Abstract: **P285**

**Novel cardioactive factors selected in vivo from an AAV library encoding the secretome**

**Authors:**
G Ruozi¹, F Bortolotti¹, A Falcione¹, S Vodret², L Zentilin¹, S Zacchigna², M Giacca¹, ¹International Centre for Genetic Engineering and Biotechnology (ICGEB), Molecular Medicine - Trieste - Italy, ²International Centre for Genetic Engineering and Biotechnology (ICGEB), Cardiovascular Biology - Trieste - Italy,

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Background: Despite marked advance in early diagnosis and prevention, therapy of cardiac disease is still largely unsatisfactory. In particular, no drugs are currently available to protect the heart against ischemic damage or promote its functional repair.

Purpose: The overall purpose of this work was the unbiased identification of novel cardioactive factors by an innovative functional selection procedure (FunSel) based on in vivo gene transfer of an AAV library of 1200 vectors coding for the mouse secretome (including all cytokines, chemokines, enzymes, extracellular matrix proteins and other secreted factors encoded by the genome) in a mouse model of myocardial infarction (MI).

Methods: The 1200 AAV vectors in the library were organized in 24 pools of 50 vectors. Each pool was injected in vivo into the left ventricle of adult CD1 mice. Then, a selective stimulus (permanent left descendvent coronary artery ligation) was applied. After 21 days, vector inserts were recovered from the survived myocardium and the frequency of each vector was determined by next generation sequencing of unique barcodes tagging each AAV genome. The most enriched factors were then generated as individual AAV9 vectors and their efficacy was validated after MI. Cardiac function was monitored by echocardiography, morphometric, histological and molecular analysis. To assess how the candidate therapeutic genes exert their effect, their activity on apoptotic and autophagic pathways was investigated.

Results: After two subsequent rounds of FunSel, a subset of factors never associated with heart function resulted enriched. The 5 top hits were identified and individually tested after MI. Three factors confirmed their beneficial effect in ischemic hearts after AAV9 cardiac delivery, leading to markedly improved cardiac function at day 60 after MI and significantly reduced infarct size. Similar results were obtained by injecting infarcted mice intraperitoneally with AAV8 vectors encoding the three factors from a liver specific promoter, thus exploiting this organ as a factory of cardioprotective molecules. The beneficial effect of the three factors correlated with their ability to act immediately after damage to preserve tissue viability. In all three cases, the number of apoptotic cells in the peri-infarctual region was significantly reduced; two of the factors also markedly stimulated cardiomyocyte autophagy.

Conclusions: These results confirm the efficacy of FunSel for the unbiased and systematic identification of new potential therapeutic hits using AAV libraries in vivo. In particular, we identified three novel and powerful secreted proteins able to significantly preserve cardiac function and reduce infarct size after MI by AAV9 cardiac delivery and after secretion from the liver. We are currently generating the corresponding recombinant proteins to test their efficacy as injectable biotherapeutics after myocardial infarction.