1 Publishable summary

1.1 Summary of project objectives

Valve replacement with mechanical or bioprosthetic prostheses is the most common intervention for valvular disease, with almost 300.000 annual replacements worldwide. There are more and more patients with grown-up congenital heart disease (GUCH) who have survived into adulthood thanks to surgical innovations and will require one or more heart valve replacements later in life. Although existing valve prostheses generally have resulted in enhanced survival and quality of life, prosthesis associated problems affect 30-35% of the patients within 10 years post operatively. In particular young recipients of current heart valve replacements have significantly reduced life expectancy (up to 50%) compared to age matched healthy individuals, and experience a high probability of serious valve-related morbidity throughout life. In patients younger than 18 years freedom from reoperation is only 58-68% at 15 years.

Heart valve prosthesis associated complications include thrombo-embolic events requiring lifelong anticoagulation in case of mechanical valves, and limited durability due to calcification and structural failure in case of biological valve substitutes. Prosthetic valves are non-viable structures and, therefore, do not have the ability to grow, repair or adjust to functional demand changes. Living, tissue engineered heart valves are expected to overcome these limitations.

In situ tissue engineering using a biodegradable synthetic scaffold that recruits endogenous cells from the bloodstream is emerging as a promising technology to create living heart valves inside the human body having the potential to last a lifetime. Compared to classical tissue engineered heart valves this new technology demonstrates off-the-shelf availability at substantially reduced cost, logistics and regulatory complexity.

The ImaValve project aims to develop intelligent materials needed for the in-situ engineering of heart valves, to process these materials into a functional heart valve scaffold that can be implanted via a minimally-invasive (transcatheter) implantation technique at the aortic position, and to take all necessary (pre-clinical) steps to enable a first-in-man clinical trial after completion of the project.

A novel approach to the biodegradable scaffold is pursued, that combines a relatively slow degrading elastomeric material with a fast degrading bioactive hydrogel material. These materials are processed into a fibrous heart valve scaffold by means of electrospinning. The elastomeric material ensures long term functionality of the valve while supporting in-vivo mature tissue formation, whereas the fast eroding hydrogel material modulates the early inflammatory host response to the scaffold and creates the necessary void space for cells and neo-tissue formation between the elastomeric fibers.

To meet our goals we have planned to:

i. Develop supramolecular slow degrading (months), durable elastomeric materials and fast eroding (week) hydrogel materials that can be rendered bioactive. Combine these materials into an electrospun heart valve scaffold.

ii. Develop a stent-scaffold combination that is suitable for transcatheter delivery of the heart valve scaffold, and that has sufficient strength and durability to sustain the pulsatile hemodynamic loads.

iii. Develop a mechanistic understanding of the human host response to the scaffold, and the effect of selected bioactives (i.e. TGF-β, MCP-1, SDF-1α) on this host response and subsequent early tissue formation

iv. Achieve sufficient alignment and associated matrix anisotropy of the in-situ deposited collagen to prevent valve leaflet retraction and to attain long-term cell and tissue homeostasis.

v. Demonstrate that the implanted heart valve scaffold in vivo will transform into a functional, living stable heart valve.

1.2 Summary of the work in the reporting period

The achievements of the project are described below. Next to successful materials development and laboratory and computational modeling to understand and predict the process of in-situ heart valve formation, we tested the first ImaValve prototypes, consisting of slow degrading elastomeric scaffolds sutured in a stent, that were implanted in a sheep model using the prototype delivery system developed within the project. While initial acute in-vivo tests where promising, new acute in-vivo tests urged us to rethink our valve/stent/delivery design; a process that we perform systematically as a team.

Polymer synthesis, processing and testing:
Several UPy-polymers based on biodegradable and biocompatible polymer backbones have been designed and synthesized. The synthesis is being optimized to be fully applicable to GMP-production standards. The biocompatibility was tested by performing a cytotoxicity test, an acute systemic toxicology study, an intracutaneous irritation test, a pyrogenicity test and complement system activation tests all according to ISO10993. All tests show that the UPy-polymers are not toxic. After extensive fatigue-testing, an optimal candidate was selected to be used as scaffold material. This material has successfully been processed into a heart-valve scaffold by electro-spinning. This scaffold was processed into a heart-valve followed by an improvement on the heart-valve design leading to excellent hydrodynamic performances.

Incorporation of bioactives into Upy polymers:
Significant progress has been made on bio-activation of the UPy-polymers to enhance specific cell-ingrowth to accelerate tissue formation. The work focused on the development of UPy-modified bioactive compounds, mainly peptides, including the design, synthesis and characterization of the bioactive compounds. Furthermore, the development of protocols for the incorporation of the UPy-bioactives into the UPy-polymer materials have been developed.

Stent design and stent-scaffold combination:
The stent for the valvular scaffold – prepared by MEDIC – is based on an original JenaValve design. Recently UZH requested to have 'imagable' markers on the stent to aid in positioning of the valve. These markers have been added, requiring no major changes to the stent design. The elastomeric scaffold is sutured in the stent using soft suture wires as advised and tested by partner XELTIS. Upon suturing, scaffolds are pre-shaped to achieve an optimal geometry for opening and closing

under systemic hemodynamic loads, as predicted by computational modelling by partner TU/e. The stent-scaffold combination is tested under conditions that mimic the in-vivo and future clinical situation. This includes: clinically relevant sterilization procedures, pre-crimping in ice, and testing under hemodynamic loading conditions in a pulse-duplicator. A purposely established "design team" consisting of partners from UHZ, TU/e, MEDIC and XELTIS oversees all required systematic steps of this procedure while providing a design and test document including all tests, failures and successes.

Design of delivery catheter:
Several versions of a prototype for a transapical delivery system were built that can implant the stent scaffold. The first prototype, of TA 23Fr, was found in testing to be too small to fit the prosthesis inside the capsule so the size was increased to 27Fr. A second prototype had a capsule made of metal, which turned out to provide excess friction with the rubber-like surface of the valve scaffold, resulting in problems with releasing the bottom part of the prosthesis.

The third generation prototype had a 28Fr capsule made of polymer, which provides a smooth inner surface, resulting in less friction between the valve and the capsule. Therefore, the prosthesis could be released easily. The fourth prototype solved an issue with temporary blocking of the flow through the aorta and associated fibrillation by an initial release of about 20% followed by a second release of about 80% of the prosthesis. Meanwhile, some details such as the ergonomics of the device were adjusted and 3D printed to be tested and to give a better experience to the surgeon.

Recruitment of cells and stimulating tissue-forming fate
In the in situ tissue engineered heart valve it is essential that the patient's own cells are recruited to the scaffold and that these cells are stimulated to form the novel heart valve tissue. The supramolecular materials allow for modular incorporation of bioactive compounds in the scaffold. We have successfully incorporated peptides into the scaffold material that can recruit macrophages and stimulate them towards the tissue-forming fate. The implementation of bioactives in the scaffold to recruit cells and stimulate tissue formation can help reduce inherent inter patient variation and improve outcomes of treatment.

Stability of tissue under mechanical loading
In later stages of tissue formation the tissue must reach homeostasis so that the tissue becomes neither hypotrophic nor hypertrophic. The mechanical stretch imposed by hemodynamic forces on the tissue engineered heart valve has an effect on differentiation and proliferation of the cells and thus on the tissue homeostasis. This process has been modelled in a mathematical model by implementing experimentally obtained values for cellular sensitivity to mechanical loading. This mathematical model can now be combined with a newly developed experimental model to develop and test hypotheses to understand and possibly control the interplay between hemodynamics, tissue formation and homeostasis.

Cell and tissue homeostasis:

After cell recruitment and differentiation (WP3), the cells within the scaffold need to produce a functional, organized matrix that upon degradation of the scaffold can bear the cyclic loads applied to the valvular tissue. The challenge will be to establish remodeling of the in situ synthesized tissue into a healthy, mature, load-bearing architecture, and to achieve cell and tissue homeostasis to maintain valve stability. In WP4 the underlying mechanisms of tissue adaptation are investigated. These mechanistic insights are essential with regard to the design of the ImaValve in terms of promoting successful growth and remodeling and tissue homeostasis. A novel bioreactor system was designed, tested and validated to measure cell traction in tissues subjected to cyclic deformation, and to track temporal changes in tissue mechanics nondestructively. The knowledge gained from this experimental platform will be used to inform a computational model of the heart valve to assess the long-term functionality of the ImaValve. Cellular traction forces and collagen remodelling have already been implemented in this model. Currently, we are working on adding tissue growth to our computational framework. Additionally, motivated by the first acute implantations of the ImaValve, our computational framework was employed to enhance the product's design.

Preclinical experiments:

Seven implantations were performed at UHZ to optimize the crimping procedure, ImaValve loading and functionality during the acute survival phase in both pulmonary (n=2) and aortic (n=5) positions. Valves delivery was optimized based on a fluoroscopy visualization and intraoperative assessment was achieved by transesophageal echocardiography (TTE) prior the sacrifice and explantation. Transapical deliveries into the pulmonary position confirmed implantation feasibility, nevertheless further optimization of the delivery device was required. Preserved valve functionality was confirmed what allowed for a transition to the aortic (high hemodynamic load) position.

First TAVI procedures confirmed the valve ability to withstand the crimping and delivery under the aortic pressure load, nevertheless, inadequate valve leaflets' coaptation behavior was noted in situ. This led to valve insufficiency confirmed by the presence of echocardiographic regurgitation jet. To minimize this undesired complication further valve design optimization was undertaken including the adjustment of valve geometry, leaflets thickness and length. Assessment of further implantations proved the changes positively influencing the valve delivery, coronary arteries flow preservation, closing-opening leaflets pattern as well as marked reduction of regurgitation accounting for the valve functionality improvement. Histological evaluation of the explants early cellular infiltration, has been started. While based on these results, a strictly defined optimal valve design was determined, some unexpected stent struts malposition was also encountered upon crimping and delivery. Further in vitro testing suggested the stent fatigue accounting for these alterations. Additional in vitro tests and acute in vivo implantations are expected to confirm these findings before starting the chronic animal survival phase.

Regulatory plan:

As decided by the consortium already in the first reporting period, a two-pronged approach was followed to accelerate development and implementation of a valvular scaffold: a) to pursue initial development of a scaffold without addition of biological factors for a first generation product, and

b) to investigate in parallel the effects of biological factors on in-situ tissue engineering. As a consequence, the heart valve of the first generation will be a classical medical device and the regulatory pathway will be CE marking by a Notified Body without involvement of medicinal Competent Authorities. Regulatory monitoring was continued throughout the period and a draft Technical File template was started.

Lastly, the design of the first-in-man study was initiated. Since long-term pre-clinical data may not become available in time due to delays in finding a stable valve- delivery device combination, a clinical study concept will be developed as basis for a later to be designed study protocol.

Dissemination and exploitation: Several scientific conferences were attended by the participants and the ImaValve concept was presented at scientific fairs (e.g. Biomedica) and in public lectures. Of particular interest are the invited presentations at the Heart Valve Society, the European Association of Cardio-Thoracic Surgery, SA HEART, the World Conference on Regenerative Medicine, and the Annual Meeting of the 21st Century Cardiothoracic Surgical Society. Notably, articles were published in *Acta Biomaterialia* and *Tissue Engineering*. All partners were engaged in the production of an ImaValve "movie" that demonstrates the goals and potential impact of the project and that can be released on public media. XELTIS, together with SUPRA, has further refined the plan of exploitation, as well as taken concrete steps towards establishing a meaningful to market for heart valve scaffolds.

1.3 Expected final results and their potential impact and use.

When successful the ImaValve project will result in a novel off-the-shelf available synthetic heart valve scaffold – suitable for transcatheter delivery via a purposely-designed stent and delivery system – which in-vivo gradually transforms into a living, durable aortic heart valve that lasts a lifetime. In addition, the project aims to provide preclinical proof of concept and a regulatory strategy including preclinical safety evaluation, to receive approval of a clinical trial by the responsible medical ethical committee and competent authorities after the project. Next to this, the project will:

- enhance our knowledge of biodegradable scaffolds and their safety and behavior in-vivo; and will deliver a novel library of degradable elastomeric supramolecular materials of interest to the biomedical field,
- substantially increase our understanding of the (inflammatory) foreign body response to degrading, bioactive materials, relevant to harnessing this response via material design.
- provide novel insights into the relationship between early tissue formation and later stage tissue maturation and organization, in order to guide neo tissue formation and maintain valve mechanical function.
- provide important information about the compliance of novel in-situ tissue engineering solutions with current clinical regulations, guidelines and standards.

We are working towards potential impacts and implications of ImaValve (end) results that include:

Novel biomaterials: The materials and material processing technologies developed in ImaValve have wide applicability in the field of regenerative medicine. The biodegradable elastomers are exceptionally versatile; their biomechanical and degradation properties can be tuned, which makes these materials particularly attractive for many tissue regeneration applications. Moreover, these biodegradable elastomeric materials provide independent control of the biomechanical and bioactive properties of the scaffold. Applications include, but are not limited to: (small) diameter vascular substitutes, venous valve replacements to treat peripheral artery disease, articular cartilage repair, myocardial regeneration, intervertebral disc regeneration, muscle regeneration, etc. Each of these applications address unmet clinical needs and represent huge market opportunities.

In-situ heart valve tissue engineering using synthetic biodegradable materials circumvents the key obstacles of traditional heart valve tissue engineering. The in-situ methodology bypasses the labor intensive, complex and costly cell and bioreactor culture phases (which require a total throughput time of about 8-10 weeks), and has off-the-shelf availability. The intrinsic manufacturing cost of the proposed technology is low. As such, the approach is attractive for commercialization.

Enhanced European collaboration: ImaValve combines the unique expertise, experience and infrastructures necessary to achieve the ambitions of the project. This combination of expertise and experience is not available in a single European member state.

Improved quality of life: The use of a living valves is expected to drastically alleviate current prosthesis associated problems that affect 30-35% of the patients within 10 years post operatively. In particular young recipients of current heart valve replacements have significantly reduced life expectancy (up to 50%) compared to age matched healthy individuals, and experience a high probability of serious valve-related morbidity (including stroke) throughout life. In patients younger than 18 years freedom from reoperation is only 58-68% at 15 years. The prevalence of aortic stenosis in Europe is 2.5% at the age of 75 years and almost 8% at 85 years. Today, it is estimated that 600,000 individuals in the EU have severe symptomatic aortic stenosis, while only a fraction of these patients is treated. Many of these patients would benefit from a transcatheter implantation of an ImaValve heart valve substitute.