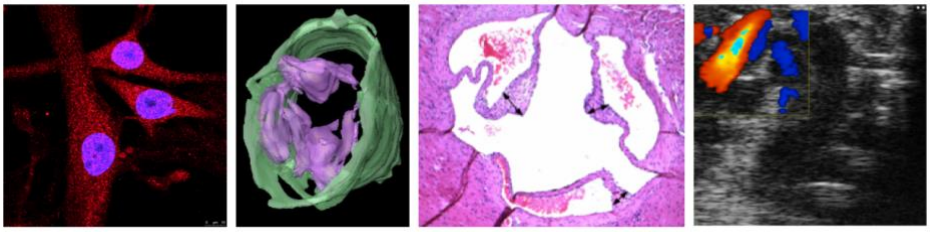
The role of Toll-like receptor 3 in radiation-induced aortic valve stenosis



Applicant:

Dr. Can Tepeköylü

Dpt. Cardiac Surgery

Medical University of Innsbruck

Anichstr.35

6020 Innsbruck

can.tepekoeylue@i-med.ac.at

project duration: 18 months

requested funding: 25.000 €

1. Deutsche Synopsis

Hintergrund:

Bestrahlung von Krebserkrankungen ist eine wichtige Stütze in der Krebstherapie. Thorakale Bestrahlung kann langfristig zu schwerwiegenden kardiovaskulären Komplikationen führen, die einer operativen Sanierung bedürfen. Eine der häufigsten Spätkomplikationen ist die Entwicklung einer Aortenklappenverkalkung. Dabei kommt es zu einer progressiven Verengung der Klappe und einer erhöhten Belastung für die linke Herzkammer. Unbehandelt führt die Erkrankung innerhalb weniger Jahre zum Tod. Die einzig derzeit verfügbare Behandlung ist der operative oder interventionelle Aortenklappenersatz. Beide Prozeduren stellen eine große Belastung für betroffene Patienten dar und können mit schwerwiegenden Komplikationen wie Schlaganfällen, Herzinfarkten, Blutungen, Prothesendysfunktionen und Herzrhythmusstörungen einhergehen. Eine medikamentöse Therapie, die die Entstehung einer Aortenklappenverkalkung nach Bestrahlung verhindern kann, wäre daher von hoher klinischer Relevanz, und könnte vielen Patienten eine invasive Herzoperation ersparen. Um ein Medikament für die Indikation zu entwickeln, wäre es von hoher Wichtigkeit, den molekularen Entstehungsmechanismus zu verstehen. Dieser ist bis dato unbekannt. Das vorliegende Projekt zielt darauf ab, dieses Grundlagenwissen zu generieren und somit eine mögliche zukünftige Therapie zu entwickeln.

Ziele:

Der Toll-Like Rezeptor 3 (TLR3) ist ein Rezeptor des angeborenen Immunsystems. Er wird durch virale RNA sowie körpereigene Nukleinsäuren, die von gestressten Zellen freigesetzt werden, aktiviert. Die Hypothese der vorliegenden Arbeit ist, dass die Bestrahlung der Aortenklappe zur Freisetzung von Nukleinsäuren und zur anschließenden Aktivierung von TLR3 führt. Die daraus resultierende inflammatorische Reaktion hat zur Folge, dass Aortenklappenzellen zu Osteoblasten mutieren und die Verkalkung vorantreiben. Das Ziel des vorgelegten Projekts ist (a) die Rolle von TLR3 in der Enstehung der strahleninduzierten Aortenklappenstenose zu untersuchen und (b) TLR3 als mögliches pharmakologisches Angriffsziel zu evaluieren, um die Entstehung der Aortenklappenstenose zu verhindern.

Methoden:

In funktionellen Zellkulturassays mit Klappenzellen aus Patientenmaterial werden Zellen bestrahlt. Die Zellen werden auf Kalkproduktion und kalzifizierende Genexpression untersucht und die Aktivierung von TLR3 untersucht. Die Morphologie und Funktion von bestrahlten Aortenklappen von Mäusen, denen der TLR3 fehlt, werden mittels Kleintierecho, microCT und histologischen Untersuchungen evaluiert.

Innovation des Projekts:

Momentan besteht keine therapeutische Option um die Entstehung einer Aortenklappenstenose zu verhindern. Betroffene Patienten müssen sich einer invasiven Herzoperation unterziehen. Im vorgelegten Projekt soll die Hypothese mittels (a) funktionellen in vitro Assays mit primären

Aortenklappenzellen, (b) morphologischer und funktioneller Charakterisierung von Aortenklappen in einem Mausmodell und (c) Analyse von Patientenmaterial evaluiert werden. Erste Ergebnisse liefern deutliche Indizien dafür, dass der identifizierte Rezeptor die Entstehung der Aortenklappenstenose mediiert. Knock-out Tiere, denen der Rezeptor fehlt, entwickelten die Erkrankung nicht. Daher scheint der Rezeptor als pharmakologischer Angriffspunkt gut geeignet.

Unser Ziel ist es nun, die genaue Rolle des Immunrezeptors bei der Entstehung der Erkrankung zu erforschen und zu untersuchen, ob eine pharmakologische Hemmung des Rezeptors tatsächlich eine Progression der Erkrankung verhindern könnte. Somit könnte erstmals die Entstehung einer Aortenklappenstenose verhindert und betroffenen Patienten eine invasive Herzoperation erspart werden.

2. Abstract

Background:

Thoracic radiation for the treatment of thoracic malignancies is associated with the development of calcific aortic valve disease (CAVD) including all its comorbidities. Currently, the pathomechanism behind this disease is not known. Toll-like receptor 3 (TLR3) is part of the innate immune system, which is involved in the recognition of nucleic acids. It has been shown recently that RNA damaged by radiation activates TLR3. This leads to subsequent inflammation and tissue damage in the skin.

Hypothesis:

RNA damage after radiation activates TLR3. Its activation and concomitant inflammation lead to the development of CAVD. Blockade of TLR3 avoids radiation induced aortic valve disease.

Methods:

Valvular interstitial cells (VICs), the predominant cell type in aortic valves, are isolated from patients undergoing heart transplantation. After radiation, cells are analyzed for TLR3 expression. In addition, RNase treatment and TLR3 knock-down is performed to investigate, whether radiation induced CAVD is TLR3 dependent. $ApoE^{-/-}$ and $ApoE^{-/-}/TLR3^{-/-}$ mice are irradiated with 14Gy and analyzed for aortic valve calcification. TLR3 antagonization prior to irradiation is applied to investigate a possible therapeutic effect. Finally, aortic valves from patients suffering from radiation induced CAVD are compared with healthy aortic valves regarding TLR3 expression and osteoblastic activity.

Expected results:

Disease associated with cancer treatment is of high relevance, as the number of cancer survivors is constantly rising due to improved therapeutic options. CAVD after thoracic irradiation is a major complication for affected patients which requires surgical valve replacement. Understanding the pathomechanism could reveal therapeutic targets for the prevention of CAVD after radiation.

3. Introduction

3.1. Radiation induced aortic valve stenosis

Thoracic radiation has significantly improved the survival of patients with breast cancer, Hodgkin's disease, lung cancer and other cancers involving the thorax[1]. However, the treatment gave rise to cardiovascular disorders due to radiation injury. Cardiovascular complications are responsible for 25% of mortality in cured cancer patients[2]. The relative risk for cardiac mortality after radiation is significantly increased ranging from 2,2. to 12,7[3-5]. Calcification of the coronary arteries and the aortic valves are mainly responsible for the cardiac complications after radiation. The development of calcific aortic valve disease (CAVD) 20 years after radiation is prevalent in up to 39 % of treated patients [6]. As usually younger patients are in need of radiation therapy, they get affected by radiation-induced CAVD and its comorbidities much earlier than from common CAVD[7].

Aortic valves mainly contain 2 celltypes: (a) valvular endothelial cells (VECs) covering the aortic and ventricular side of the valve leaflets producing nitric oxide (NO) and avoiding thromboembolic events and (b) valvular interstitial cells (=VICs, fibroblasts) responsible for the production of extracellular matrix proteins including collagen, elastin and glycosaminoglycans ensuring mechanical resistance and elasticity of the valve leaflets. During the pathogenesis of calcific aortic valve disease, VICs undergo a phenotype switch from fibroblasts to osteoblasts resulting in progressive calcification[8]. Inflammation, oxidative stress and renin-angiotensin signaling have been described to promote the phenotype switch. Valvular cells start producing bone tissue leading to consecutive calcification of the aortic valve. Progressive osteoblastic activity of valvular cells results in narrowing of the aortic valve orifice impeding regular blood flow during systole. Progression of the disease causes increased workload for the left ventricle resulting in heart failure including all its comorbidities[9]. Without treatment, 50% of patients with symptomatic aortic valve stenosis die within 2 years[10]. Currently, there is no pharmacological treatment available, affected patients need to undergo surgical aortic valve replacement. Patients undergoing surgery are at risk for all complications associated with open heart surgery including thromboembolism, size mismatch, paravalvular leakage, infection, bleeding and organ failure[11-13]. Experimental data showed possible promising effects of statin therapy in CAVD patients, however, in several clinical trials statin therapy revealed no benefit in reduction of cardiovascular events or progression of the disease[14]. Therefore, there is a strong need for the development of novel promising therapeutic targets.

It has been shown only recently that radiation treatment of VICs leads to the production of osteoblastic proteins[7]. However, it remains unknown how the physical stimulus of radiation results in activation of osteoblastic activity of VICs.

3.2. Toll-like receptor 3 and radiation

Toll-like receptors (TLRs) are part of the innate immune system and capable to bind highly conserved sequences expressed by microorganisms. Ten different TLRs have been described in humans. Every TLR recognizes a specific pattern of a microorganism. TLR3 is involved in the

recognition of double stranded RNA (dsRNA) to identify viruses. Its activation results in antiviral immune responses, namely the production of type I interferon and inflammatory cytokines[15].

However, as TLR ligand selectivity is relatively low, TLRs can recognize body-own molecules which are released during inflammation and tissue damage. Evidence is increasing that TLR activation via self-molecules can contribute to disease[15,16]. An efficient immune system might protect from infections in early years, but might come at the cost of increased risk for atherosclerosis and cardiovascular disease. Cellular damage by physical stimuli causes release of danger associated molecular patterns (DAMPs) including proteins and nucleic acids. Released molecules bind to TLRs initiating a pro-inflammatory response contributing to the onset and progression of cardiovascular disease. We could identify recently TLR3 as a receptor mediating the cellular response upon mechanical stress[17]. TLR3 is involved in the recognition of double stranded RNA (dsRNA) to identify viruses. Its activation results in antiviral immune responses, namely the production of type I interferon and inflammatory cytokines and has been linked to atherosclerosis[15,16]. It was shown recently that TLR3 recognizes endogenous nucleic acids released upon injury[18,19]. In a previously published study it was shown that UVB radiation damage of the skin is detected by TLR3[20]. Authors could show that radiation lead to RNA damage. This damage was recognized via TLR3 and induced a proinflammatory response including IL-6 and TNF-a upregulation. This response was completely missing in $TLR3^{-/-}$ mice. Another group could show that TLR3 blockade protected mice from lethal radiation-induced gastrointestinal syndrome [21]. Whole-body irradiation of wild-type (WT) mice resulted in death of all treated mice, whereas $TLR3^{-/-}$ mice showed no change whatsoever in survival[21].

We therefore hypothesized that TLR3 plays a major role in the pathogenesis of radiation induced CAVD.

4. Clinical & Public Impact of the Project

Disease associated with cancer treatment is of high relevance, as due to improved treatment alternatives many patients survive their cancer. The development of CAVD after thoracic irradiation is a significant burden for affected patients. Currently, the only treatment option is aortic valve replacement. This invasive procedure includes all risks of open heart surgery. Understanding the pathophysiology of the disease could provide novel therapeutic targets. Pharmacological treatment before or immediately after radiation therapy which mitigates the effect of radiation could therefore prevent many patients from invasive open heart surgeries. The expected basic research findings in the described project will impact on the international scientific community and on the general public as well, thereby reflecting Austria's international competitiveness in biological science, health care and medical technologies. By gaining robust evidence for the mechanism of radiation induced CAVD the proposed project could develop a powerful tool for a possible treatment. This could not only improve the quality of life of the affected patients, but also reduce the socio-economic health burden of this disease.

5. Preliminary results

Isolated human aortic valve cells (VICs) showed ubiquitous expression of TLR3 **(Figure 1A)**. TLR3 expression was significantly increased in patients with history of radiation compared to healthy aortic valves (from patients after heart transplantation without valve pathology, HTX). (**Figure 1B**).Radiation of VICs resulted in increased TLR3 expression **(Figure 1C)**. Aortic valves of radiated mice showed higher TLR3 expression compare to untreated controls **(Figure 1D).** Radiation of VICs lead to significantly increased production of calcific nodules and enhanced alkaline phosphatase activity. The osteoblastic effects of radiation were abolished after pretreatment of cells with a TLR3-Inhibitor (**Figure 1E**). These results give us a strong hint that radiation results in TLR3 activation in aortic valves.

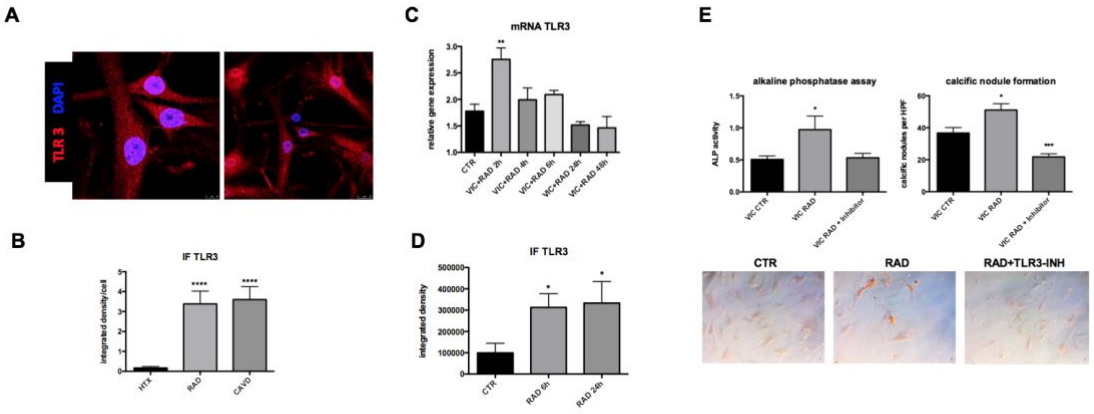


Figure 1: A Human aortic valve cells show high expression of TLR3 (TLR3=red, nuclei=blue). **B** Human aortic valves with thoracic radiation history (RAD) show increased TLR3 expression compared to healthy aortic valves (HTX) (TLR3=red, nuclei=blue). **C** Radiation leads to increased TLR3 expression in radiated VICs isolated from healthy aortic valves. **D** Aortic valves of radiated mice (RAD) showed higher TLR3 expression compare to untreated controls (CTR). **E** Radiation of VICs resulted in increased osteoblastic activity with increased production of calcific nodules (alizarin red staining) and increased activity of the alkaline phosphatase. Osteoblastic activity after radiation was abolished upon TLR3 inhibition.

Next, we aimed to analyze whether TLR3$^{-/-}$ mice show a different phenotype concerning the development of CAVD. Wild-type aged mice (WT) showed thickened aortic leaflets, increased valve area and numerous fibrotic plaques, hence early-stage CAVD**.** However, these changes were completely missing in age-matched senescent TLR3$^{-/-}$ animals seen in histological analysis and microCT analysis **(Fig.2a)**. We found the same results in functional echocardiographies: Aged WT mice showed significantly increased mean gradients (mmHg: 3.14±0.64 vs. 1.16±0.22, p=0.025), mean velocity (mm/s: 796±77 vs. 527±55, p=0.029) and valve thickness all indicating early-stage CAVD. However, all these changes were completely missing in age-matched TLR3$^{-/-}$ mice **(Fig. 2b)**.

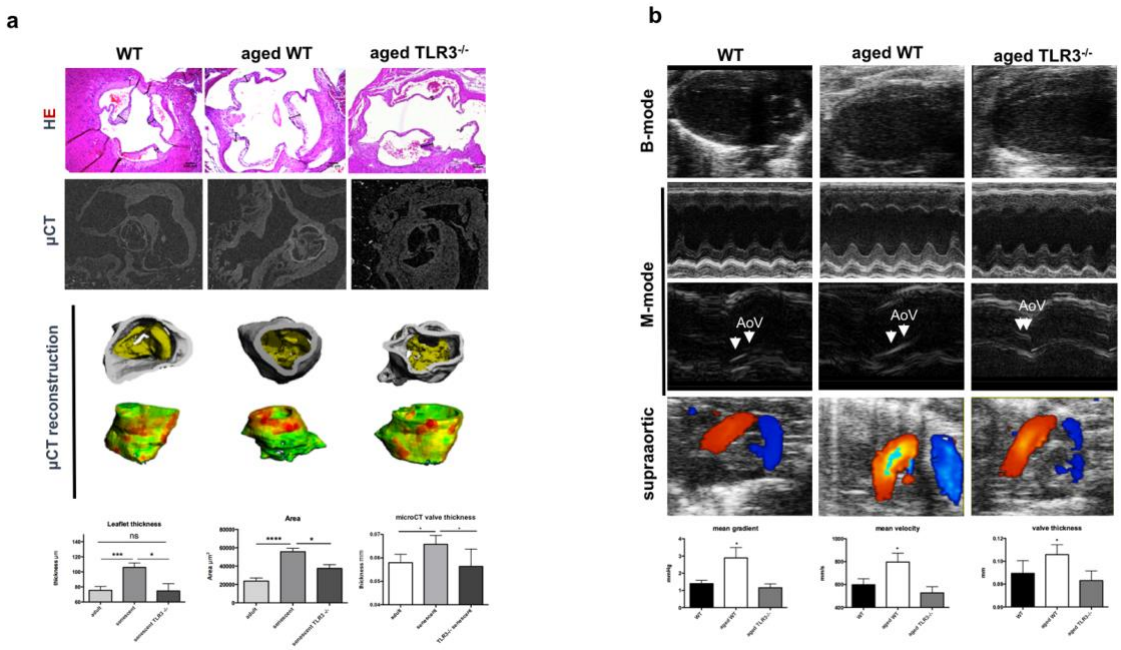


Figure 2: TLR3$^{-/-}$ mice show no morphological and hemodynamic phenotype of CAVD. Aged wild-type (WT) mice showed clear signs of beginning CAVD with increased leaflet thickness, leaflet area and multiple plaques. However, these morphological changes were missing completely in age-matched TLR3$^{-/-}$ mice. These findings were confirmed in ex vivo microCT scans of the murine aortic valves (a).Functional analysis of the aortic valves showed clear signs of CAVD in aged WT mice including increased pressure gradient, velocity and aortic valve thickness. Again, these changes were missing completely in age-matched TLR3$^{-/-}$ mice.

6. Objectives

The proposed study aims to investigate

(a) involved pathomechanisms in aortic valve calcification following radiation treatment.

(b) whether RNA damage and consequent recognition by TLR3 plays a role in radiation-induced CAVD.

(c) whether treatment with TLR3 antagonist can prevent the development of radiation induced CAVD

7. Working plan

The experimental design is depicted in **Figure 3**. The hypothesis will be tested in valvular interstitial cells isolated from patients, in a mouse model and in immunohistological stainings from human aortic valves.

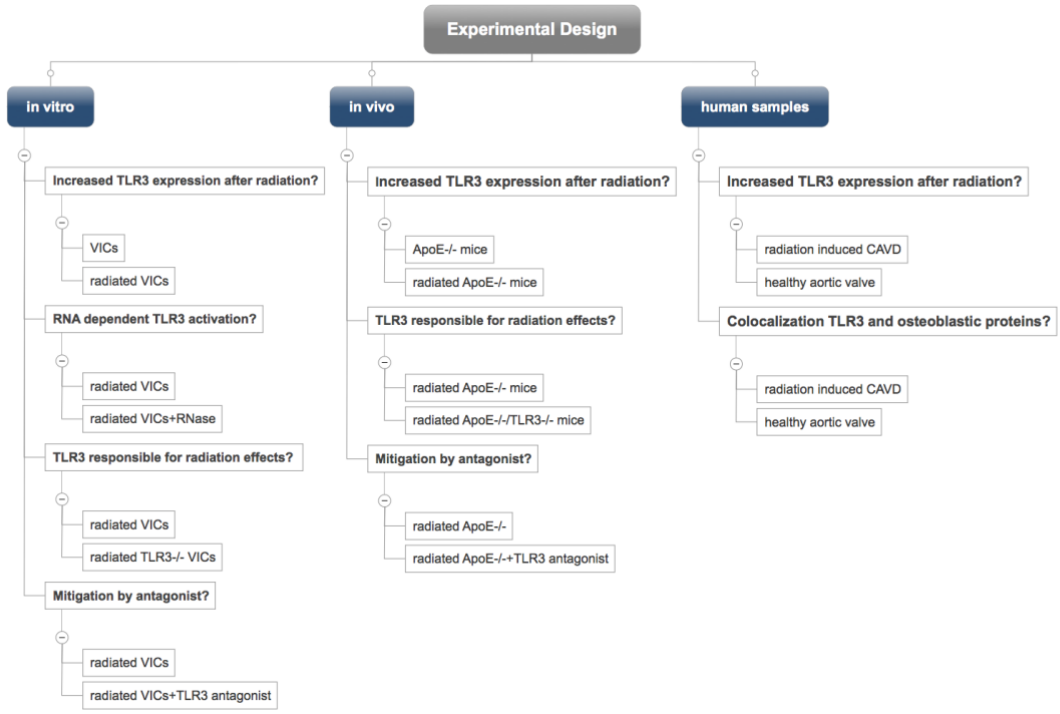


Fig.3

7.1. In vitro model

Valvular interstitial cells (VIC), the predominant cell type in aortic valves, are isolated from aortic valves from patients undergoing heart transplantation after informed consent by collagenase digestion as described previously[22]. Cells are cultured in M199 medium containing 10% fetal calve serum und Penicillin, Streptomycin and Glutamat. Transfection is performed using the Lonza Nucleofector Kits. Isolation and cultivation of VICs is well established in our laboratory. Ethical permission from the Medical University of Innsbruck has been obtained. Cells from 5 patients are used for the experiments. VICs are radiated after a standard protocol as described previously[7]. A total irradiation of 10 Gy (175rad/min for 2,85 minutes twice) will be applied. Osteoblastic activity of cells is analyzed via Western Blot for the osteoblastic protein bone morphogenetic protein 2 (BMP2), transcription factor Runt-related transcription factor 2 (Runx2), alkaline phosphatase activity (kit, abcam) and alzarine red staining. TLR3 expression and its pathways (TRIF, TRAF3, TBK1, IRF3, pNFKB) are analyzed via Western blot. To investigate whether RNAs play a role in the pathogenesis and whether radiation effects are TLR3 dependent, the experiment is repeated with RNase and after a

TLR3 knock-down in VICs. Finally, radiated VICs are incubated with TLR3 antagonist (TLR3/dsRNA Complex Inhibitor, Millipore) to analyze whether a mitigative effect can be achieved.

7.2. In vivo model

Ethical permission for all animal experiments has been obtained. All required mice are available to the PI. For the in vivo investigation of radiation induced CAVD, $ApoE^{-/-}$ mice are radiated as described previously[23]. Aortic valves are harvested 24h and 30 weeks after radiation and analyzed for TLR3 expression and its pathways (TRIF, TRAF3, TBK1, IRF3, pNFKB) via Western blot. In addition, inflammatory cytokines IL-6 and TNF-a are measured via RT-PCR. Aortic valve thickness is analyzed in H.E. stainings. Calcifications are quantified in von Kossa stainings. Inflammatory infiltrates are analyzed via immunofluorescence staining. Atherosclerotic plaques are visualized via Oilred O staining. Hearts are scanned for morphological evaluation using a micro CT and Vevo small animal echocardiography device. In a next step, it is analyzed whether the radiation effect is TLR3 dependent. Therefore, the double knock-out mice $ApoE^{-/-}/TLR3^{-/-}$ are radiated and their aortic valves are compared with radiated $ApoE^{-/-}$ mice for morphological stenotic changes like valve thickness, calcifications, inflammatory infiltration and atherosclerotic plaques several weeks after radiation. In a final step, radiated mice are treated with TLR3 antagonist to analyze whether mitigation of radiation induced CAVD can be achieved.

7.3. Human samples

Human aortic valves are obtained from (a) patients undergoing heart transplantation (HTX) for other reasons that valve pathology and will serve as controls and (b) patients undergoing heart valve replacement after radiation induced CAVD. Ethical permission from the Medical University of Innsbruck has been obtained. Five patients per group are investigated. TLR3 expression, concomitant inflammation and osteoblastic activity via immunofluorescence staining (TLR3, F/480, BMP-2, Runx2) western blotting (TLR3, IL-6, TNF-a, BMP2, Runx2) and RT-PCR (TLR3, IL-6, TNF-a, BMP-2, Runx2). In addition, colocalization of TLR3 and osteoblastic markers BMP-2 and Runx2 are performed via immunofluorescence staining and in situ hybridization. Colocalization of TLR3 and osteoblastic proteins is analyzed.

8. Project schedule

The project is structured into workpackages as depicted in **Fig.4**.
Expected duration: 18 months

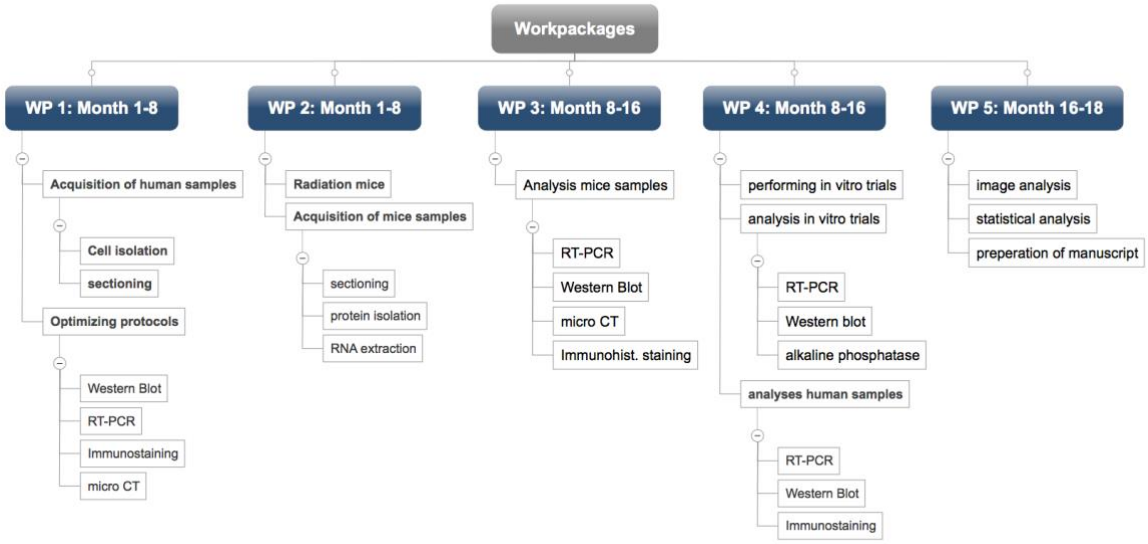

Workpackages
WP 1: Month 1-8
Acquisition of human samples
Cell isolation
sectioning
Optimizing protocols
Western Blot
RT-PCR
Immunostaining
micro CT
WP 2: Month 1-8
Radiation mice
Acquisition of mice samples
sectioning
protein isolation
RNA extraction
WP 3: Month 8-16
Analysis mice samples
RT-PCR
Western Blot
micro CT
Immunohist. staining
WP 4: Month 8-16
performing in vitro trials
analysis in vitro trials
RT-PCR
Western blot
alkaline phosphatase
analyses human samples
RT-PCR
Western Blot
Immunostaining
WP 5: Month 16-18
image analysis
statistical analysis
preperation of manuscript

Fig.4

9. Itemization of the requested funding

Enzymes & consumables for cell isolation & culture	5.000,00 €
kits for in vitro functional tests	3.000,00 €
RT-PCR reagents	4.500,00 €
antibodies	4.000,00 €
western blot consumables	1.500,00 €
costs for animal experiments	2.000,00 €
TLR3-antagonist	1.500,00 €
consumables for immunohistochemistry	2.000,00 €
in vivo imaging consumables	1.500,00 €
TOTAL	**25.000,00 €**

10. Project team and infrastructure

Our working group, based in the cardiac surgical lab is granted access to all facilities of the Medical University of Innsbruck. Innsbruck Medical University provides its scientists with outstanding research possibilities. Besides extensive support programs for young scientists, there are several platforms for scientific exchange and collaborative research. High-end equipment in clinical as well as basic science areas supports the scientific environment. The Center for Biochemistry and Biology was founded to enforce excellence in basic research. It accommodates well-equipped core facilities and divisions in bioinformatics, biological chemistry, cell biology, clinical biochemistry, developmental immunology, experimental pathophysiology and immunology, genetics and RNomics, molecular biology and neurobiochemistry. The divisions are led by top-qualified scientists. Innsbruck also has a long tradition of renown clinical science especially in the fields of neurology, cardiovascular medicine, intensive care, clinical imaging, cancer research, transplantation medicine and regeneration. In addition, the university provides a Clinical Trial Center, which supports scientists at planning, execution and evaluation of clinical trials. Our team comprises at present one PostDoc, two PhD-students, one biomedical analyst and five graduants. Dr. Can Tepeköylü will act in this project as principal investigator. He has broad experience with the methods applied in this project: Cell culture as well as various animal models and analysis are well established in the lab. In addition, molecular biological analysis tools like rtPCR, western blots, Immunofluorescence, FACS etc. are available and well established. The group has broad experience with animal models and functional assessment of animal trials. The research group was awarded numerous national as well as international research prizes in the last years. The applicant can rely on the support of Priv.-Doz. Dr. Johannes Holfeld and Prof. Dr. Michael Grimm (Department of Cardiac Surgery).

11. References

1. Jaworski, C., Mariani, J.A., Wheeler, G. & Kaye, D.M. Cardiac complications of thoracic irradiation. *J. Am. Coll. Cardiol.* **61**, 2319-2328 (2013).
2. Lee, C.K., Aeppli, D. & Nierengarten, M.E. The need for long-term surveillance for patients treated with curative radiotherapy for Hodgkin's disease: University of Minnesota experience. *Int. J. Radiat. Oncol. Biol. Phys.* **48**, 169-179 (2000).
3. Aleman, B.M.*, et al.* Late cardiotoxicity after treatment for Hodgkin lymphoma. *Blood* **109**, 1878-1886 (2007).
4. Swerdlow, A.J.*, et al.* Myocardial infarction mortality risk after treatment for Hodgkin disease: a collaborative British cohort study. *J. Natl. Cancer Inst.* **99**, 206-214 (2007).
5. Castellino, S.M.*, et al.* Morbidity and mortality in long-term survivors of Hodgkin lymphoma: a report from the Childhood Cancer Survivor Study. *Blood* **117**, 1806-1816 (2011).
6. Wethal, T.*, et al.* Valvular dysfunction and left ventricular changes in Hodgkin's lymphoma survivors. A longitudinal study. *Br. J. Cancer* **101**, 575-581 (2009).
7. Nadlonek, N.A.*, et al.* Radiation induces osteogenesis in human aortic valve interstitial cells. *J. Thorac. Cardiovasc. Surg.* **144**, 1466-1470 (2012).
8. Rajamannan, N.M.*, et al.* Calcific aortic valve disease: not simply a degenerative process: A review and agenda for research from the National Heart and Lung and Blood Institute Aortic Stenosis Working Group. Executive summary: Calcific aortic valve disease-2011 update. *Circulation* **124**, 1783-1791 (2011).
9. Jessup, M. & Brozena, S. Heart failure. *N. Engl. J. Med.* **348**, 2007-2018 (2003).
10. Rosenhek, R.*, et al.* Natural history of very severe aortic stenosis. *Circulation* **121**, 151-156 (2010).
11. Genereux, P.*, et al.* Paravalvular leak after transcatheter aortic valve replacement: the new Achilles' heel? A comprehensive review of the literature. *J. Am. Coll. Cardiol.* **61**, 1125-1136 (2013).
12. Cannegieter, S.C., Rosendaal, F.R. & Briet, E. Thromboembolic and bleeding complications in patients with mechanical heart valve prostheses. *Circulation* **89**, 635-641 (1994).
13. Généreux, P.*, et al.* Bleeding Complications After Surgical Aortic Valve Replacement Compared With Transcatheter Aortic Valve Replacement. *J. Am. Coll. Cardiol.* **63**, 1100-1109 (2014).
14. Hutcheson, J.D., Aikawa, E. & Merryman, W.D. Potential drug targets for calcific aortic valve disease. *Nat. Rev. Cardiol.* **11**, 218-231 (2014).
15. Kawai, T. & Akira, S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* **11**, 373-384 (2010).
16. Cole, J.E.*, et al.* Unexpected protective role for Toll-like receptor 3 in the arterial wall. *Proceedings of the National Academy of Sciences* **108**, 2372-2377 (2011).
17. Holfeld, J.*, et al.* Toll-like receptor 3 signalling mediates angiogenic response upon shock wave treatment of ischaemic muscle. *Cardiovasc. Res.* **109**, 331-343 (2016).
18. Kariko, K., Ni, H., Capodici, J., Lamphier, M. & Weissman, D. mRNA is an endogenous ligand for Toll-like receptor 3. *J. Biol. Chem.* **279**, 12542-12550 (2004).
19. Cavassani, K.A.*, et al.* TLR3 is an endogenous sensor of tissue necrosis during acute inflammatory events. *J. Exp. Med.* **205**, 2609-2621 (2008).
20. Bernard, J.J.*, et al.* Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. *Nat. Med.* **18**, 1286-1290 (2012).
21. Takemura, N.*, et al.* Blockade of TLR3 protects mice from lethal radiation-induced gastrointestinal syndrome. *Nature communications* **5**, 3492 (2014).
22. Meng, X.*, et al.* Expression of functional Toll-like receptors 2 and 4 in human aortic valve interstitial cells: potential roles in aortic valve inflammation and stenosis. *Am. J. Physiol. Cell Physiol.* **294**, C29-35 (2008).
23. Stewart, F.A.*, et al.* Ionizing radiation accelerates the development of atherosclerotic lesions in ApoE-/- mice and predisposes to an inflammatory plaque phenotype prone to hemorrhage. *Am. J. Pathol.* **168**, 649-658 (2006).

12. CV Can Tepeköylü, M.D.

born 02.09.1986 in Oberpullendorf, Austria; Nationality: Austria

Department of Cardiac Surgery , Medical University of Innsbruck
Anichstraße 35, 6020 Innsbruck, Austria
T: +43 512 504 80466 , E: can.tepekoeylue@i-med.ac.at, F: 0043 512 504 22502

Current positions:
- Resident at the Department of Cardiac Surgery, Medical University of Innsbruck
- PostDoc at the CARE Lab, research group on cardiac regeneration

Academic education:
- PhD-program "Molecular cell biology" at Medical University of Innsbruck, Austria
- MD from the Medical University of Vienna, Austria

Advanced training:
- Echocardiography diploma (DEGUM), Charité Berlin, Germany 2018
- Clinical Investigation Training, Diploma on Good Clinical Practice – 2017
- Diploma for leading animal experiments according to the European Union (FELASA B)
- Research Fellowship in Kaohsiong, Taiwan 2010
- Research Visit at the Children's Mercy Hospital in Kansas City, MO 2012
- Research Fellowship in Bern, Switzerland 2011

Funding:
- Tyrolean Medical Science Fund: Vasculogenesis in Infarcted Myocardium via Recruitment of Endothelial Progenitor Cells by cardiac shock wave treatment (Co- Investigator)
- Tyrolean Medical Science Fund: The Role of Nucleic Acid Toll-like Receptors in the Development and Progression of Calcific Aortic Valve Disease (Principal Investigator)
- Bayer Grants4Targets: Innate immunity and Calcific Aortic Valve Disease (Principal Investigator)
- Research Grant of the Austrian Workers Compensation Board (AUVA): Molecular Working Mechanisms of Shock Wave Therapy (Co- Investigator)
- Medical University of Innsbruck – START Grant: Characterizing the link between innate immunity and solid organ transplantation (SOT): The role of Toll-like receptor (TLR)-3 in ischemia reperfusion injury (Co-Investigator)
- Tyrolean Medical Science Fund : The Role of Toll-like Receptors in the Development and Progression of Aortic Aneurysms (Principal Investigator)

Awards (selection):
- Best Scientific Paper Award: 16th Congress of the International Society for Medical Shockwave Treatment, Salzburg, Austria 2013
- Best Abstract Award: Integrated Management of acute and chronic cardiovascular disease, Innsbruck, Austria 2014
- Wolfgang Denk Award, Austrian Society for Cardiothoracic Surgery: Annual Meeting of the Austrian Society for Cardiac Surgery, Salzburg, Austria 2016
- Young Investigators Award, European Society of Cardiology (ESC) Acute Cardiovascular Care Geneve, Switzerland 2014
- Young Investigator Award, Kardiologie Innsbruck 2016, Austria
- EuRegio Young Scientists' Award, European Forum Alpbach, Austria 2016
- Selection as ESC Basic Science Research Highlight 2017, ESC Congress. Barcelona, Spain 2017
- Selection as European Society of Solid Organ Transplantation (ESOT) Research Highlight 2017, ESOT Congress. Barcelona, Spain 2017
- Young Investigator's Award, Austrotransplant, Zell am See, Austria 2017.
- Young Investigator's Award 2017, Austrian Society for Radiation Protection in Medicine, Vienna 2017

Functions in Scientific Societies:

- Task force member „European Society for Cardiothoracic Surgery“, Heart Failure
- Nucleus Member „European Society for Cardiothoracic Surgery“, Residents
- Nucleus Member „Cardiologists of Tomorrow Austria“
- Nucleus Member „Austrian Society for Cardiac Surgery, Young chapter“
- Organizing Faculty: Focus:Valve Training for minimally invasive valve surgery, Innsbruck, Austria, 2017 + 2018
- Organizing Committee: Basic Research Meeting of the International Society for Medical Shockwave Treatment, Vienna 2010, Innsbruck 2012, Frankfurt 2014

Memberships:
European Association for Cardio-Thoracic Surgery (EACTS), European Society of Cardioolgy (ESC), International Society for Medical Shockwave Treatment (ISMST), Austrian Society for Surgical Research, Austrian Society for Surgery, Austrian Society for Cardiothoracic Surgery

Key Papers:
Tepeköylü C, Lobenwein D, Urbschat A, Graber M, Grimm M, Pechriggl E, Fritsch H, Paulus P, Holfeld J. Shock wave treatment after hindlimb ischemia results in increased perfusion and M2 macrophage presence. T Tiss Eng Regen Med. 2016 Sep 30. **IF 4.71**

Tepeköylü C, Primessnig U, Pölzl L, Graber M, Lobenwein D, Nägele, Kirchmair E, Pechriggl EJ, Grimm M, Holfeld J. LL37/ RNA complexes released via shockwave treatment prevent from heart failure after acute myocardial ischemia. J Cell Mol Med. 2016 Dec 20.**IF 4.9**

Blaas I, Heinz K, Würtinger P, Türkcan A, **Tepeköylü C,** Grimm M, Doppler C, Danzl K, Messner B, Bernhard D. Vein Graft Thrombi, a Niche for Smooth Muscle Cell Colonisation-A Hypothesis to Explain the Asymmetry of Intimal Hyperplasia. J Thromb Haemost. 2016 Feb 14. **IF 4.99**

Holfeld J, **Tepeköylü C,** Reissig C, Lobenwein D, Scheller B, Kirchmair E, Kozaryn R, Albrecht-Schgoer K, Krapf C, Zins K, Urbschat A, Zacharowski K, Grimm M, Kirchmair R, Paulus P. Toll-like receptor 3 signalling mediates angiogenic response upon shock wave treatment of ischaemic muscle. Cardiovasc Res. 2016 Feb 1;109(2):331-43. **IF 5.99**

Lobenwein D*, **Tepeköylü C*,** Kozaryn R, Pechriggl EJ, Bitsche M, Graber M, Fritsch H, Semsroth S, Stefanova N, Paulus P, Czerny M, Grimm M, Holfeld J. Shock Wave Treatment Protects From Neuronal Degeneration via a Toll-Like Receptor 3 Dependent Mechanism: Implications of a First-Ever Causal Treatment for Ischemic Spinal Cord Injury. J Am Heart Assoc. 2015 Oct 27;4(10):e002440. **IF 4.3**

Tepeköylü C, Lobenwein D, Blunder S, Kozaryn R, Dietl M, Ritschl P, Pechriggl EJ, Blumer MJ, Bitsche M, Schistek R, Kotsch K, Fritsch H, Grimm M, Holfeld J. Alteration of inflammatory response by shock wave therapy leads to reduced calcification of decellularized aortic xenografts in mice†. Eur J Cardiothorac Surg. 2015 Mar;47(3):e80-90. **IF 3.3**

Tepeköylü C, Wang FS, Kozaryn R, Albrecht-Schgoer K, Theurl M, Schaden W, Ke HJ, Yang Y, Kirchmair R, Grimm M, Wang CJ, Holfeld J. Shock wave treatment induces angiogenesis and mobilizes endogenous CD31/CD34-positive endothelial cells in a hindlimb ischemia model: implications for angiogenesis and vasculogenesis. J Thorac Cardiovasc Surg. 2013 Oct;146(4):971-8. IF **4.17**