## Gene Therapy, Cell Therapy

### Guido Tarone award - engineered adeno-associated viral vectors with an improved in vivo targeting of cardiac fibroblasts

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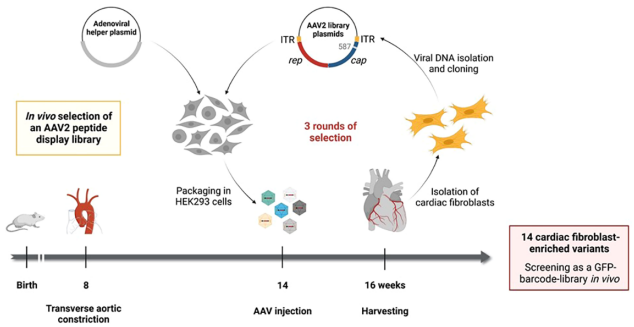
**Background:** Cardiac remodelling processes are closely associated with fibrosis, a pathological mechanism leading to the stiffening of the myocardium and therefore, to a deteriorating heart function. Therapeutic strategies are urgently required since current treatments are not addressing the critical over-activation of fibroblasts that contributes to the progression to heart failure. We developed a novel gene therapy approach based on adeno-associated viral (AAV) vectors to specifically target these disease-associated fibroblasts and identified three promising AAV variants with an improved tropism for cardiac fibroblasts.
**Purpose:** Adeno-associated viruses present optimal features for a long-term gene delivery in vivo. Their safety and efficacy has already been demonstrated in diverse clinical trials that resulted in five market approvals in Europe to date. However, the available serotypes are not suitable for the targeting of cardiac fibroblasts. Thus, our aim was to retarget AAV2 variants derived from a large peptide display library.
**Methods:** The AAV2 library is based on a random 7mer peptide insertion into the viral capsid proteins. This library was selected in mice with pressure-overload induced cardiac hypertrophy with severe cardiac fibrosis. After three rounds of selection, cardiac fibroblast-enriched variants were identified via next generation sequencing. For further in depth analysis, fourteen variants were combined to a GFP-expressing barcoded sub-library that was once screened in vivo. Here, three variants were chosen for a subsequent in vitro characterization including transduction of several on and off target cells, natural AAV2 receptor affinity assays and in vitro neutralization with serum from immunized mice. Finally, the three AAV variants were examined in a proof-of-principle study in mice.
**Results:** The results of the GFP-barcode-library screening indicated an enrichment of AAV-Var1, Var4 and Var14 vector genomes as well as the highest relative eGFP expression in cardiac fibroblasts compared to cardiomyocytes, endothelial cells and the major off target organ, the liver. In vitro studies confirmed an increased transduction efficiency of murine and human cardiac fibroblasts while off target cells were less transduced. The retargeting is accompanied by a reduced binding affinity to an AAV2 primary receptor analogue. One major challenge for clinical translation is the high prevalence of neutralizing antibodies. Therefore, the diminished recognition by AAV2 antibodies is beneficial for future studies. Finally, the enhanced tropism for cardiac fibroblasts was validated in a proof-of-principle approach in vivo.
**Conclusions:** The three AAV variants are highly interesting novel gene therapy vectors for the treatment of cardiac fibrosis and potentially additional fibrotic diseases. Future studies will, therefore, include an in-depth analysis of their tropism, immune responses and therapeutic potential in a murine animal model.




### Atrial natriuretic peptide conjugated spermine-acetalated dextran nanoparticles for targeted miRNA delivery to the heart

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**Introduction:** Myocardial infarction (MI) is a life-threatening condition characterized by irreversible cell death. During the past decades, several therapeutic strategies have held the promise of restoring the full functionality of a damaged heart after MI. However, MI approved therapies, to date, only ameliorate the state of care of these patients. Therefore, new therapeutic approaches need to be explored. Herein, we have developed nanoparticles (NPs) for MI treatment. Atrial natriuretic peptide (ANP) conjugated spermine-acetylated dextran (AcDXSp) NPs were loaded with miRNA-145. The NPs modulated the immunoenvironment toward cell repair and regeneration, induced cardiac fibroblasts differentiation to myofibroblasts and reduced mature cardiomyocyte death after hypoxia.
**Methods:** AcDXSp NPs were prepared by double emulsion technique and functionalized with PEG and ANP. Cytocompatibility and cell–nanoparticle interactions were studied using embryonic STEM cell derived maturated cardiomyocytes and fibroblasts. An in vitro hypoxia model was developed to mimic cells response after MI. Cells response to the NPs treatment was evaluated through qPCR, Western blot, and flow cytometry.
**Results:** The peptide-functionalization of the NPs led to an increased uptake of the NPs in the cardiac cells . The pH-dependent NPs degradation and miRNA release was evaluated at pH 7.4 and 5.5. The expression of hypoxia markers was assessed in the in vitro hypoxia model by qPCR. The NPs showed no cytotoxicity in the tested concentrations and miRNA delivery was confirmed.
**Conclusions:** The preliminary data show that the final system was biocompatible and biodegradable and successfully delivered miRNA in the in vitro hypoxia model, highlighting the potential of this system toward improved gene delivery to the heart, and thus, potential cardiac regeneration therapy.

## Biomarkers

### Role of matrix metalloproteinase 9 in prediction composite one-year endpoint in st-segment elevation myocardial infarction

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Acute myocardial infarction remains significant in mortality, due to difficulties in predicting early complications. Therefore, it is important to assess the risk in patients with ST-segment elevation myocardial infarction to improve long-term survival. For STEMI patients, biomarkers play a beneficial confirmatory role in diagnosis and prognostic utility. Matrix metalloproteinases (MMPs) are a component of the extracellular matrix and are closely associated with the instability of atherosclerotic plaque. MMP-9 can serve as a biomarker to evaluate the severity of coronary artery lesions and predict long-time of poor outcomes and mortality of STEMI.
The aim of our study was to determine the role of MMP-9 markers in predicting one-year outcomes in STEMI patients who underwent primary percutaneous coronary intervention (PCI).
**Materials and Methods:** The study included 165 patients admitted with STEMI within 12 hours of the onset of symptoms. All patients underwent primary PCI according to the guidelines, followed by standard examinations and treatment at the hospital. Blood samples for biomarker analysis (MMP-9) and other routine tests were taken at admission (≤ 12 hours). At six months after the event, all patients underwent clinical follow-up. Patients were contacted by telephone, family members or their physicians 1 year after the event. Primary composite endpoint was determined as the composition of all-cause death, new myocardial infarction; ischemic stroke, heart failure decompensation with or without hospitalization, hospitalization due to any cardiovascular disease decompensation.
**Results:** The primary composite endpoint reached 9% of patients at one-year follow-up. ROC analysis showed association of MMP-9 level >194.6 pg/ml with the one-year composite endpoint ($p = 0.0037$), area under curve (AUC) is 0.711, with 91.7% sensitivity (Sen), and 47.4% specificity (Spe), 95% confidence interval (CI) - 0.604 to 0.802. We used Kaplan–Meier analysis and found that higher MMP-9 levels were associated with a marked increase in the one-year cardiovascular composite endpoint, Cox's F-Test, $p = 0.04487$. A logistic regression analysis proved that the composite endpoint at one year after STEMI depend on MMP-9 level (OR = 1.0151, 95% CI: 1.0001–1.0304, $p = 0.0481$).
**Conclusions:** The biomarker MMP-9 predicts major adverse cardiovascular events during a 1-year follow-up in STEMI patients after primary PCI. Future studies are required to clarify this result.

### Regional levels of tenascin-C in patients with heart failure and atrial fibrillation

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**Background:** Tenascin-C (TNC) is an extracellular matrix glycoprotein that is upregulated at high levels during active tissue remodeling. The serum concentration of TNC has been reported to be increased in patients with several cardiac diseases. The aim of this study was 1) to measure the TNC plasma level in heart failure (HF) and atrial fibrillation (AF) patients compared to patients without structural heart disease and 2) to search for a local production in the heart.
**Methods:** This study included 13 patients with HF who underwent CRT- implantation (CRT- group), 13 patients with persistent AF who underwent left atrial catheter ablation (AF- group) and 13 patients without structural heart disease or AF, who underwent electrophysiological study (Control- group). Blood was collected at the beginning of the procedures from the coronary sinus (CS), superior vena cava (SVC) and aorta (Ao). Level of TNC-B (ng/dl) in the CS (TNC-CS), SVC (TNC-SVC) and aorta (TNC-Ao) was measured.
**Results:** Patients characteristics: CRT- group: mean age 74 y, 8 male, LV- EF 32%, 3 with a history of atrial fibrillation. AF- group: mean age 68 y, 9 male, LV- EF 55%. Control- group: mean age 54 y, 9 male, LV- EF 65%.
The TNC level in the SVC was in the CRT group significant elevated ($6125 \pm 2030$ ng/ml vs $475 \pm 238$ ng/ml in controls ($p < 0.01$). In the AF group the TNC level in the SVC as well as significantly higher $4983 \pm 1814$ ng/ml vs $475 \pm 238$ ng/ml in controls ($p < 0.01$). The TNC level of the CRT- and the AF- group were not significant different ($p = 0.219$) (*Figure 1*). No significant differences were noted at the various collection sites (CS, SVC and aorta) between the 3 groups ($p = 0.38$).
**Discussion:** The TNC serum levels in HF- and AF- patients are elevated compared to a control group without structural heart disease and without a history of AF. We could not determinate, the source of serum TNC, differences in the level of TNC between CS and SVC and aorta were not visible. Whether an effective treatment of HF and AF will lower TNC levels and slow the disease progression needs to be evaluated in further studies.

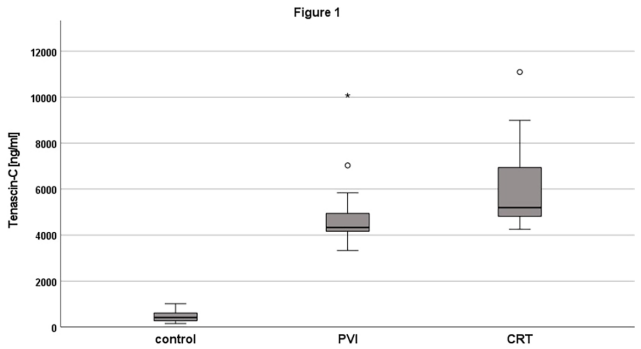


Figure 1

### Assessment of fibrosis in heart failure and atrial fibrillation using TIMP-1 serum levels

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**Background:** Tissue inhibitor of metalloproteinase- 1 (TIMP-1) levels are strongly associated with cardiac extracellular matrix accumulation and atrial fibrosis. The aim of this study was 1) to compare the TIMP-1 plasma levels in heart failure (HF) and atrial fibrillation (AF) patients and 2) to search for a local production in the heart.
**Methods:** This study included 13 patients with HF who underwent CRT implantation (CRT- group), 13 patients with persistent AF who underwent left atrial catheter ablation (AF- group) and 13 patients without structural heart disease or AF, who underwent electrophysiological study (control group). Blood was collected at the begin of the procedures from the coronary sinus (CS), superior vena cava (SVC) and aorta (Ao). Level of TIMP-1 in the CS (TIMP-1-CS), RA (TIMP-1-SVC) and aorta (TIMP-Ao) was measured.
**Results:** Patients characteristics: CRT- group: mean age 74 y, 8 male, LV- EF 32%, 3 with a history of atrial fibrillation. AF- group: mean age 68 y, 9 male, LV- EF 55%. Control- group: mean age 54 y, 9 male, LV- EF 65%.
The levels of TIMP-1 in the SVC was in the CRT group significantly higher $213 \pm 70$ ng/ml vs $160 \pm 23$ ng/ml in controls ($p = 0.03$). The level of TIMP-1 in the AF- group ($191 \pm 73$ ng/ml) was not significantly different from the control group ($p = 0.195$) (*Figure 1*). At the various collection sites (CS, SVC and aorta), no significant differences between the 3 groups were noted.
**Discussion:** TIMP-1 serum levels in HF patients are significantly elevated compared to a control group without structural heart disease. No significant differences in levels of TIMP-1 were seen between AF patients and the control group. This is an expression of the advanced disease in the HF patients. The source of serum TIMP-1 are unclear as differences in the level of TIMP-1 between CS, SVC and aorta were not detectable.

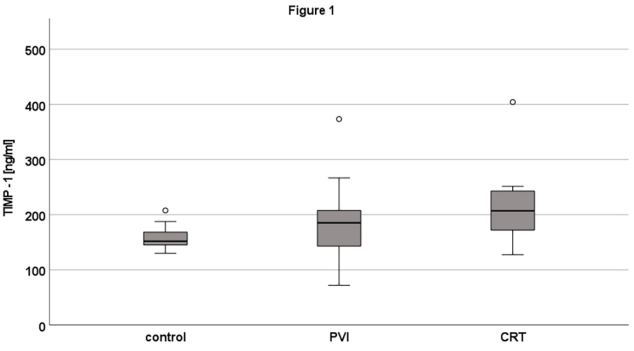


Figure 1

### Influence of therapy in patients with CAD and type 2 diabetes on inflamation biomarkers P-selectin and Galectin-3 changes

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**Funding Acknowledgements:** Type of funding sources: None.

The aim of this study was to evaluate the influence of therapy in patients with CAD and type 2 diabetes on inflammation biomarkers P-selectin and Galectin-3 levels.
**Methods:** In this study, all 121 subjects were defined into 3 groups: CAD with type 2 diabetes (group 1), CAD without type 2 diabetes (group 2) and type 2 diabetes without CAD (group 3). We used 'Human sP-selectin Platinum ELISA', 'Human Galectin- ELISA' and 'CRP-enzyme immunoassay-Best (highly sensitive)' to determine the levels of P-selectin, Galectin-3 and hs-CRP.
**Results:** In the subgroup of angina patients treated with aspirin at a dose of 75 mg/d, the level of P-selectin did not differ significantly compared to patients who did not receive antiplatelet therapy. In patients treated with clopidogrel at a dose of 75 mg/d, the level of P-selectin was significantly lower compared with patients who did not receive antiplatelet therapy and with patients receiving aspirin monotherapy (($66.4 \pm 25.6$) ng/ml, ($97.2 \pm 19.3$) ng/ml and ($81.5 \pm 29.1$) ng/ml, respectively, $p < 0.05$). In patients receiving DAT, the level of P-selectin ($57.9 \pm 28.1$ ng/ml) was significantly lower than in patients who did not receive antiplatelet drugs or received aspirin monotherapy. Unlike P-selectin and Galectin-3, the level of hs-CRP did not depend on antiplatelet drugs and was the same in all groups of patients.
In the group of patients with angina who received anticoagulant therapy, the level of P-selectin was significantly lower compared to patients who did not receive anticoagulant therapy (($73.1 \pm 21.1$) ng/ml and ($95.6 \pm 22.3$) ng/ml, respectively, $p < 0.05$). At the same time, the levels of Galectin-3 and hs-CRP did not differ in the groups of patients who were and were not treated with anticoagulants.
In the group of patients treated with statins, the level of P-selectin was not significantly lower compared to patients not treated with statins (($85.35 \pm 19.23$) ng/ml and ($92.12 \pm 37.34$) ng/ml, respectively, $p < 0.05$). At the same time, statin therapy significantly affected the level of hs-CRP, which was significantly lower in the group of patients receiving statins.
In the group of patients with CAD treated with metformin, the level of P-selectin was not significantly higher compared to patients who did not receive metformin therapy (($99.1 \pm 24.3$ ng/ml and ($84.6 \pm 23.6$) ng/ml, respectively, $p < 0.05$). Galectin-3 levels in plasma also did not differ significantly. At the same time, metformin therapy at a dose of 1000 mg twice daily led to a significant decrease in hs-CRP levels.
**Conclusion:** Therapy with clopidogrel and anticoagulant rivaroxaban is associated with a decrease in P-selectin levels, which reflects a decrease in the activity of the platelet component of the systemic inflammatory response in atherosclerosis. Unlike clopidogrel, acetylsalicylic acid does not affect the level of P-selectin. Therapy with statins and metformin led to a decrease in hs-CRP levels.

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