis. The major *technological objectives* of PRESTIGE were (1) to develop novel imaging technologies allowing for early diagnosis and a better risk prediction of late ST and (2) to evaluate optimized stent designs that support vascular healing.

Figure 1: Organization of the work of the PRESTIGE consortium



Specifically, PRESTIGE focused on the following aims:

(1.) to gain a better mechanistic understanding of the molecular and cellular events triggering late ST (WP1): Currently, three predominant mechanisms are thought to lead to stent thrombosis: (a.) failure of re-endothelialisation or coverage of stent struts with dysfunctional endothelial cells (characterized by impaired thrombo-resistance), (b.) platelet adhesion and aggregation, as well as (c.) activation of clotting factors with subsequent fibrin formation. Leukocytic infiltration of the arterial wall, triggered in particular by polymers used for DES coating, are considered important, however, the exact cascade of cellular and molecular events initiating thrombus formation within coronary stents resulting in stent remains to be delineated. Therefore, a major objective of PRESTIGE was to dissect the basic pathophysiology leading to stent thrombosis. To achieve this PRESTIGE generated a collaborative platform integrating the expertise of excellent European groups with a major focus on platelet and endothelial biology and coagulation. By combining their different scientific and technological knowledge, PRESTIGE aimed to gain fundamental insight into the pathophysiology of the early steps initiating stet thrombosis (WP1).

(2.) to develop and validate novel strategies to reduce stent thrombosis (WP2): In a multidisciplinary approach combining basic science, preclinical research and small- and medium-sized enterprises (SMEs), PRESTIGE aimed to leverage the mechanistic information gained in WP1 into the development and validation of new therapeutic strategies including novel stent devices with reduced thrombotic risk and improved healing characteristics (WP2). In doing so, PRESTIGE targeted the development of novel treatment options that reduce the risk of stet thrombosis in patients undergoing DES implantation but have only minor effects on bleeding.

(3.) to develop and evaluate novel imaging technologies (WP3): A detailed characterisation of arterial healing (including thrombotic processes) after DES implantation in man remains a key scientific gap and is central to the development of techniques targeted at reduction of the burden of late stent thrombosis. The third specific objective of PRESTIGE was to advance novel imaging technologies by utilizing a multidisciplinary approach bringing together imaging engineers (HMGU/TUM), basic researchers (DHM), and clinical imaging specialists (BER, K.U.LEUVEN). PRESTIGE focused on two imaging technologies: (1.) optical coherence tomography (OCT) and (2.) near-infrared fluorescence molecular imaging (NIRF). It was expected that novel imaging technologies would critically contribute to reduction in the burden of stent thrombosis (a.) through identification of surrogate parameters indicating the risk of late ST and hence allowing individualized anti-thrombotic therapy and (b.) via the identification of early markers of stent thrombosis.

(4.) to perform a multi-stranded characterisation of patients with late ST (WP4): Because stent thrombosis is a relatively rare event, it is impossible to investigate *clinically* using

single-centre approaches. Furthermore, given the low event rate, we estimated that a prospective cohort study would need to enrol, comprehensively investigate (including imaging evaluation) and follow in the region of 50,000 patients in order that approximately 500 patents with subsequent late stent thrombosis might be captured. Consequently it was our contention that in order to address this shortcoming, a multi-centre case-control study (with a recruitment phase of 3 years) was the best method to clinically address the issue of stent thrombosis. Therefore the fourth specific objective of PRESTIGE was to coordinate the establishment of a pan-European stent thrombosis registry – the PRESTIGE Registry – through a collaborative network of centres from Central, Southern and Eastern Continental Europe and the UK. Over a period of 3 years, PRESTIGE Registry was expected to recruit at least 500 patients presenting with stent thrombosis. It was planned that all patients would undergo a multi-stranded analysis, including an in-depth description of patient-demographic and procedure-related factors, analysis of genetic and bio-markers, platelet function testing, histopathologic analyses of the thrombus (retrieved from the involved coronary artery) and intracoronary imaging of the involved segment of the coronary artery using optical coherence tomography (OCT) and intravascular ultrasound (IVUS).

PRESTIGE: Main scientific results

WP1: Assessment of basic mechanisms leading to ST

Task 1:	**Development of a mouse model of DES implantation (Participants: DHM, INSERM, BIO)**

Our goal was to dissect the molecular and cellular mechanisms of stent thrombosis *in vivo*. To reach this objective, we proposed to establish a mouse stent implantation model. To achieve this aim, BIO together with DHM/LMU developed and tested mouse-dedicated stent devices. Stent design and implantation has been a challenge due to the size constraints. Several steps have been necessary as follows

Stent design: Several prototypes have been developed and tested and the major difficulties to be overcome were the size of the stent and of the balloon. In addition, we had to come up with a reliable technique to gain vascular access for stent placement. To achieve this, we developed an atraumatic inserter. In brief, stents of 2.5mm in length and with diameters of 1.0–2.0 mm were laser cut from a L605 cobalt-chromium (CoCr) tube and electro-polished. This resulted in surface properties similar to the CoCr surfaces for clinical applications. The second major component of the mouse stenting system was the stent delivery system

composed of four distinct elements: (I) a luer lock enabling connection of a dilatation device, (II) a shaft (5-10cm length) to provide enough push force transmission to advance the balloon/stent segment with a central lumen to allow passage of a medium for balloon inflation, (III) a distal balloon section carrying the crimped stent, designed to dilate the stent to a diameter of 1.0–1.2mm, and (IV) a tip to provide a shape allowing for entry across an incision in the femoral or aortic artery and subsequent non-traumatic advancement of the delivery catheter within the vessel. Five generations of delivery system have been necessary to achieve the development of an atraumatic inserter. The size of the 5th generation inserter is 0.68 mm in diameter.

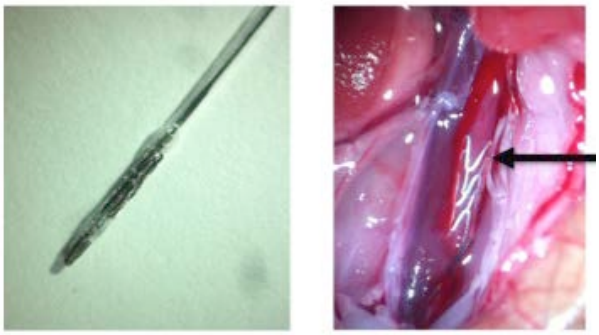


Figure 2: Mouse model of stent implantation.
Left, the 5th generation of the mouse dedicated stent and implantation device.
Right, a view of the inflated stent placed in the abdominal aorta of the mouse (arrow).

Stent implantation: Due to the size of the stent inserter, the stents could not be implanted in the femoral artery as originally planned. Instead, stents were implanted into the abdominal aorta as shown in **Figure 2**. Mice receiving stents were protected from stent thrombosis (ST), while on dual antiplatelet therapy (DAPT). However, discontinuation of DAPT results in reproducible ST with histological features consistent with those found in human ST patients.

It should be acknowledged that the technique of implantation needs considerable practice, but is now established and may be used to confirm *in vivo* the relevance of novel targets including those identified in Task 2. Currently, we are evaluating the role of distinct leukocyte subsets and their procoagulant machinery (including neutrophil extracellular traps; NETs) in the development of ST in our novel model of mouse stent placement (see below). In addition, we will further investigate define the contribution of platelets and coagulation cascades to the development of ST in this model. The experiments will be finalized within the next months and we expect that the findings will lead to several publications in high-ranked international journals.

Task 2:	**Molecular and cellular events leading to thrombotic events within DES (Participants: DHM, INSERM, ULEIC, BIO)**

In vitro studies: For this purpose, BIO developed a new model of bare metal discs (cobalt-chromium, CoCr) with diameter of 6 mm adapted for use in micro-titration plates. Utilizing

these devices several approaches were set up to dissect the cellular and molecular mechanisms that could lead to stent thrombosis (see below).

Platelet interaction with bare metal

Platelets adhere to CoCr through a GPIb-dependent mechanism

Human and mouse washed platelets efficiently adhered to the CoCr surfaces in a time-dependent manner. Adhesion was prevented when CoCr was first passivated with a PBS-Human Serum Albumin-1% solution.

CoCr induces platelet activation

Fluorescence microscopy showed that platelets adhering to CoCr discs became spherical and extended filopodia. These morphological changes are typical signs of early platelet activation. Interestingly, at longer time points most of the platelets spread over the surface indicating that integrins undergo fully-fledged activation on CoCr.

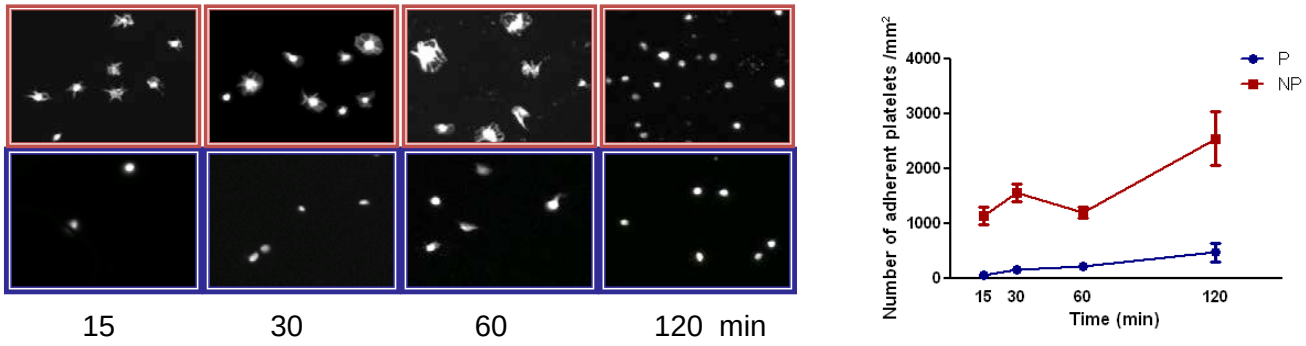


Figure 3: Platelet adhesion to CoCr. Left: scanning electron microscopy of human washed platelets adherent to CoCr (top) or HAS blocked CoCr (bottom). Right: quantification of adherent platelets (NP: bare CoCr, P: HAS passivated CoCr)

Development of a flow-based assay to study platelet adhesion to CoCr under flow

Beside the static adhesion assay, we have developed a microfluidic flow chamber to study platelet recruitment to CoCr under rheological conditions. Chambers with channels of different geometries and sizes were tested in order to identify flow conditions under which platelets adhere to stents. With these new chambers we were able to observe platelet adhesion and shape change on CoCr (**Figures 3 and 4**). The extent of adhesion increased with the time of perfusion while pre-coating of CoCr with albumin reduced the pro-adhesive properties of CoCr. At low shear a homogenous layer of platelets was observed; at higher shear, platelets formed small aggregates (**Figure 3**). The use of antibodies to different platelet receptors indicated that GPIb (CD42b) and GPIIbIIIa (CD41) are involved in the interaction of platelets with CoCr (**Figure 4**).

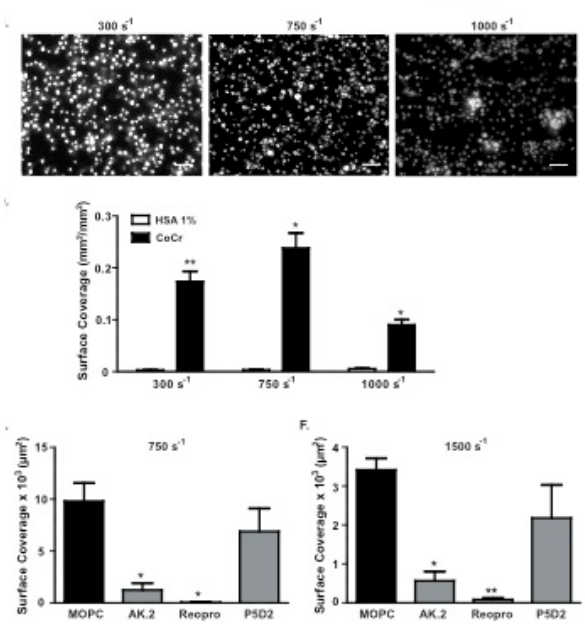


Figure 4: Platelet adhesion to CoCr in flow conditions. Whole anticoagulated blood was perfused over CoCr discs at increasing shear rates. After 5 min flow chambers were disassembled and analysed by scanning electro microscopy. Representative images are shown (top). Quantification of the surface occupied by platelets on bare CoCr vs HAS-coated CoCr (middle). The experiments were performed in the presence of an irrelevant antibody (MOPC), an blocking anti –GPIb (SZ2), Reopro (Abxicimab) that blocks GPIIBIIIa or an anti-a2 integrin (bottom panels).

Effect of CoCr on coagulation

<u>CoCr activates the intrinsic pathway of coagulation</u>

Thrombin generation was measured in platelet poor plasma (PPP) after insertion of CoCr discs into the microwell titration plates. CoCr significantly increased the rate and the extent of thrombin generation. Interestingly, this does not require activation of the extrinsic pathway of coagulation, since CoCr also triggered thrombin formation in the absence of tissue factor. Instead, activation of the intrinsic (contact) pathway is critical, as CoCr-triggered thrombin generation was blocked in the presence of Corn trypsin inhibitor that blocks FXII activation. Data were confirmed by using FXII deficient plasma.

<u>CoCr promotes thrombin generation in platelet rich plasma</u>

Since activated platelets act as a catalyst for thrombin generation by providing the phospholipid surface required for the assembly of the tennase and prothrombinase complexes and by delivering FV, we measured the effect of CoCr on thrombin generation in platelet rich plasma (PRP). As observed in PPP, CoCr increased the rate and extent of thrombin generation in PRP in the presence and in the absence of tissue factor.

We investigated whether CoCr-triggered platelet procoagulant activity was dependent on platelet activation. When experiments were performed in the presence of the P2Y12 ADP receptor competitive inhibitor ticagrelor or of a blocking anti-glycoprotein VI antibody, the rate and extent of thrombin generation returned to normal values indicating that at least P2Y12 and GPVI are involved in CoCr-induced platelet procoagulant activity. We have further studied the involvement of GPVI in the growth of the thrombus and unexpectedly found that GPVI behaves as a receptor for polymerized fibrin with as consequences an increased extent

of platelet procoagulant activity and the recruitment of platelets by fibrin rich clots. The Fab fragment of the antibody 9O12 counteracts this effect of GPVI.

CoCr promotes inflammation

Inflammation and coagulation are closely linked in particular at the level of the contact pathway. Indeed, inflammatory cells could be a critical element triggering FXII activation and there is increasing evidence that the release by neutrophils of their nuclear content lead to the formation of extracellular traps (NETs) that could trigger thrombosis. We observed that isolated granulocytes when incubated on CoCr discs, displayed morphological changes indicating activation. NET-like structures were formed upon co-incubation of isolated granulocytes and platelets on CoCr; labelling of histone H1 and measurement of cell free DNA in the cell supernatants indicated that CoCr behaves as a scaffold for NET formation.

In vivo studies

Developing a mouse dedicated stent and an implantation procedure has been a great challenge taking longer than was originally anticipated (see above). However, both objectives were achieved and this has allowed us to **develop a mouse model of stent thrombosis (ST)**. After stent placement, mice treated with aspirin plus P2Y12 inhibitor did not develop ST. In contrast, in mice without dual antiplatelet therapy, the stent was patent at the end of the implantation procedure, however a large occluding thrombus was observed 24 to 48h later (**Figure 5**).

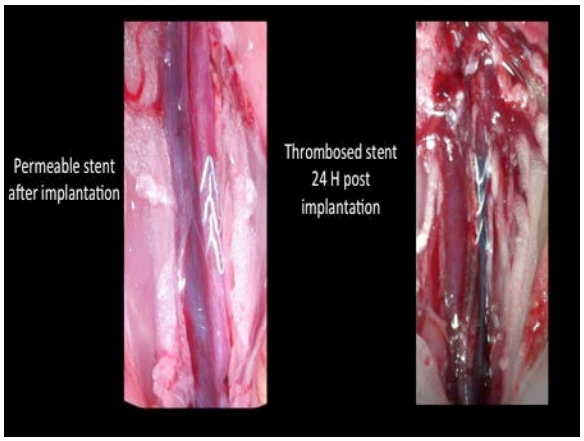


Figure 5: Example of mouse stent thrombosis (for details see text).

Mouse stent thrombosis was further characterized by histological analysis. The lumen of the aorta was completely occluded at the level of the stent by clots rich in red blood cells and in fibrin with the presence of leucocytes (**Figure 6**). Histological analysis revealed that ST in mice resembles that observed in human patients.

The availability of stent devices and a dedicated model of mouse stent placement (see above) is currently used in several models of genetically modified mice to address the molecular and cellular mechanisms of ST (see above). The resulting in vivo data will be analysed with respect to the in vitro data described above.

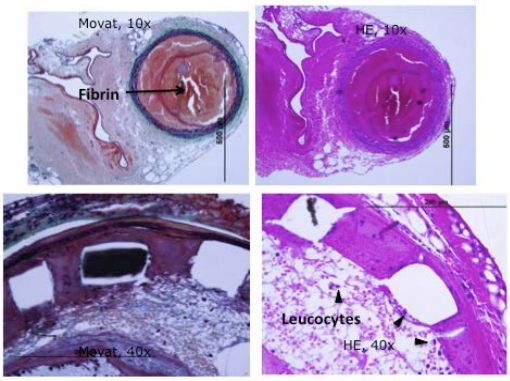


Figure 6: Histological analysis of stent thrombosis. Histological sections of the mouse abdominal aorta at the level of the thrombotic stent were stained with movat (left) or hematoxylin-eosin (right).

Task 3:	**Molecular and cellular processes regulating endothelialisation during DES healing (Participants: (DHM, INSERM, ULEIC)**

ULEIC established a model for measuring the growth of endothelial cells on cobalt chromium discs and observed that EaHy926 cells grew slightly less well on the bare metal stent discs than on culture plates, but the difference was not statistically significant. Pre-coating the discs with human plasma did not affect the ability of the EaHy926 cells to grow. They also observed that VEGF and BIQ, both pro-angiogenic factors, inhibited the growth of the cells significantly reflecting the reprogramming of the ECs to differentiate rather than proliferate. This suggests that pro-angiogenic factors are not a suitable stimulus for re-endothelialisation of stent materials.

Endothelial cells grown on CoCr acquire a procoagulant phenotype

INSERM further progressed in characterizing the capacity of CoCr to be endothelialized and the phenotype of endothelial cells covering stent material. Human coronary artery endothelial cells adhere, grow and survive on CoCr forming a layer of confluent cells (**Figure 7**).

Importantly, healthy endothelial cells are protective against thrombosis due to the fact that (i) they do not express tissue factor (TF) but (ii) express thrombomodulin and the protein C receptor (EPCR) supporting protein C (PC) activation by thrombin, APC in turn shutting off the coagulation cascade. Indeed, endothelial cells grown on culture plates inhibit thrombin generation, whereas smooth muscle cells trigger thrombin generation. When HAEC were grown on CoCr, thrombin generation was accelerated and increased compared to cells grown on plastic (**Figure 7**). This observation was independent of the absence or presence of tissue factor indicating that endothelial cells grown on CoCr lose their protective role and

acquire a procoagulant phenotype. Indeed, an increased TF activity was measured in cells grown on CoCr as compared to controls and PC activation was significantly decreased. These data further indicated that CoCr constitutes a surface that impacts the endothelial cells phenotype.

Endothelial cells grown on CoCr acquire an inflammatory phenotype

We further characterized the phenotype of endothelial cells by immunofluorescence using antibodies to PECAM1 ICAM1 and VCAM1. Only cells grown on CoCr were positive for ICAM and VCAM indicating that they tend to acquire an inflammatory phenotype (**Figure 7C**)

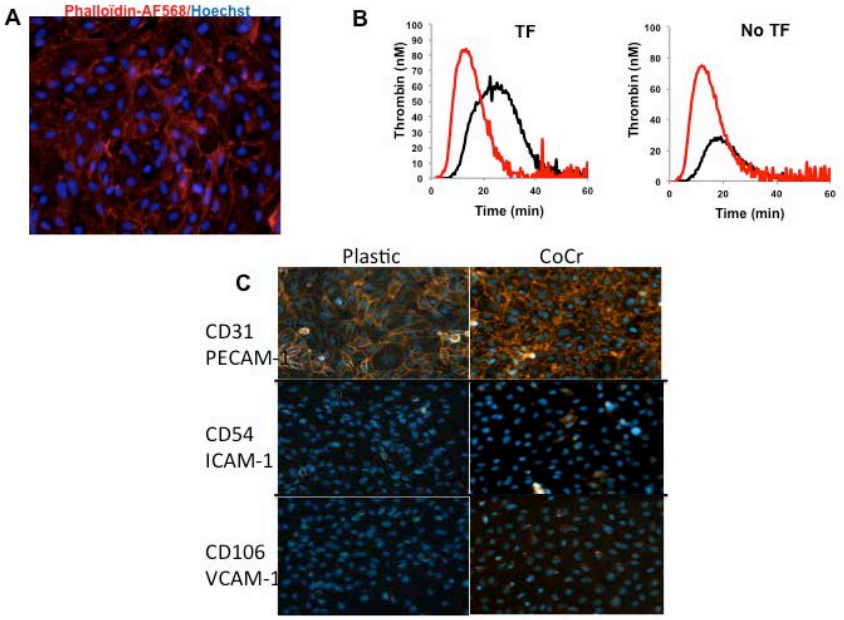


Figure 7: Abnormal CoCr Healing
A: HAEC grown to confluence on CoCr discs
B: Thrombin generation was measured on HAEC grown on plastic or on CoCr in the presence (left) or absence (right) of TF
C: PECAM, VCAM and ICAM expression of HAEC cultured on plastic or Cocer was analyzed by immune-cytochemistry using specific antibodies.

Summary: Significant results and overall achievements

1.) Successful development of a mouse stent thrombosis model. The design of the mouse stents (BIO) closely resembles that of stent platforms used in patients. This novel approach overcomes limitations of previously described mouse stenting models. Stents are implanted in the abdominal aorta (DHM/LMU) and thrombosis occurs in the first 24 hours following the implantation in the absence of dual antiplatelet therapy. The histology of the thrombi is consistent with that reported in humans and supports the in vitro data pointing to the concomitant activation of platelets and coagulation with the participation of inflammatory cells.

2.) CoCr alloy is a potent activator of coagulation (INSERM) with activation of FXII being a key regulator of thrombin generation in response to CoCr.

3.) Platelets adhere and aggregate on CoCr under static conditions and more importantly at different shear rates. We identified that the GPIb-IX complex, as well as the αIIbβ3 integrins participate in platelet attachment to the CoCr surface. Antiplatelet agents (P2Y12 and GPVI antagonist) are capable to reduce the CoCr-triggered platelet procoagulant activity.

Altogether, these observations strongly suggest that traces of thrombin formed via the activation of the contact phase are capable to activate platelets that in turn amplify the rate and efficacy of coagulation.

4.) CoCr provides a scaffold for neutrophil recruitment and formation of NETs suggesting that stent thrombosis could result from the coordinated activation of coagulation and inflammation.

5.) Quite unexpectedly, endothelial cells were found to grow on the stent material in vitro. However the characterisation of the endothelial surface indicated that endothelial cells loose their protective properties and acquire a proinflammatory and prothrombotic phenotype. This very important observation indicates that endothelialization is not synonymous of healing.

Manuscript describing the findings outlined under 1-5 are in preparation and will be submitted to high-ranked journals in the field.

6.) The successful establishment of a GPVI humanized (hGPVI) mouse model (Mangin P et al. JPET. 341;156) provides a useful tool allowing (i) evaluation of the role of GPVI in the pathophysiology of stent thrombosis and (ii) *in vivo* analysis of the efficacy of antagonists targeting human GPVI as novel strategy to prevent stent thrombosis.

7.) Studies performed to get further insight into the mechanism of thrombus growth show that GPVI represents a yet unidentified receptor for polymerized fibrin, the interaction between fibrin and GPVI being involved in the amplification of thrombin generation and in the recruitment of platelets by fibrin-rich clots (manuscript submitted).

Hence, the results obtained so far clearly point towards prothrombotic properties of CoCr stent platforms both in vitro and in vivo. Indeed, our present finding indicate that CoCr may activate the coagulation cascade, platelets and leucocytes and may open new avenues to treat and prevent thrombotic events in patients with advanced coronary artery disease receiving stents.

WP2: Bio-engineering approaches to reduce ST risk

Task 1:	**Approaches to foster endothelialization of stents**

A number of approaches have been investigated to enhance endothelialisation of stents in vitro and in vivo.

DHM in cooperation with **NEO, RTU** and **KIZ** established a proof-of-principle for the coupling of RGD peptidomimetics on functionalized surfaces exhibiting increased density of NH_2 groups on polymer-coated discs with the help of plasma-treatment. RGD peptidomimetics

were shown to bind to aminogroup-functionalized discs in vitro, which resulted in an increased adhesion of endothelial cells compared to control discs without RGD peptidomimetics. In the next step, the chemical conformation of RGD peptidomimetics was further refined to increase binding efficiency to plasma-treated and aminogroup-functionalized surfaces. This technology was successfully transferred to Co-Cr stents by NEO and was subsequently tested in animal studies for their ability to attract endothelial cells.

In addition, a total of 10 stents have been coated by a monolayer of the co-polymer of chitosan and polylactic acid (PLA) developed by **KIZ**. It has been shown by **NEO** that the content of aminogroups for RGD peptidomimetic coupling after plasma treatment is greatest on this polymer monolayer (see task 2). In a further step, a total of 20 stents were tested in a novel animal model of neoatherosclerosis; 10 stents were coated with the above mentioned chitosan/PLA copolymer followed by plasma treatment and coupling of specific endothelial cell attracting RGD peptidomimetics. Another 10 control bare metal stents without coating were also delivered. These stents were implanted into the above-mentioned animal model and results are expected in February 2015.

In cooperation with the sub-contractor **Dr. Soldani** (Laboratory of Biomaterials and Graft Technology – Institute of Clinical Physiology of the National Research Council, Pisa, Italy), Co-Cr discs were modified with aminosilane or poly-urethane polymers to provide a substrate for the coupling of novel aptamers (short oligomer sequences) attracting endothelial progenitor cells. Aminosilanization increased endothelial cell adhesion to Co-Cr discs in vitro.

Aminosilanized discs were then incubated with single-strand oligonucleotides. The binding of oligonucleotides at the disc surface was confirmed by laser scanning confocal microscopy, after incubation with complementary oligonucleotide labelled with fluorescent dyes. Two cell types were used to assess cell viability and binding to oligonucleotides: human umbilical vein endothelial cells (HUVEC) and porcine EPC (pEPC). Preliminary tests were performed demonstrating the importance of an appropriate disc coating and that an early dynamic step did not increase cell adhesion and viability. Oligonucleotides significantly increased cell adhesion and viability on Co-Cr discs. The use of a specific cell-capturing oligonucleotide further increased cell adhesion and viability, showing more pEPC clones. Flow cytometry confirmed cell binding to this oligonucleotide. Co-Cr stents were functionalized using the selected oligonucleotide; oligonucleotide presence and pEPC binding were confirmed by laser scanning confocal microscopy. Functionalized stents were delivered for *in vivo* testing.

In a final step Co-Cr stents coated with selected oligonucleotides will be implanted into porcine coronary arteries in 5 animals and compared to uncoated control stents. As oligonucleotides specific to porcine pEPC clones were fabricated from the subcontractor, healthy porcine will need to be used for testing of the functionalized stents. First results are expected in July 2015.

Task 2:	Investigation of stent design to modulate platelet-stent interactions and coagulation

Plasma treatment involves surface modification with plasma to impart energy changes which alter the properties of the treated surface. Plasma treatment of different polymeric coatings was achieved in the first reporting period and resulted in a substantial increase in reactive aminogroups on the surface of different polymers. The desired surface functionality was determined in concordance with **DHM** to be NH_2-groups. As objective, a target value of 2 % NH_2-groups was specified, to enable coupling of a sufficient amount of the target RGD peptidomimetics on the surface. It was reported by **NEO** that among 6 monolayer and 2 multilayer chitosan/heparin coatings the target value of 2 % NH_2-groups was reached for 3 monolayer coatings made of carboxymethylchitosan (fabricated by **KIZ**, NH_2/C= 2,4 %), low molecular weight chitosan (fabricated by Primex, NH_2/C= 2,7 %) and ***copolymer chitosan-polylactic acid*** (fabricated by **KIZ**, NH_2/C= 5,0 %).

This coating technology was subsequently refined in the second reporting period to produce highly efficient reactive aminogroups on stent surfaces followed by coupling of RGD peptidomimetics. This task was therefore executed in parallel to **task 1** as plasma treatment with subsequent aminogroup-functionalization turned out to be the ideal coating technology for coupling of RGD peptidomimetics on the stent surface with the goal to foster vascular healing.

Chitosan polymers are polysaccharides derived from crustacean shells, which have a number of biomedical uses. Different chitosan polymers were investigated for their ability to promote cell adhesion and tested for their water contact angle, which has been shown to be associated with biocompatibility and coagulation pathways. Both monolayer and multilayer chitosan polymers were assessed. For the preparation of **chitosan sulfate** coating, with intrinsic anticoagulant properties similar to heparin thanks to the sulfate groups along chitosan chains, **KIZ** has evaluated two synthesis routes for chitosan sulfate, starting from mushroom-derived ultrapure chitosane. The best route was further developed for ensuring tight control of the sulfation degree. The purification downstream process was also developed. KIZ has developed the proton NMR method in order to confirm the substitution degree and purity.

In subsequent steps using **ULEIC** investigated the plasma-treated stents with RGD peptidomimetics coupled to the surface that have been developed in **Task 1**.

In a next step, functionalized stents were delivered to **DHM** and **ULEIC** for subsequent animal testing. The following stents were distributed: **5 stents** were coated with 3 bilayers of chitosan/sulfated chitosan CS60. Increasing the number of layers increased surface roughness and at the same time improved surface coverage with the polymer **(A)**; **5 stents** were coated by a monolayer of sulfated chitosan CS60 developed by **KIZ**. Sulfated chitosan

CS60 has a degree of acetylation of 20.2 mol% and a sulfate content of 41% **(B)**; **10 stents** were coated with the above mentioned chitosan/PLA co-polymer followed by plasma treatment and coupling of specific endothelial cell attracting RGD peptidomimetics **(C).** The work package leader and co-leader decided to designate the limited number of coated stents to in vivo animal testing rather than structural, ultra-structural and in vitro analysis. In this regard, **ULEIC** successfully developed a rabbit model of iliac artery stent implantation and the stents described under **A** and **B** above were implanted for 28 days; first histopathology results are expected in February 2015. Stents described under **C** were tested in an animal model of neoatherosclerosis (see task 1)

Task 3:	**Investigation of stent design to modulate inflammation**

INSERM investigated novel dextran-based co-polymeric stent coatings – dextran-polybuthylmethacrylate (Dex-PBMA) – that showed decreased platelet adhesion in vitro and in vivo (**figure 8**). The dextran-graft-butymethacrylate (Dex-PBMA) copolymer is a new polymer with a balance of hydrophilic/hydrophobic properties. The Dex-PBMA copolymer exhibits elastic properties and therefore is used as a coating for stents. Data shows that Dex-PBMA coating is homogeneous and shows excellent mechanical properties after deformation both on discs and stents. Interestingly, our in vitro data of endothelialization of stents reveal a major difference between the polymer-coated and the bare metal stents. The Dex-PBMA copolymer thus displays the ability to stimulate endothelial cell proliferation while limiting vascular smooth muscle cell growth ([1] SM Derkaoui et al., Acta Biomat 2012).

Amphiphilic co-polymers were investigated based on the co-polymerization of hydrophilic and hydrophobic moieties. The copolymer was made of dextran-graft-polybutylmethacrylate. The Dex-PBMA co-polymer exhibits elastic properties and therefore is used here as a coating for stents.

In addition, **DHM** investigated the biocompatibility and inflammatory response of completely bioabsorbable magnesium stents that are aimed at decreasing inflammatory reactions after stent implantation at the long term. Extended investigation shows excellent biocompatibility of bioabsorbable magnesium stents at long-term in rabbits during the absorption process, with absence of toxic vessel reactions.

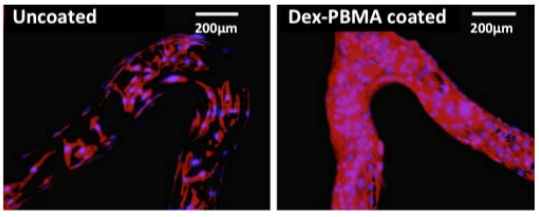


Figure 8: Fluorescence images of ECs, 5 days after being seeded directly onto stent

Following *in vitro* tests performed on Dex-PBMA co-polymers, the first *in vivo* evaluation was carried out in male Wistar rats according to two protocols.

INSERM investigated the coating of Dex-PBMA co-polymer on metallic stents via plasma discharge. Following a multi-step grafting of the specific polymer, Fourier transform spectroscopy (FTIR) and nuclear magnetic resonance analysis (1H-NMR) helped to characterize polymeric compounds. Finally polymer coatings were examined by X-Ray Photoelectron Spectroscopy (XPS) to confirm the grafting and determine the composition. Further biological assessment will be performed in the following setting: (i) Implantation in rat abdominal aorta for 30 days of Dex-PBMA coated and bare CoCr stents (n=6); and (ii) Implantation in hypercholesterolemic rabbit aorta of Dex-PBMA coated or bare CoCr stents. Grafting of CMD-PBMA on stents and implantation in hypercholesterolemic rabbit aorta of CMD-PBMA coated or bare CoCr stents. First results are expected in June 2015.

Task 4: Evaluation of stents in relevant preclinical animal models

Both small and large animal models have been employed in testing functionalized pro-healing stents fabricated by the participants involved in WP2. **DHM** implemented a previously well-characterized rabbit model of iliac artery stent implantation to test RGD peptidomimetic coated stents. Furthermore, a novel model of neoatherosclerosis was implemented to better characterize the pro-healing nature of the fabricated stents. **ULEIC** established a rabbit model of acute and chronic stent implantation into the iliofemoral arteries of New Zealand White Rabbits. **INSERM** employed a rat model of aortic stent implantation and a hypercholesterolemic rabbit model of aorta stent implantation.

Summary: Significant results and overall achievements

Task 1:

a) Fabrication and characterization of RGD peptidomimetic to promote endothelial healing

b) Establishment of grafting technology to provide anchorage of RGD peptidomimetics to Chitosan/PLA copolymer surface by applying plasma treatment

c) Delivery of 20 Chitosan/RGD peptidomimetic coated stents for testing in rabbit model of iliac artery stenting and neoatherosclerosis, respectively

d) Transfer of aminosilanization coating with aptamer technology from discs to Co-Cr stents. Delivery of a total of 10 coated stents for further testing in animal studies (subcontracting)

Task 2:

a) this task was executed in parallel with **task 1.1** as plasma treatment of stent surface turned out to be the most efficient method for coupling of RGD peptides (see **task 1**)

b) Detailed assessment of different monolayer or multilayer chitosan polymers to modulate platelet-stent interactions and coagulation pathways by water contact angle (see section *additional significant results*)

Task 3:

a) Establishment of appropriate animal models to investigate the in vivo behaviour of different stent prototypes

b) Initial in vivo assessment of novel dextran-based copolymers to reduce platelet-stent interactions (see additional significant results)

c) Execution of a dedicated long-term animal study to investigate the inflammatory response and biocompatibility of completely bioabsorbable magnesium stents

WP3: Novel imaging approaches to assess healing

Task 1:	**In vitro imaging**

HMGU designed and constructed a fully operational NIRF imaging catheter system (see **Figure 9**), capable of 2D intravascular imaging. The system was optimized for resolution and sensitivity and was tested in custom-designed phantoms in vitro. This optimization included improvement in quantification secondary to blood attenuation, beam broadening and catheters positioning.

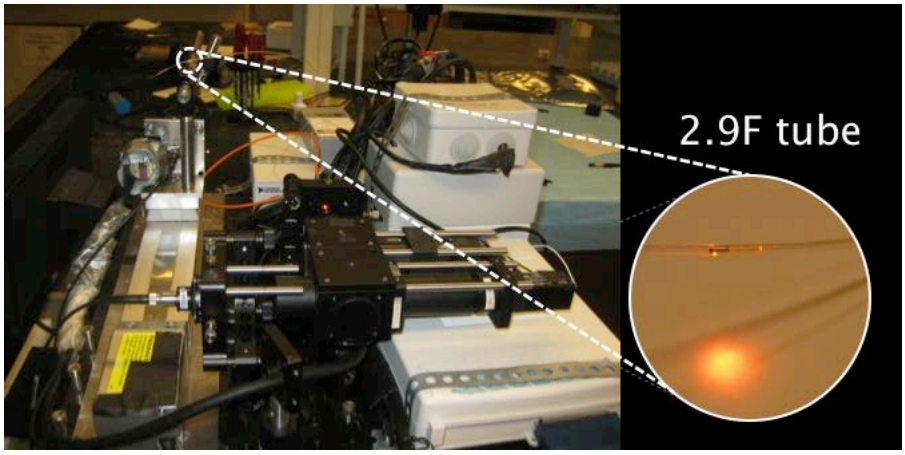


Figure 9. Experimental set-up of NIRF catheter

Task 2:	Preclinical imaging in animal models

A preclinical animal study was performed to investigate endothelial healing surrogate parameters among healthy and atherosclerotic diseased animal models. As a first step, a total of 18 stents (5 BMS, 13 DES) were implanted in a rabbit iliac model for 28 days and OCT imaging performed prior to termination. Histological sections were cut every 1mm and correlated with corresponding OCT frames. There was a very good overall correlation among OCT-derived and histological morphometric measurements, while OCT resulted in slightly greater area dimensions. The ratio of uncovered to total stent struts (RUTTS) was a reliable predictor for stent strut endothelialization where a RUTTS score of 0.3 correlated with endothelialization of stent struts in the range of 75 to 80% (see **Figure 10**). Importantly, RUTTS score by OCT underestimated the true coverage rate of stent struts (RUTTS by histology) in the range of 60-80% endothelialization, while below that level of endothelialization OCT RUTTS score generally overestimated histological strut coverage. Both phenomena are explained by the limited resolution of OCT imaging, which in the range of 60-80% endothelialization is not capable to detect every endothelial cell covering stent struts, while in the higher range of endothelialization OCT tends to over-diagnose endothelial cells, which, by histology, can be diagnosed as fibrin or thrombus coverage and cannot be distinguished by OCT.

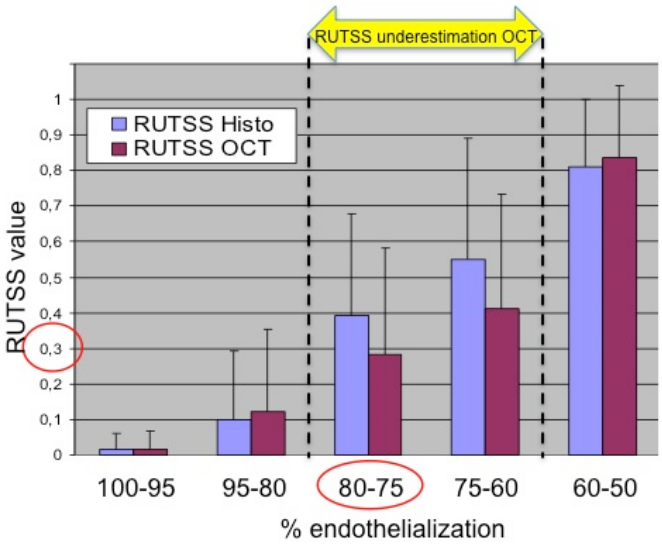


Figure 10. Correlation of endothelialisation by histology and OCT-derived RUTTS score. In the range of 60-80% of endothelialisation OCT RUTTS score tends to underestimate stent strut coverage in comparison to histological assessment; below 60% of endothelialisation, OCT RUTTS tends to overestimate histological strut coverage.

In a separate study, it was our goal to establish a novel surrogate parameter applying tissue characterization using OCT-based grey-scale signal intensity analysis. Owing to the limited capability of OCT in detecting differences at the level of tissue or cellular components, an attempt was made to define immature neointimal tissue frequently observed in human autopsy studies in the setting of late stent thrombosis. Immature tissue was composed of proteoglycans with presence of fibrin, absence of endothelial cells and sparse smooth muscle cell coverage, while mature tissue represented the opposite. This basic distinction was considered to be of clinical relevance, as it simply resembled the main pathological features of impaired vascular healing following DES

implantation. The principle of defining and detecting immature neointimal tissue was established in a preclinical correlation study, in which stented iliac arteries of atherosclerotic rabbits were investigated with OCT and histology at 28 and 42 days after stent implantation (see **Figure 11**). Following co-registration of OCT frames and histopathological cross sections, immature tissue was distinguished form mature with high sensitivity and specificity using this novel method of OCT imaging analysis based on grey scale signal intensity. These findings were subsequently confirmed in a number of human autopsy samples, which confirmed the previously established validity to detect immature tissue using OCT. Finally, the same methodology and definition of immature tissue was prospectively applied in patients at 6 to 8 months of invasive follow-up after DES implantation. Interestingly, only 28% of stent struts represented mature neointimal tissue in this study.

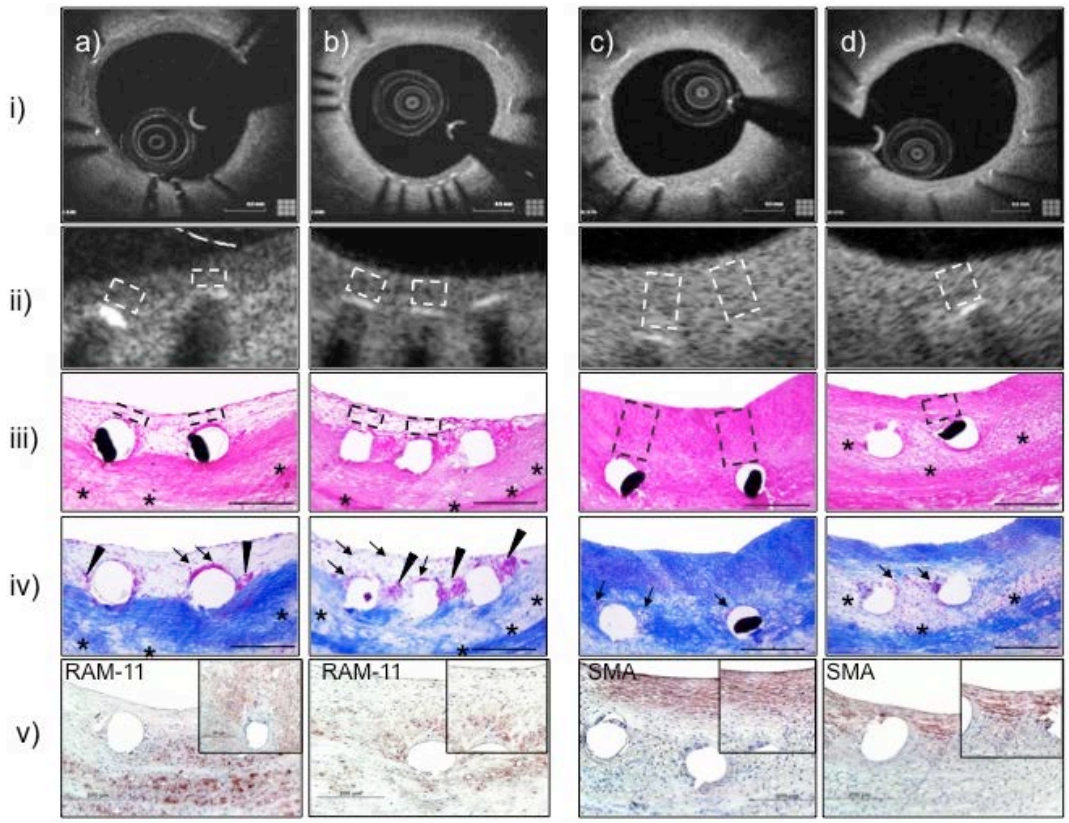


Figure 11. OCT based tissue characterization of mature (c and d) and immature (a and b) tissue following implantation of drug eluting stents. By definition, immature tissue is rich in fibrin, devoid of smooth muscle cells and proteoglycans. Endothelial cells are mostly absent in immature tissue.

Task 2 also focused on imaging of stained endothelial cells (ECs) in animal models. Staining was performed using a 2-antibody procedure in which the primary antibody would have affinity to the ECs whereas the secondary antibody would be conjugated to a fluorescent probe and would have affinity to the primary antibody. Two fluorescent probes were identified that were compatible with both the imaging catheter system and staining process: Alexa Fluor 790 and CF660C. The probes were tested on excised rabbit aortas, which were imaged *ex vivo* using a fluorescence microscope and the intravascular NIRF system. Staining

was performed on denuded and healthy aortas to assess the ability to detect ECs. Control vessels were either without the primary antibody or native (completely unstained). For each fluorescent probe, the NIRF system was optimized to enable maximum sensitivity. This included optimization of the optical filters used as well as the choice of excitation laser. Both specificity and sensitivity were assessed in the imaging experiments.

Two sets of experiments were performed. In the first set, the focus was on identifying the most appropriate fluorescent probe. Both probes were tested on healthy rabbit aortas and compared to the control. Out of the two probes, Alexa Fluor 790 demonstrated the higher sensitivity, which was higher by an order of magnitude than the one achieved by the CF660C. Nonetheless, the background signal obtained with the Alexa Fluor 790 for the control vessels was considerably higher than the one achieved for CF660C, leading to reduced specificity. Since the system's detection sensitivity was sufficient for both probes, albeit not equal, the decision on the most appropriate probe for EC detection was based solely on specificity. Since the CF660C probe demonstrated an excellent signal to background ratio, it was chosen as the preferred probe.

The second set of experiments was designed to test whether the staining process may be used for distinguishing between healthy and denuded arteries. The hypothesis was that denuded arteries, in which the endothelium was damaged, would not be properly stained. To test the hypothesis, staining with the CF660C fluorescent probe was performed for both healthy and denuded rabbit aortas. Imaging was performed using the NIRF system and compared to the control. The results showed a significantly weaker fluorescent signal in the denuded blood aortas in comparison to the healthy aortas. These findings were corroborated by a fluorescence microscopy performed on small sections of the aortas (**figure 12**).

In the final step of WP3, the promising approach of endothelial staining will be thoroughly investigated. The performance of endothelial staining will be tested in stented blood vessel with the goal of determining whether stent damage to the endothelium may be detected using this method. Additionally, imaging will be performed in time series and compared to histology. This animal study will be performed in February 2015 with results available in July 2015.

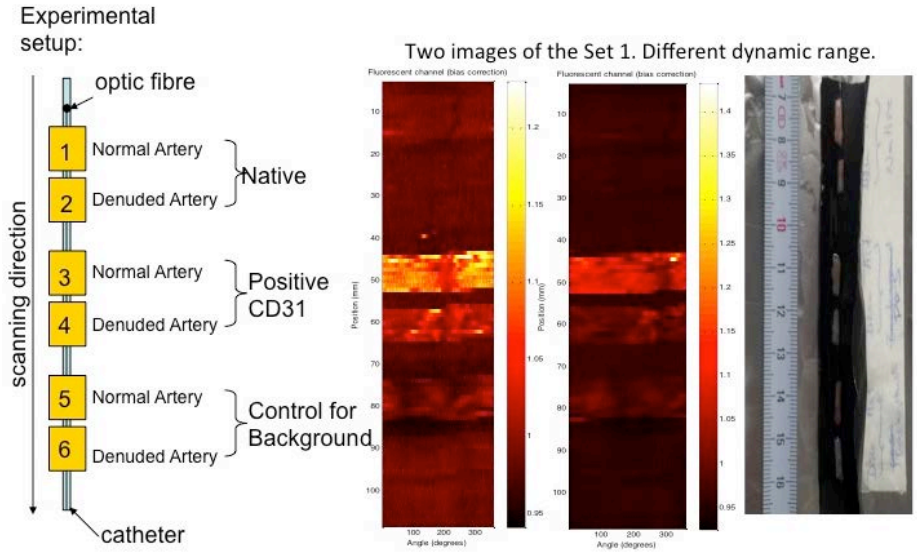


Figure 12. NIRF imaging of rabbit aorta following denudation procedure (area 2, 4 and 6) showing expected decreased fluorescence signal for endothelial cells as compared to normal artery (area 3). Area 1 and 5 represent control areas.

Task 3:	**Ex-vivo OCT imaging and histopathological analysis of clinical autopsy specimens**

A total of 8 fresh human hearts of patients suffering sudden cardiac death were submitted from medical examiners for diagnostic consultation. Hearts were grossly examined and digital radiographs taken in different position. Following X-ray examination, coronary angiography was performed to assess the presence or absence of coronary atherosclerotic lesions. A total of 9 stented coronary lesions (5 DES and 5 BMS lesions, 1 had both DES and BMS implanted) were identified. All stented lesions were interrogated by OCT imaging and subsequently processed for histopathological assessment. OCT images were co-registered to histologic sections and 420 struts from 61 sections could be reliably co-registered with appropriate OCT-detected stent struts in 61 frames. Stent struts coverage by histology was determined to be the gold-standard and referred to the presence of at least 2 complete layers of smooth muscle cells/endothelial cells above stent struts. Detection of uncovered stent struts by OCT achieved a specificity of 100%, while sensitivity was 78.9 % owing to 4 stent struts that were judged to be covered by OCT but remained uncovered by histology.

Task 4:	**Morphology of healing in patients: In vivo imaging of limus-eluting DES using OCT and NIRF fluorescence imaging**

In Task 4 imaging studies were performed to identify markers of stent failure. The stented arteries of patients suffering late stent thrombosis were evaluated with OCT and dedicated novel software to identify the main differences in signal textile characteristics and segmental

heterogeneity in coverage (see **Figure 13**). Stented segments exhibiting thrombus deposition were compared with uninvolved stented areas with competent tissue and normal reference segments. Optical signal properties of tissue coverage across these segments were evaluated for backscattering, attenuation and peak/mean intensity at 1 mm intervals. A novel OCT software displaying a cut-open view of the vessel was developed and tested for feasibility in patients with current generation limus DES to map longitudinal segmental heterogeneity of strut coverage at different time points. Results I stent thrombosis patients were compared with those of natively occurring STEMI.

A total of 620 frames were assessed in DES patients presenting with stent thrombosis. Mean signal intensity decreased from normal reference vessel to competent neointima and significantly more in presence of incompetent tissue (6.8 ± 0.85 vs 6.58 ± 0.81 vs 5.98 ± 0.89, $p < 0.001$) where the thrombus was forming, with high signal attenuation only observed at the level of incompetent tissue stent segment. At the stented segment involved by late thrombosis strut coverage has different light signal properties compared with segments unaffected by thrombus and normal reference segments.

In a second project 10 patients with current generation DES (5 with permanent polymer, 5 with reabsorbable abluminal polymer) were evaluated at 3 months follow-up for detecting heterogeneity in early vascular response. Longitudinal diversity of OCT tissue properties (intensity, attenuation, homogeneity, correlation, variance) can be effectively assessed at different stages of DES follow-up through a novel software, displaying en-face open view around 360 degrees of the stented region.

In a final project assessing the thrombus signal properties, OCT of the infarct related artery was performed in 6 patients with stent thrombosis compared with 12 patients presenting with spontaneous myocardial infarction. In patients presenting with stent thrombosis, signal intensity and backscattering of occlusive thrombus were significantly lower, and signal attenuation was significantly higher, compared with optical properties of thrombus forming in natively occurring STEMI.

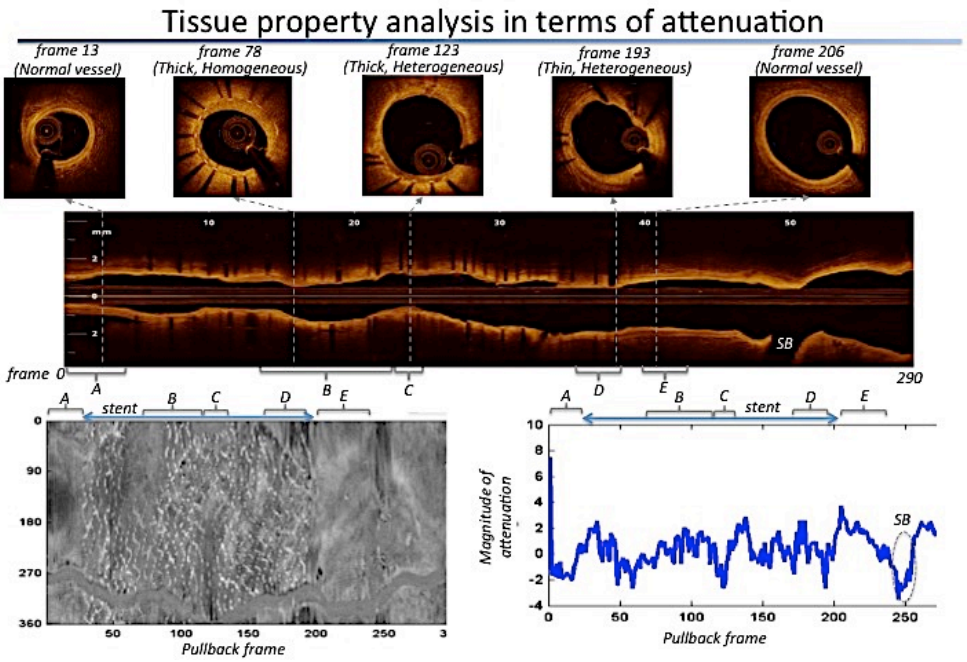


Figure 13. Tissue property analysis by OCT including textile analysis at surveillance after stenting with DES

Summary: Significant results and overall achievements

Task 1. A novel NIRF imaging catheter system was developed and fine-tuned to help establish novel surrogate parameters for endothelial healing of stent struts following implantation of DES. NIRF was shown to be capable of detecting differences in endothelialisation among denuded and intact rabbit aortas. Results of extension of testing to stented rabbit iliac arteries will be available in July 2015.

Task 2. Preclinical animal studies were performed in healthy and diseased animal models to characterize surrogate parameters of endothelial healing following implantation of DES in vivo. The ratio of uncovered to total stent struts (RUTTS) was established as a reliable surrogate parameter for endothelial healing with some limitations owing to the limited resolution of OCT imaging. A novel method for tissue characterization of stented arteries was developed and fully validated in a large translational study ranging from preclinical assessment to prospective clinical testing.

Task 3. A total of 8 human hearts were investigated by OCT imaging and histopathology to establish and validate strut coverage as a surrogate marker of endothelial healing following DES implantation.

Task 4. Signal intensity was characterized in patients with stent thrombosis presenting with stent thrombosis and found to be lower in areas of stent thrombosis compared with normal stented vessel. Moreover, in patients presenting with stent thrombosis, signal intensity and backscattering of occlusive thrombus were significantly lower compared with optical

properties of thrombus forming in natively occurring STEMI. This methodology can be applied to the established cohort of patients with stent thrombosis and will allow for a highly exploitable and innovative evaluation of these patients.

WP4: Multimodal characterisation of ST patients

Work package 4 involved the close collaboration of all PRESTIGE clinical centres including centres in central, north-western, southern and eastern Europe.

Task 1: Data Collection

An OPENCLINICA database was created by K.U.LEUVEN as an e-CRF application, used for electronic data capture and clinical data management. This database complies with all legal requirements regarding privacy of patients and quality control and is open for coded access to all PRESTIGE clinical centres. Enrolment of patients with data entry at the different sites has continued throughout the project and has been regularly monitored by the data manager at the Leuven site. Overall, 649 stent thrombosis cases have been entered into the database. Accordingly the target of patient recruitment for the WP4 clinical registry (n=500) was met and exceeded within time.

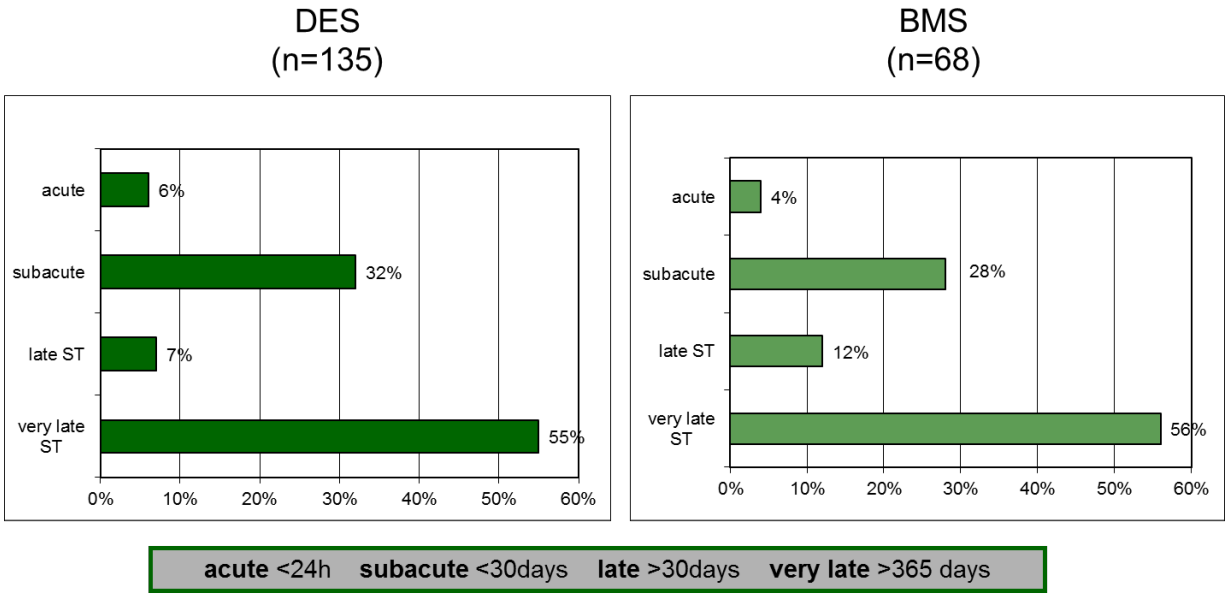
A core lab for assessment of platelet function in patients with stent thrombosis was established in NIE. Data on more than 200 patients was available with testing performed in the acute phase as well as at 30 days. In the acute phase platelet reactivity testing in patients with stent thrombosis showed that high platelet reactivity (HPR) was seen mainly at the time of presentation with ST, decreasing by 24 hours regardless of the administrated P2Y12 inhibitor. HPR in the acute phase of ST appears to be inversely associated to the available time for the antiplatelet drugs to become effective. At 30 days, At 30 days, HPR was seen most often in patients treated with clopidogrel, and more often in patients who had suffered from an early stent thrombosis. Moreover HPR rates are different when measured by each of the 2 main assays used in clinical practice, making the choice of test unclear when determining whether a patient needs another antiplatelet drug.

A core lab for the histopathologic analysis of thrombi was established in DHZ. After retrieval using aspiration catheter at individual PRESTIGE clinical centres, the thrombus was fixed in formalin and immediately shipped to the core lab in DHZ. By end November 2014, a total of 237 histopathologic samples of good quality had been collected at the core lab. This makes PRESTIGE PRESTIGE is the largest stent thrombosis registry with histological analysis of thrombus aspirates. A breakdown of the collected samples according to stent type and age of the thrombus is shown in **Figure 14**.

The main findings were that leukocyte recruitment, particularly that of neutrophils, is a hallmark of human stent thrombosis. Moreover we found more leukocytes in stent thrombi

compared to thrombi from non-stented coronary arteries. We also showed that NETs, central effectors of immunothrombosis, can also be detected in human stent thrombosis. Finally, we showed that eosinophils are recruited in ST as well as in thrombi from non-stented arteries, indicating that eosinophils might play a particular role in thrombosis.

Figure 14. Classification of thrombus samples according to type of stent and time course of stent thrombosis.



A core lab for analysis of frequency domain OCT pullbacks was established in DHM in collaboration with BER and K.U.LEUVEN. In total, 240 patients have undergone OCT imaging in the setting of stent thrombosis primary percutaneous intervention. Qualitative analysis of the pullbacks was performed by an expert panel (10 experts from the consortium, who met in Munich on 5 different occasions. all OCT pullbacks were assessed on a frame-by-frame level in the core lab, with a methodology dictated by a dedicated protocol convened among the experts prior to analysis. A major mechanism for the development of ST (e.g. uncovered struts, malapposition, neoatherosclerosis, stent thrombus alone) was ascribed to each patient and to each image run. Contributing factors were also adjudicated and documented. Each OCT acquisition was judged on image completeness and quality. Quantitative analysis was done on a frame-by-frame base in the core laboratory. Strikingly, a larger than expected proportion of neoatherosclerosis with plaque rupture was detected as a cause of stent thrombosis, a phenomenon that has never been so well characterized to date from any other database. As such this will be elucidated as a new phenomenon in much greater detail during further analysis of the results of the OCT registry.

A DNA bank of patients with stent thrombosis has been established in DHZ, NIE, ULEIC. In the majority of ST-cases (80%) that have been entered into the database to date, DNA-samples have been collected and sent or are stored under optimal conditions locally to be sent to the nearest DNA bank. A total of around 400 patients with ST have a DNA sample

stored. ULEIC has started the coordination of a proceeding for central storage and further analysis of these samples. Analysis of DNA was not allocated for funding under 260309 PRESTIGE and additional funding for this task will be sought.

Task 2:	**Data Consolidation**

Data consolidation has been performed in a highly successful manner. Electronic data capture has been used to compile a central European PRESTIGE Registry database. All participating PRESTIGE clinical centres have entered their own ST-cases as well as those provided to them by their satellite centres. Feedback to all centres and co-ordinating units has taken place on a regular and frequent basis. The data analysed in the core lab analyses for platelet function testing, thrombus histopathology and OCT analysis has also been entered into the database, to be correlated with the collected clinical data. In addition completeness of data entry and quality control of the entered data has been performed.

Construction of a late stent thrombosis predictive risk factor algorithm is planned after complete collection and analysis of all clinical data, as well as all results of platelet function testing, histopathologic analysis of thrombi, OCT and IVUS examination, and analysis of mutual correlations.

Task 3:	**Application of imaging predictors of late ST to imaging surveillance of DES-treated patients – Imaging evaluation by frequency domain OCT of polymer-modified DES platforms**

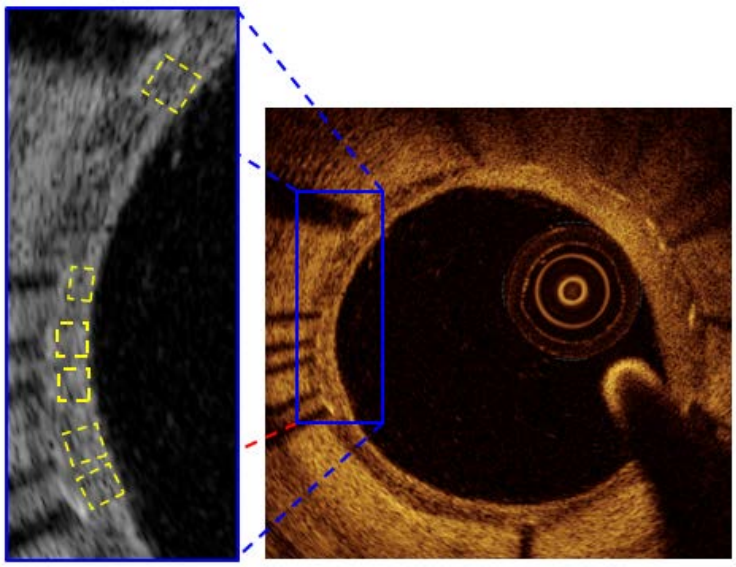
Imaging correlates of immature healing and late stent thrombosis on surveillance of DES-treated patients was done using parameters delineated in WP3. Imaging evaluation by frequency domain OCT of polymer-modified DES platforms was done by DHM. In WP3 we showed that using histopathology as gold standard, OCT is capable of characterizing mature from immature neointimal tissue after coronary stenting. In this next step in order to validate this approach in man we first performed prospective tissue characterization in 10 patients that underwent invasive surveillance 6 months after coronary DES implantation (**Figure 15**).

We found that while stents were covered with neointimal tissue, most analysed areas were categorized as immature tissue type (62.3%), likely indicative of on-going heightened risk of stent thrombosis 6 months after stenting (Malle et al. ATVB 2013). This established proof-of-concept that OCT is capable of distinguishing between mature and immature neointimal tissue which is found covering stent struts following coronary stent implantation. In addition an analysis of healing after implantation of limus-eluting stents with biodegradable polymer

versus durable polymer has been completed (Tada et al. Int J Cardiovasc Imaging 2014). OCT qualitative analysis was assessed in 34 patients (**Figure 16**). The incidence of peri-strut low-intensity area (PLIA) – another potential marker of delayed arterial healing and of risk for stent thrombosis – was evaluated in both groups. Patients with PLIA had higher levels of neointimal hyperplasia (**Figure 17**). The proportion of patients who had any frames with PLIA was similar in both groups (41.2 % in BP-BES and 36.4 % in PP-EES, P = 0.>99). Frames with PLIA as compared to those without had higher percent hyperplasia obstruction. In a second randomized study an analysis of 59 patients treated with drug-eluting stents coated with sirolimus and biodegradable polymer (BP-SES) versus everolimus and permanent polymer (PP-EES) including tissue characterization (GSI) methodology has been completed. Interestingly while tissue coverage was higher with PP-EES, coverage with mature tissue was higher with BP-SES. Long-term follow-up of these patients will be undertaken to determine the clinical significance of these findings.

Finally parallel collection of OCT data in ST cases has been a cornerstone of task 1 of WP4. As of 30/11/2013, data on 140 cases was collected and analysed (see above).

Figure 15.OCT pullback after DES implantation illustrating identification of regions of interest for grey-scale signal intensity analysis.



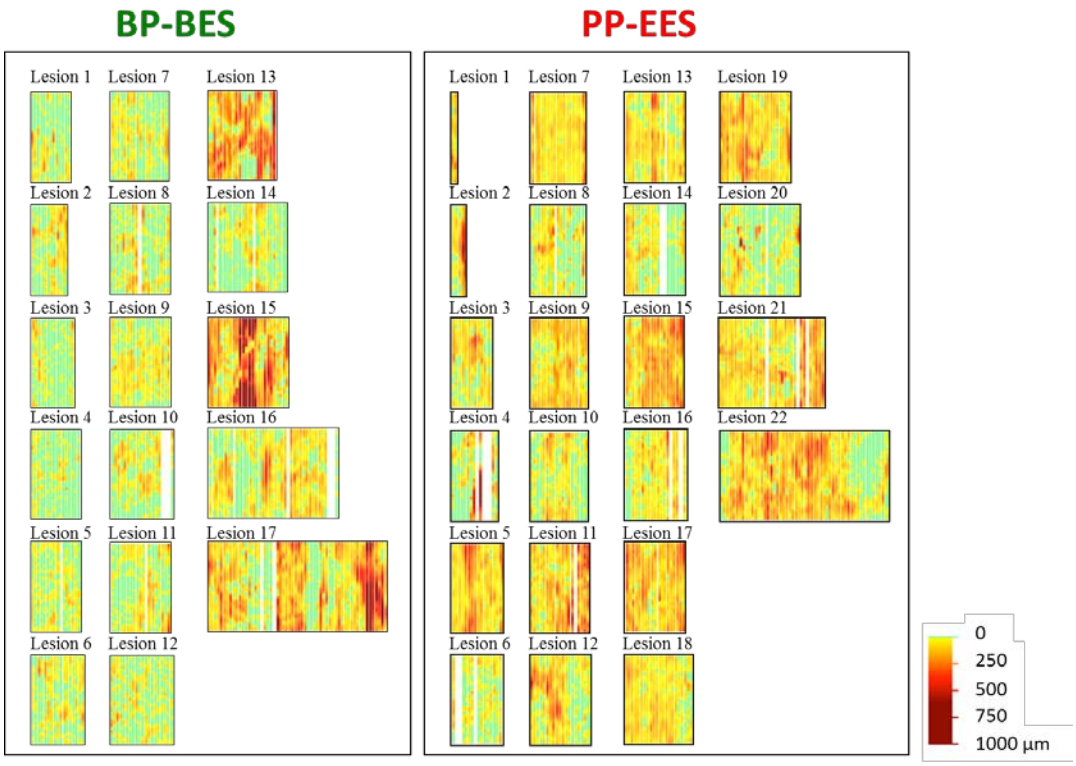


Figure 16. Strut-level neointimal thickness from OCT surveillance at 6-8 months after randomized comparison of drug-eluting stent coated with biolimus and biodegradable polymer (BP-DES) versus everolimus and permanent polymer (PP-DES)

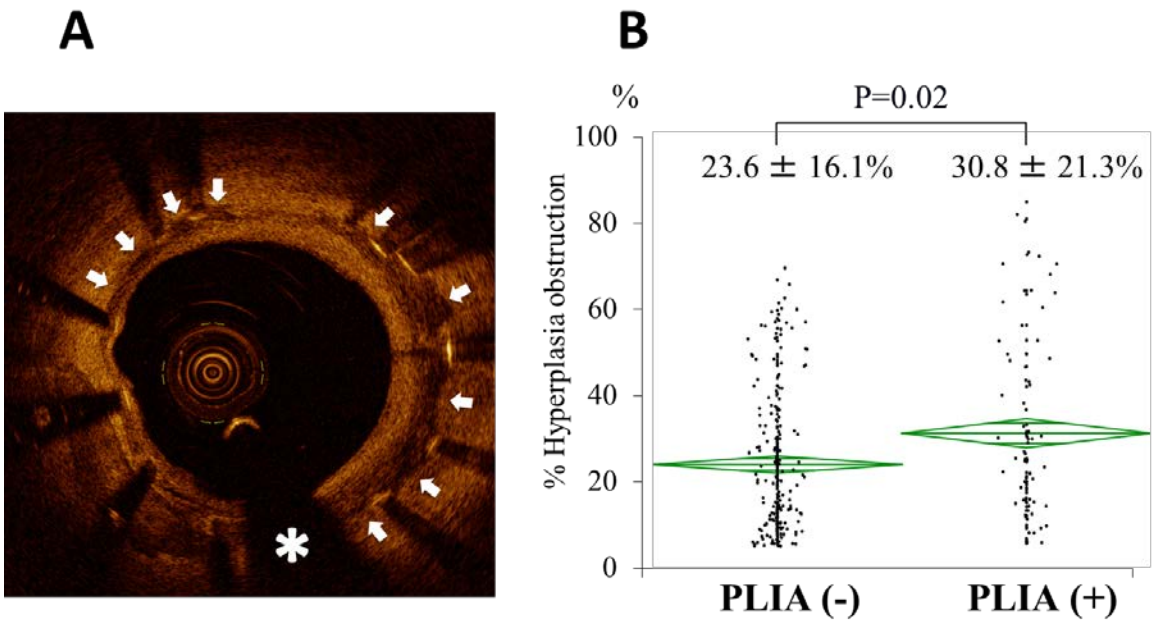


Figure 17. (A) Evaluation of peri-strut low-intensity areas (PLIA)(arrows) by OCT surveillance at 6-8 months after randomized comparison of drug-eluting stent coated with biolimus and biodegradable polymer (BP-DES) versus everolimus and permanent polymer (PP-DES); *=guide-wire artefact; (B) Percentage hyperplasia obstruction in patients with and without PLIA

Summary: Significant results and overall achievements

Task 1 and Task 2: With the current status of collection of stent thrombosis cases (>640 cases in total and 240 cases of OCT data in stent thrombosis), we met the objectives set out at the initiation of this project. In addition complete data entry, data consolidation in the database and quality control of these data has been performed.

In terms of platelet function, in the acute phase of stent thrombosis platelet is high, decreasing by 24 hours regardless of the administrated P2Y12 inhibitor. HPR in the acute phase of stent thrombosis appears to be inversely associated to the available time for the antiplatelet drugs to become effective. At 30 days, At 30 days, HPR was seen most often in patients treated with clopidogrel, and more often in patients who had suffered from an early stent thrombosis.

In terms of histopathology of thrombus aspirate the main findings were that leukocyte recruitment, particularly that of neutrophils, is a hallmark of human stent thrombosis. Moreover more leukocytes were found in stent thrombi compared to thrombi from non-stented coronary arteries, suggesting differential pathophysiological mechanisms between stent thrombosis and native coronary thrombosis.

The collection of a large number of OCT image acquisitions in the setting of acute stent thrombosis is also unique. With 220 analysable and high quality cases collected, this is by far the largest dataset of this kind worldwide. The main finding was that in a larger than expected proportion of cases neoatherosclerosis was the dominant cause of stent thrombosis.

This has been a truly collaborative European research enterprise that can act as a model for shared research in patients with cardiovascular and other diseases. The data collected are very substantial and efforts will continue to integrate all results and analyses obtained in order to allow genuine patient benefit. Integration of all data obtained will allow us to understand on a per patient basis the issues that have led to the life-threatening stent thrombosis event. These understandings will pave the way for improvement in stent implantation technique, stent manufacturing and medical therapy after stent implantation. Given the important efforts made to build the electronic database, to set up core labs for different subtasks, and the good understanding between consortium partners, it was decided to continue the established clinical register of patients with ST, beyond FP7 funding.

PRESTIGE: Potential Impact and Main Dissemination Activities

The overall impact of our project on the reduction of in-stent thrombosis will result from both the scientific knowledge acquired and the new technologies realised over the course of the project.

The impact on prevention of late stent thrombosis

All work packages of the current project provide novel insights into the mechanistic understanding of stent thrombosis, thereby generating a basis for innovative preventive strategies. Prevention of stent thrombosis is of utmost importance owing to its high case mortality rate. Future progress will be realised through the development of novel anti-thrombotic coatings for stent devices as well as novel molecular targets for antithrombotic therapies. A number of areas with potential key impact should be highlighted.

Firstly, the successful development of a mouse model of stenting with the successive design, production and testing of five generations of stents was central achievement of PRESTIGE. The design of the mouse stents closely resembles that of stent platforms used in patients. The novel approach utilized overcomes limitations of previously described mouse stenting models. Subsequent investigation showed that this model is capable of inducing stent thrombosis inside 24 hours and that thrombus morphology shares many similar features with stent thrombus sin humans. The impact of this model is likely to be significant for researchers in cardiovascular medicine. This model will allow consortium members and other researchers to investigate additional molecular pathways involved in stent thrombosis. Moreover, this model will facilitate study of the impact of genetic mutations on haemostasis and thrombosis in the setting of stent implantation.

Secondly, the elucidation of the mechanisms underlying interactions between Co-Cr stents and platelets as well as Co-Cr and coagulation pathways presents opportunities for future research targeting these pathways. We dissected the molecular and cellular mechanisms of stent thrombosis in vitro using a newly established model of CoCr disks. The results obtained so far clearly point towards prothrombotic properties of CoCr stent platforms. Indeed, our present finding indicate that CoCr may activate both the coagulation cascade, platelets and leucocytes and is in line with the recent clinical observation that additional long-term anticoagulant treatment has superior anti-ischemic efficacy in patients undergoing stent placement compared to antiplatelet therapy alone. This may open new avenues to treat and prevent thrombotic events in patients with advanced coronary artery disease receiving stents.

Thirdly, quite unexpectedly, endothelial cells were found to grow on the stent material in vitro. This is likely to be an important finding for future studies as well as for other researcher sin the field of stent development. Moreover the successful establishment of a GPVI humanized (hGPVI) mouse model provides a useful tool allowing future *in vivo* analysis of the efficacy of antagonists targeting human GPVI as novel strategy to prevent stent thrombosis. In addition, studies performed to get further insight into the mechanism of thrombus growth show that GPVI represents a yet unidentified receptor for polymerized fibrin, the interaction between fibrin and GPVI being involved in the amplification of

thrombin generation and in the recruitment of platelets by fibrin-rich clots. This represents a potentially important target for pharmaceutical therapy.

Fourthly, the realization of stent coatings that foster endothelialization and promote vascular healing has potential for direct clinical application. In particular, fabrication and characterization of RGD peptidomimetic coatings and investigation of chitosan/PLA co-polymers and dextran-based copolymers show particular promise. Establishment of appropriate animal models to investigate the in vivo behaviour of different stent prototypes has enabled further pre-clinical evaluation of these devices. Moreover, execution of a dedicated long-term animal study to investigate the inflammatory response and biocompatibility of completely bioabsorbable magnesium stents furthers the development of novel stent platforms designed to address the problem of stet thrombosis very late after coronary intervention.

The impact on prediction of late stent thrombosis

Considerable progress was also made on impacting the prediction of stent thrombosis. In terms of novel imaging approaches investigated to identify patients at risk, significant impact is expected from at least 3 developments.

Firstly, a novel NIRF imaging catheter was to enable two-dimensional intravascular in vivo imaging of fluorescent probes in arteries with similar dimensions to those of human coronary arteries. This catheter system was then further refined and fine-tuned to help establish novel surrogate parameters for endothelial healing of stent struts following implantation of DES. NIRF was shown to be capable of detecting differences in endothelialisation among denuded and intact rabbit aortas. Results of extension of testing to stented rabbit iliac arteries will be available in July 2015.

Secondly, reliability of OCT imaging in assessing amount, distribution and type of tissue covering the stent struts was compared with co-registered histopathology in animal and autopsy studies. Dedicated preclinical and human autopsy studies were conducted to correlate intravascular OCT imaging with histopathology. Innovative tissue characterization software based on grey-scale signal intensity (GSI) analysis was validated to distinguish mature from immature neointimal tissue after stent implantation and the validated algorithm was subsequently tested in a first-in-man clinical study.

Thirdly, regarding the detection of neoatherosclerotic change after stenting, a preclinical model has been established by investigators of the consortium. Foam cell accumulation around stent struts was identified as an important disease hallmark. This development has already led to subsequent histopathological/imaging correlation studies which will play an important role in defining the key imaging characteristics required to identify this important disease substrate.

In terms of specific clinical data, a number of findings with direct clinical impact have already been reported.

Firstly, a unique database comprising data from detailed multimodal assessment of patients presenting with stent thrombosis to the nine participating clinical centres (>640 cases in total and 240 OCT datasets) has been acquired and consolidated in line with the identified work plan. To the best of our knowledge this represents the largest collection of stent thrombosis cases acquired to date worldwide. Future exploitation of this data by consortium partners and other researchers will permit construction of a patient level risk stratification model including clinical data, platelet function testing and bioimaging data and will provide a basis for a comprehensive predictive approach to the problem of stent thrombosis. The primary results of the data analysis will be presented at the European Society of Cardiology meeting in London in August 2015.

Secondly, in terms of platelet function, in the acute phase the finding that platelet reactivity appears to be inversely associated with the available time for the antiplatelet drugs to become effective is important.

Thirdly, in terms of histopathology of thrombus aspirate the main findings were that leukocyte recruitment, particularly that of neutrophils, is a hallmark of human stent thrombosis. Moreover more leukocytes were found in stent thrombi compared to thrombi from non-stented coronary arteries, suggesting differential pathophysiological mechanisms between stent thrombosis and native coronary thrombosis.

Finally, the collection of a large number of OCT image acquisitions in the setting of acute stent thrombosis is also unique. With 220 analysable and high quality cases collected, this is by far the largest dataset of this kind worldwide. The main finding was that in a larger than expected proportion of cases neoatherosclerosis was the dominant cause of stent thrombosis.

The role of future genetic analyses

Although not directly funded in the grant agreement the collection of genetic data in patients with stent thrombosis was provided for in the grant agreement. The precise phenotypic characterization of the 640 stent thrombosis cases and the availability of DNA samples in a high proportion of cases provide an ideal platform for a highly exploitable genetic analysis. The first steps have already been initiated in drawing up a follow-on agreement between the participating clinical centres to collaborate on analysis and exploitation of this data.

The social impact

Addressing concerns regarding the late safety of drug-eluting stents is vital to the further widespread adoption of these devices. The impact of drug-eluting stents on enhancing quality of life, reducing patient morbidity (and possibly mortality), and reducing economic costs is very sizeable and optimizing DES utilization and reducing the burden of ST will result in significant societal benefit.

The impact on European cardiovascular research networks

Meeting the challenges involved in reducing the burden of ST is best accomplished utilizing a pan-European approach, drawing on the proximity and interrelationship of numerous excellent researchers in the respective fields of medicine and industry. A European approach not only facilitated the interaction of all consortium partners but also provides a broad spectrum of outstanding research expertise drawn from a range of different European countries. The establishment of such networks between researchers and industrial partners may also provide great future potential and flexibility to meet the changing needs of clinical research in interventional cardiology.

In relation to the clinical work package (WP4) in particular, this has been a truly collaborative European research enterprise that can act as a model for shared research in patients with cardiovascular and other diseases. The data collected are very substantial and efforts will continue to integrate all results and analyses obtained in order to allow genuine patient benefit. Integration of all data obtained will allow us to understand on a per patient basis the issues that have led to the life-threatening stent thrombosis event. These understandings will pave the way for improvement in stent implantation technique, stent manufacturing and medical therapy after stent implantation. Given the important efforts made to build the electronic database, to set up core labs for different subtasks, and the good understanding between consortium partners, it was decided to continue the established clinical register of patients with ST, beyond FP7 funding.

Overall the work of the consortium has already stimulated pan European cooperation on a number of related research topics outside of the scope of PRESTIGE. We envisage that this will strengthen the European pre-eminence in cardiovascular research.

The impact of the multidisciplinary approach

The overall aim of the current proposal was the development of novel strategies to reduce the burden of late ST. Given the multi-factorial and complex character of this disease, a close collaboration between industrial and research partners including basic researchers, developmental scientists, as well as preclinical and clinical researchers is of highest importance. For the successful translation of laboratory findings into clinical interventions, an interactive interplay between independent and complementary competencies in the field of vascular biology, polymer design as well as pre-clinical and clinical evaluation was of

critical importance. In the current consortium, experts in the field of platelet research and coagulation were involved in the establishment of novel targets for the inhibition of stent thrombosis. For this investigation, extensive collaborative research in the field of vascular biology, stent design and developmental science was needed. For the experimental development of the stent prototypes intended for preclinical and clinical deployment, all consortium partners involved in technical stent design cooperated to achieve this goal. For the assessment of clinical risk factors and findings from bioimaging studies will also facilitate the design of optimized anti-thrombotic treatments. This network of expert investigators is now well established and will facilitate future multidisciplinary collaboration across a variety of projects related to cardiovascular disease

The impact on other vascular diseases

The results of the PRESTIGE project are expected to have additional impact on several vascular disease entities apart from the impact on late stent thrombosis.

De novo coronary artery disease – General impact on coronary artery disease may be expected through the optimisation and refinement of invasive and non-invasive imaging and treatment strategies. The identification of novel targets that selectively interfere with platelet-stent interactions and coagulation pathways are also likely to offer significant benefit to patients selected for non-invasive treatment. Several clinical trials have already shown that an optimized interference with pro-thrombotic pathways has a clear beneficial effect on major adverse outcomes of patients presenting with CAD throughout a large variety of patient populations.

Cerebrovascular and peripheral artery disease – The promising data observed with novel stent platforms leveraging plasma treatment of stent surfaces, attachment of peptidomimetics to attract endothelial cells, and passivation of stent-blood interactions with chitosan polymer and dextran-based copolymers might of wide-reaching impact for the percutaneous treatment of obstructive vascular disease in the cranial and peripheral vascular bed. Moreover the identification and characterization of OCT parameters indicative of mature and immature tissue healing after stenting might conceivably be applied to clinical imaging in cerebral and peripheral vascular disease.

Main dissemination activities and exploitation of results

The dissemination of results arising from the work of the consortium has been particularly successful. Some of the key dissemination activities were as follows:

1. A public website was created and successfully used to co-ordinate actions of the consortium and to disseminate its work.
2. A public information workshop was organised in August 2012 in Munich.
3. A Flyer with information about the project was developed, designed and printed.

4. Dedicated scientific presentations outlining the work of the consortium took place at: the CRT meeting in Washington DC in February 2014, European Society of Cardiology meeting in Amsterdam 2014 and the Transcatheter Cardiovascular Therapeutics meeting in San Francisco 2014.
5. Popular scientific articles highlighting the work of the consortium were published in Circulation and the European Heart Journal (see **Figure 12**).
6. A final scientific workshop was organized at Freising, near Munich in November 2014, including a popular scientific component targeted at general public.
7. Press releases on new stent technology and novel methods of intravascular imaging were prepared and disseminated.
8. Approximately 15 manuscripts reporting results from the work of the consortium have already been published in the peer-reviewed scientific literature.

Publications

A list of the key scientific publications published in peer-reviewed literature to date is included here:

- **Tissue Characterization After Drug-Eluting Stent Implantation Using Optical Coherence Tomography Significance**

  C. Malle, T. Tada, K. Steigerwald, G. J. Ughi, T. Schuster, M. Nakano, S. Massberg, J. Jehle, G. Guagliumi, A. Kastrati, R. Virmani, R. A. Byrne and M. Joner

  Published in: Arteriosclerosis, Thrombosis, and Vascular Biology, Vol. 33, No. 6, June 2013, pp. 1376-1383.

- **A new dextran-graft-polybutylmethacrylate copolymer coated on 316L metallic stents enhances endothelial cell coverage**

  S.M. Derkaoui, A. Labbé, P. Chevallier, S. Holvoet, C. Roques, T. Avramoglou, D. Mantovani, D. Letourneur.

  Published in: Acta Biomaterialia, Vol. 8, No. 9, September 2012, pp. 3509-3515.

- **Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo**

  von Brühl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, Khandoga A, Tirniceriu A, Coletti R, Köllnberger M, Byrne RA, Laitinen I, Walch A, Brill A, Pfeiler S, Manukyan D, Braun S, Lange P, Riegger J, Ware J, Eckart A, Haidari S, Rudelius M, Schulz C, Echtler K, Brinkmann V, Schwaiger M, Preissner KT, Wagner DD, Mackman N, Engelmann B, Massberg S.

Published in: The Journal of Experimental Medicine, Vol. 209, No. 4, April 2012, pp. 819-835.

- **A Humanized Glycoprotein VI (GPVI) Mouse Model to Assess the Antithrombotic Efficacies of Anti-GPVI Agents**

  Mangin PH, Tang CJ, Bourdon C, Loyau S, Freund M, Hechler B, Gachet C, Jandrot-Perrus M.

  Published in: The Journal of Pharmacology and Experimental Therapeutics, Vol. 341, No. 1, January 2012, pp. 156-163.

- **Polymer-free sirolimus- and probucol-eluting vs. new generation zotarolimus-eluting stents in coronary artery disease**

  Massberg S, Byrne RA, Kastrati A, Schulz S, Pache J, Hausleiter J, Ibrahim T, Fusaro M, Ott I, Schömig A, Laugwitz KL, Mehilli J.

  Published in: Circulation, Vol. 124, Issue 5, 2011, pp. 624-632.

- **Polymer-free immobilization of a cyclic RGD peptide on a nitinol stent promotes integrin-dependent endothelial coverage of strut surfaces**

  Michael Joner , Qi Cheng , Sabine Schýnhofer-Merl , Monica Lopez , Stefanie Neubauer , Carlos Mas-Moruno , Burkhardt Laufer , Frank D. Kolodgie , Horst Kessler , Renu Virmani

  Published in: Journal of Biomedical Materials Research - Part B Applied Biomaterials, Vol. 100B, Issue 3, pp. 637-645

- **Functional Comparison of Induced Pluripotent Stem Cell- and Blood-Derived GPIIbIIIa Deficient Platelets**

  Mathias Orban , Alexander Goedel , Jessica Haas , Kirstin Sandrock-Lang , Florian Gýrtner , Christian Billy Jung , Barbara Zieger , Elvira Parrotta , Karin Kurnik , Daniel Sinnecker , Gerhard Wanner , Karl-Ludwig Laugwitz , Steffen Massberg , Alessandra Moretti

  Published in: PLoS One, Vol. 10, Issue 1, e0115978

- **Critical Role of Platelet Glycoprotein Ibý in Arterial Remodeling**

  S. Chandraratne, M.-L. von Bruehl, J.-I. Pagel, K. Stark, E. Kleinert, I. Konrad, S. Farschtschi, R. Coletti, F. Gaertner, O. Chillo, K. R. Legate, M. Lorenz, S. Rutkowski, A. Caballero-Martinez, R. Starke, A. Tirniceriu, L. Pauleikhoff, S. Fischer, G. Assmann, J. Mueller-Hoecker, J. Ware, B. Nieswandt, W. Schaper, C. Schulz, E. Deindl, S. Massberg

  Published in: Arteriosclerosis, Thrombosis, and Vascular Biology

- **Crossroads of coagulation and innate immunity: the case of deep vein thrombosis**

  C. Schulz , B. Engelmann , S. Massberg

  Journal of Thrombosis and Haemostasis, Vol. 11, pp. 233-241

- **Thrombocytosis as a Response to High Interleukin-6 Levels in cGMP-Dependent Protein Kinase I Mutant Mice**

  L. Zhang, R. Lukowski, F. Gaertner, M. Lorenz, K. R. Legate, K. Domes, E. Angermeier, F. Hofmann, S. Massberg

  Published in: Arteriosclerosis, Thrombosis, and Vascular Biology, Vol. 33, Issue 8, pp. 1820-1828

- **Sphingosine kinase 2 (Sphk2) regulates platelet biogenesis by providing intracellular sphingosine 1-phosphate (S1P)**

  L. Zhang , N. Urtz , F. Gaertner , K. R. Legate , T. Petzold , M. Lorenz , A. Mazharian , S. P. Watson , S. Massberg

  Published in: Blood, Vol. 122/Issue 5, pp. 791-802

- **Thrombosis as an intravascular effector of innate immunity**

  B. Engelmann , S. Massberg

  Published in: Nature Reviews Immunology, Vol. 13, Issue 1, pp. 34-45

- **Capillary and arteriolar pericytes attract innate leukocytes exiting through venules and 'instruct' them with pattern-recognition and motility programs**

  Konstantin Stark, Annekathrin Eckart, Selgai Haidari, Anca Tirniceriu, Michael Lorenz, Marie-Luise von Brühl , Florian Gärtner, Alexander Georg Khandoga, Kyle R Legate, Robert Pless, Ingrid Hepper, Kirsten Lauber, Barbara Walzog, Steffen Massberg

  Published in: Nature Immunology, Vol. 14, Issue 1, pp. 41-51

- **Randomized comparison of biolimus-eluting stents with biodegradable polymer versus everolimus-eluting stents with permanent polymer coatings assessed by optical coherence tomography**

  Tomohisa Tada, Adnan Kastrati, Robert A. Byrne, Tibor Schuster, Rezarta Cuni, Lamin A. King, Salvatore Cassese, Michael Joner, Jürgen Pache, Steffen Massberg, Albert Schömig, Julinda Mehilli

  Published in: International Journal of Cardiovascular Imaging, Vol. 30, Issue 3, March 2014, pp. 495-504

Figure 12. Popular scientific articles highlighting the work of the consortium from the European Heart Journal

PRESTIGE: Address of the project public website

Further details of the PRESTIGE project are available at a public-access website **(Figure 13**) at www.prestige-fp7.eu

Figure 13. PRESTIGE project home page