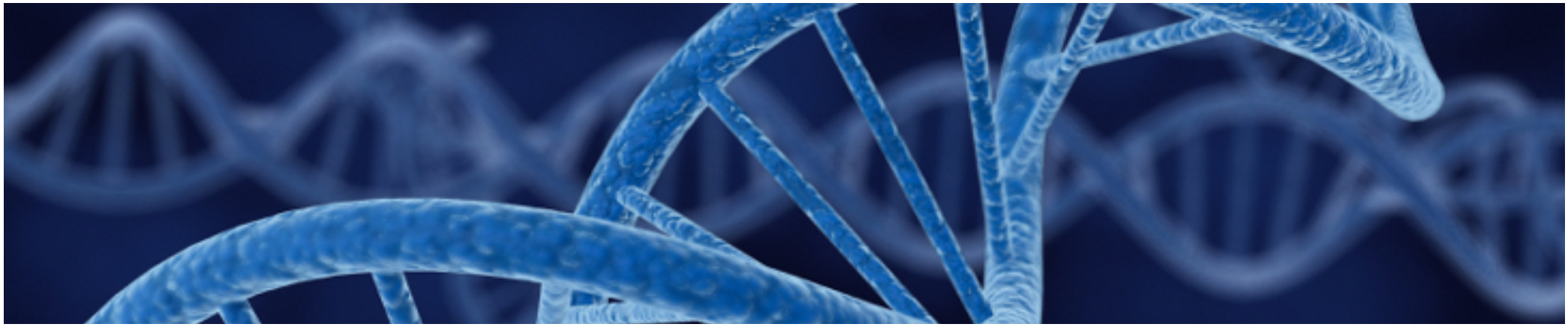

LMNA-related dilated cardiomyopathy

Lentiviral vector strategy to cure dilated cardiomyopathy

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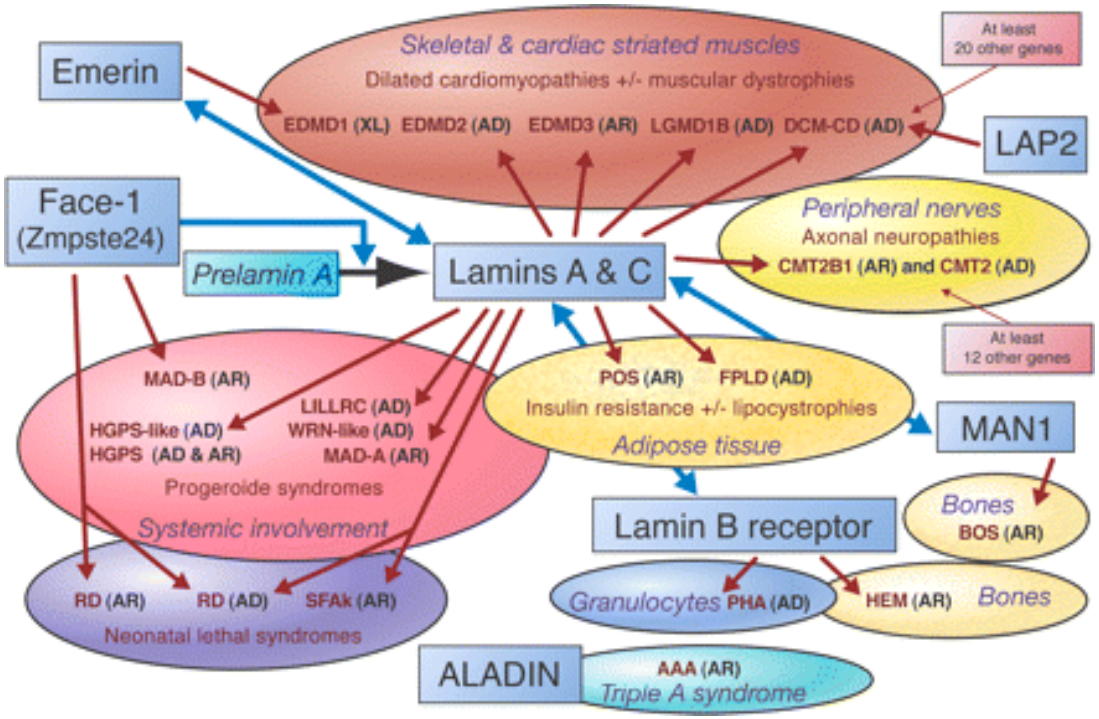
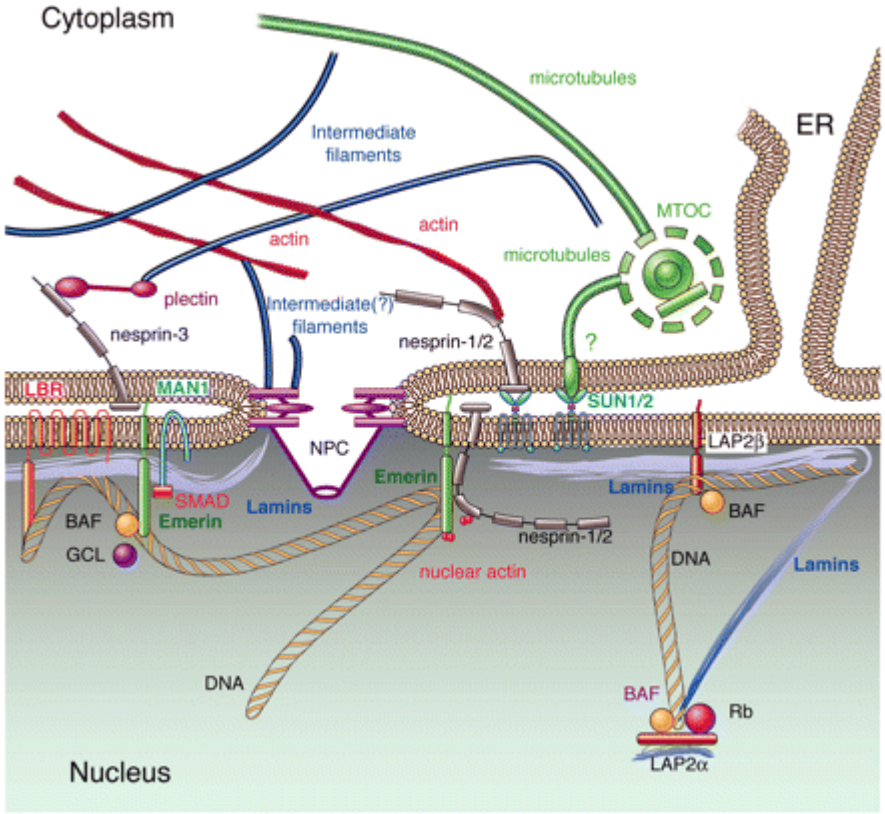
AA 2014/2015
Gene therapy
Pr. Isabella Saggio

Nuclear lamins and laminopathies

- Lamins A and C are intermediate filament proteins
- Major structural proteins of the nucleus
- Essential for nuclear integrity and organization of nuclear functions
- Located in the nuclear lamina and interact with a lot of actors of the nuclear envelope





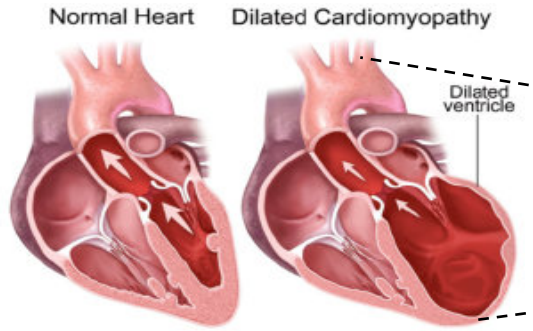
- Laminopathies is caused by abnormalities in the structure or processing of the lamin A/C (mutations in *LMNA* gene)
- 66% of laminopathies involve deffects in skeletal and striated muscle

J. L. V. Broers *et al.,* 2006

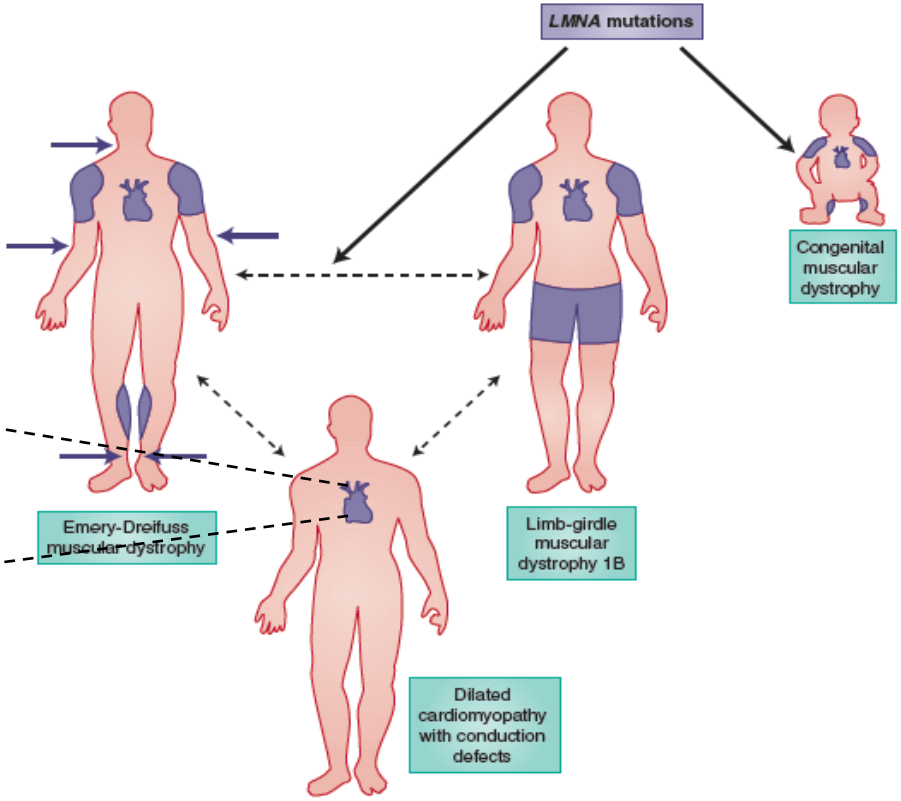
LMNA-related dilated cardiomyopathy (DCM)

DCM is the most common feature in laminopathies

Severe symptomes:
- Dilation of the heart chambers
- Arrythmias and/or conduction abnormalities

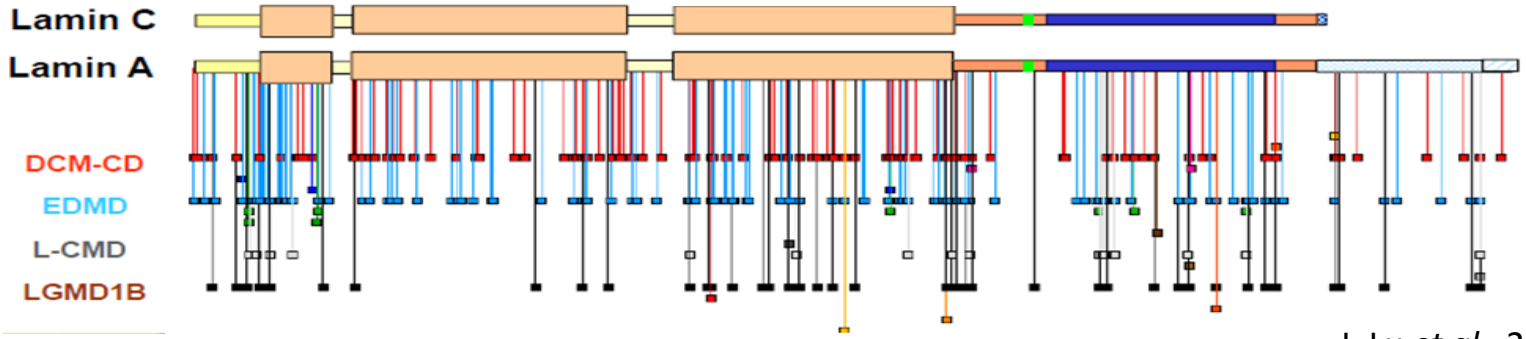


www.transgenomic.com



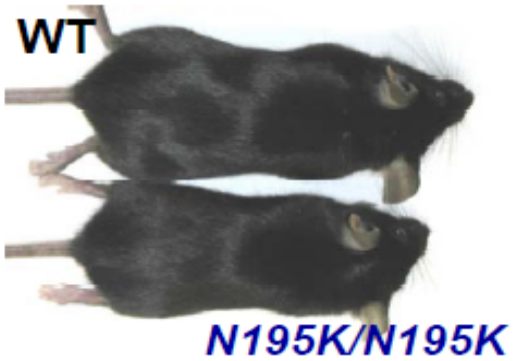
Eventually results in **heart failure**

Heterogeneous mutations → Strategy with a mutation independant therapy



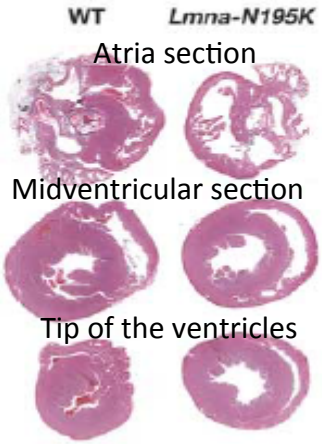
J. Lu *et al.*, 2011

Lmna$^{N195K/N195K}$ mutant mice

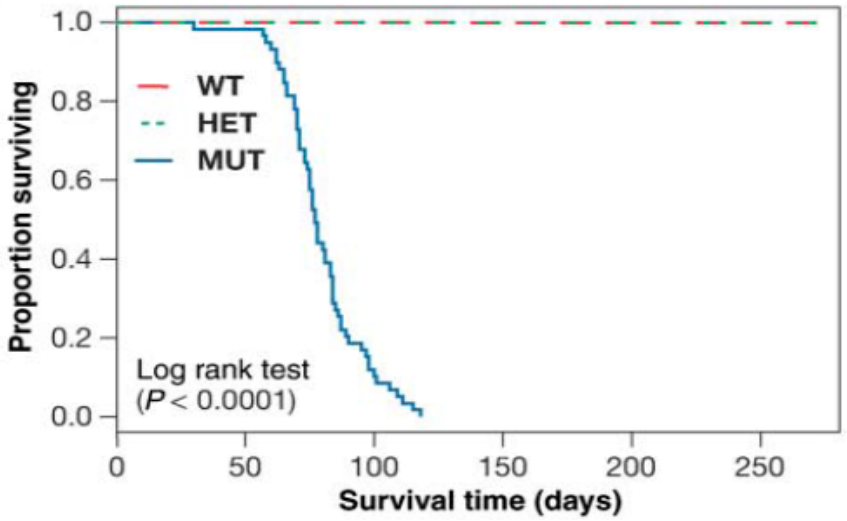


Features:

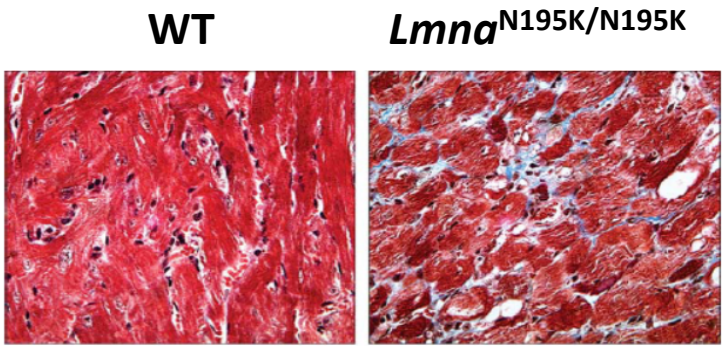
- Model is smaller than WT
- Mild DCM at 10 weeks → Ventricles and atria
- Die from arrhythmia, conduction defects by 16 weeks
- *Lmna*$^{N195K/N195K}$ hearts have increased interstitial fibrosis



Starting of dilatation at 10 weeks. Ventricles and atria of 10-weeks-old mutant hearts show mild dilatation when compared with WT hearts.



Survival rate. Survival of homozygous mutant males (n = 38) was compared with females (n = 22).

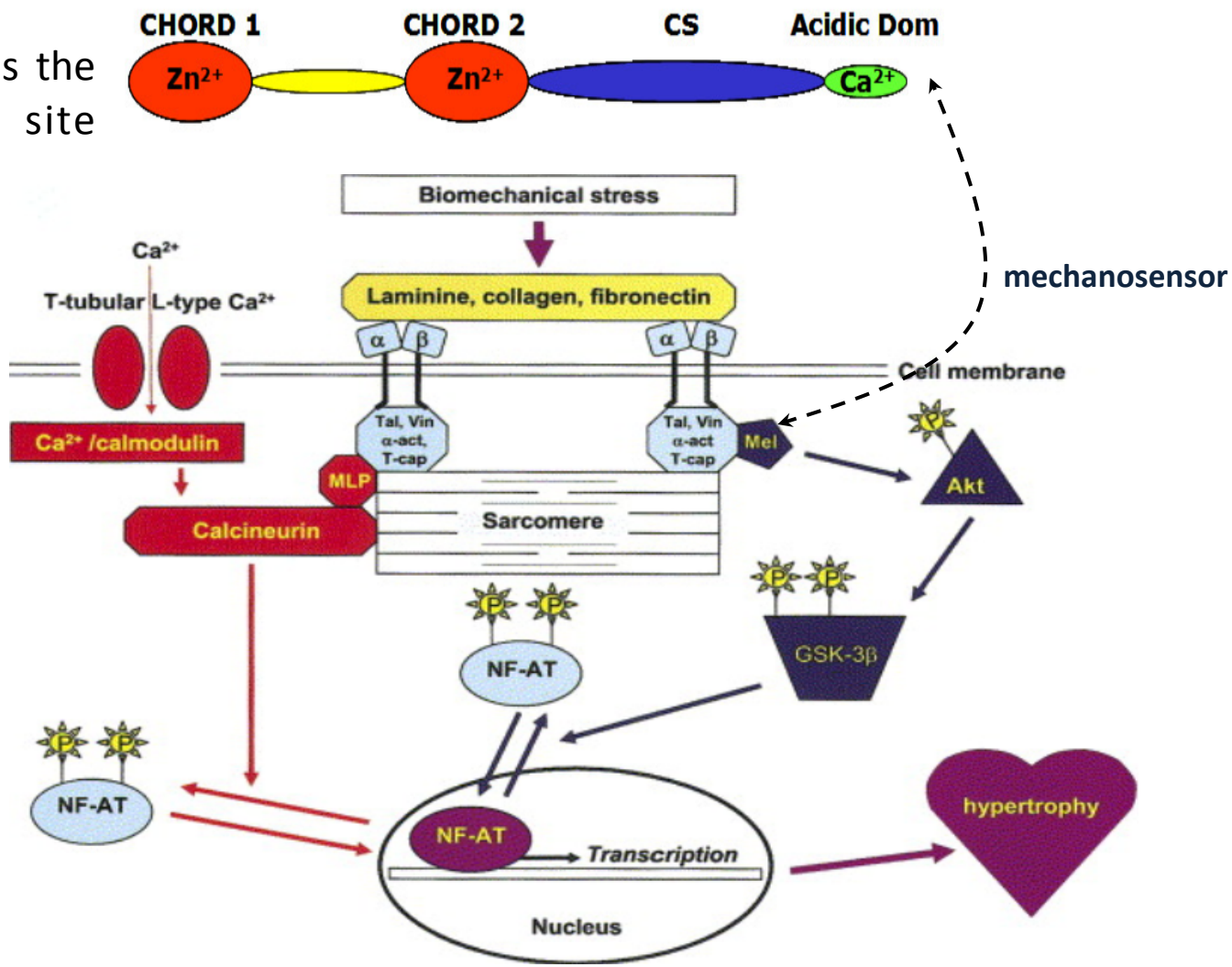


Sections of ventricular tissue from a WT and mutant heart . Increased interstitial fibrosis was observed in LmnaN195K/N195K heart tissue; Collagen were stained with Masson's trichrome .

L. Mounkes *et al.,* 2005

Melusin roles

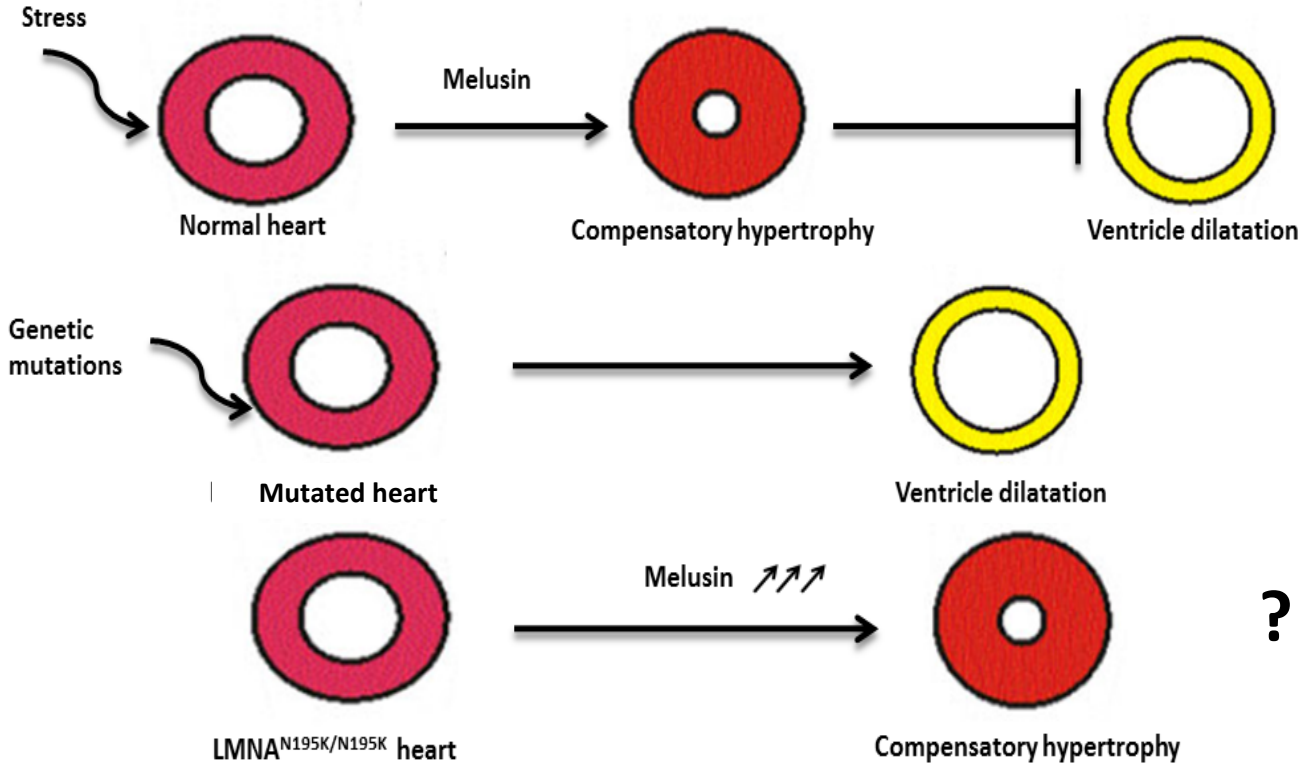
Melusin structure:
CS domain contains the integrin binding site
(Brancaccio *et al*., 1999)



Melusin signaling pathway and mechanism of action: Melusin controls the phosphorylation of AKT and GSK3β in response to mechanical overload (Brancaccio *et al.,* 2003; Brokat *et al.,* 2007)

Melusin as a mutation-independent approach

Cardioprotective effects of melusin preventing cardiac dilation and failure



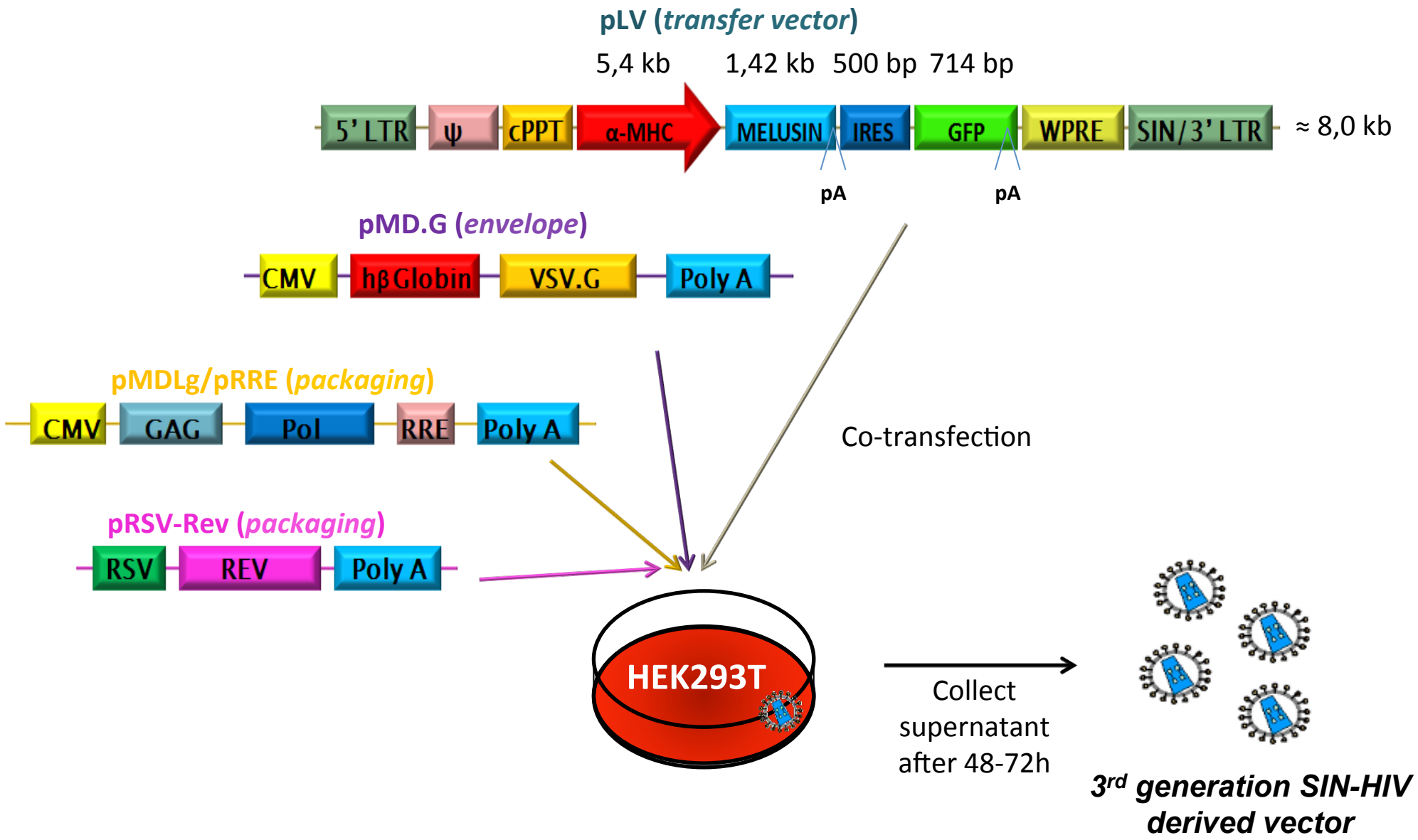
AIM

Induction of cardioprotective effects by a mutation-independent approach

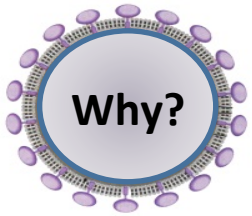
STRATEGY

Selective melusin overexpression through lentiviral vector delivery

Vector production



Lentiviral vector



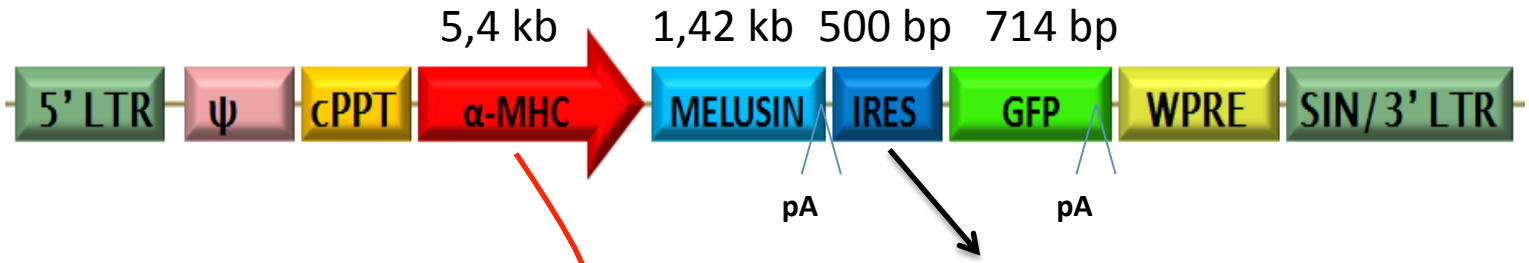
Capacity:8-10 kb

Efficient integration into non-dividing cells

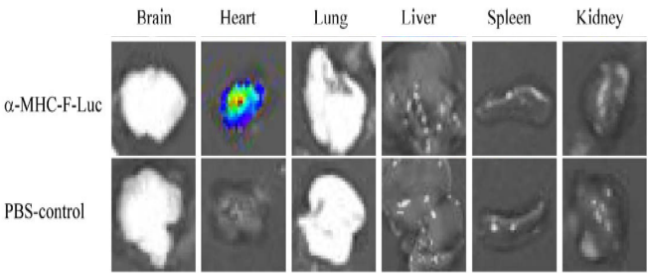
Low immunogenicity

Construct:

- **SIN-HIV-1 derivated vector**
- **Third generation:**
  - Packaging and REV plasmids
  - VSV-G pseudotype envelope plasmid
  - pLV transfer vector :

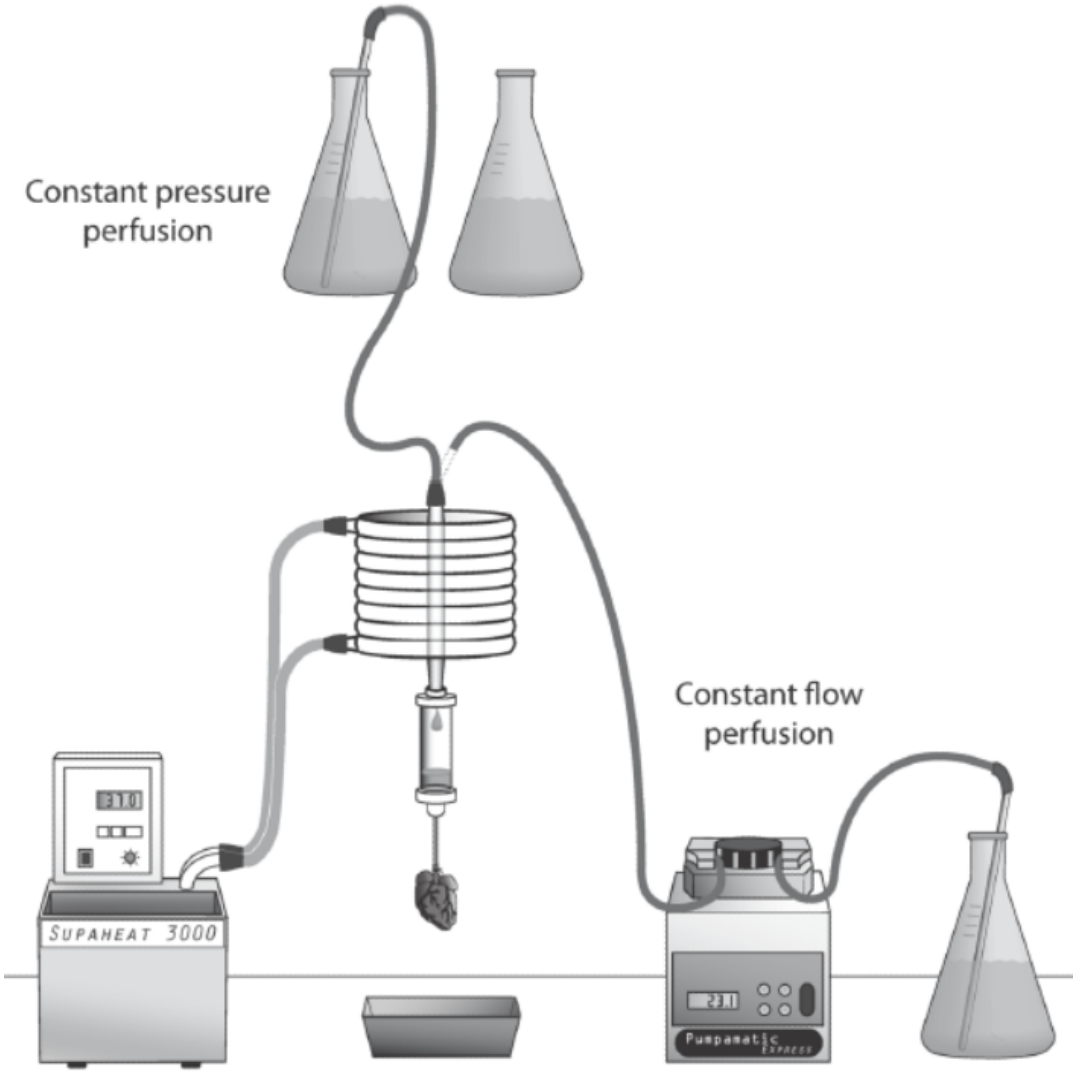


Bicistronic expression

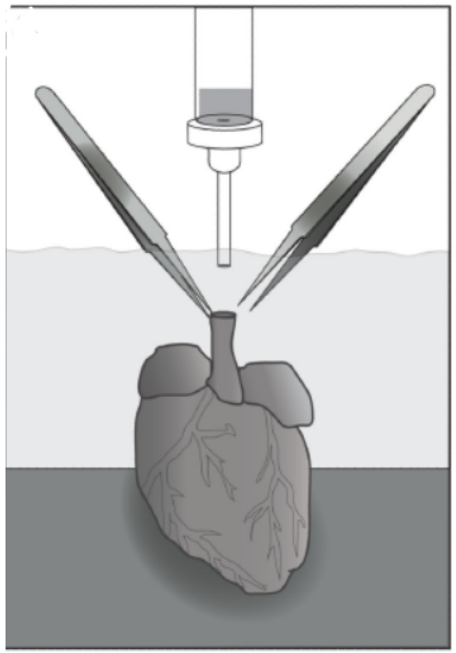


(Chyan-Jang Lee et al., 2010)

Cardiomyocytes isolation



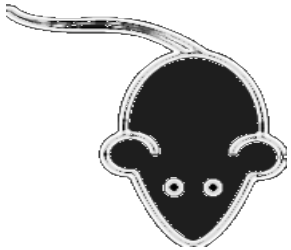
Langendorff apparatus



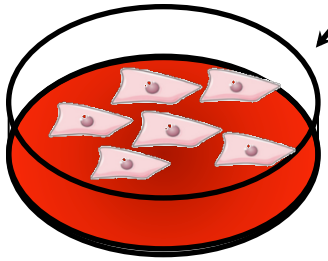
Cannulation of the aorta. The heart and tip of the cannula are immersed in low-Ca2+ solution.

William E. Louch *et al.*, 2005

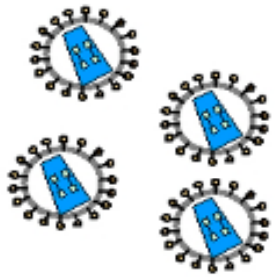
In vitro experiments



Cardiomyocytes isolation



Transduction

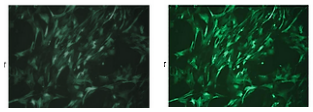


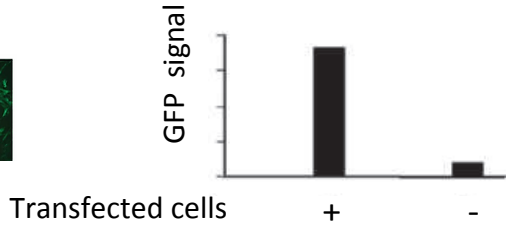
Experiments

Expected results

Is the vector integrated?

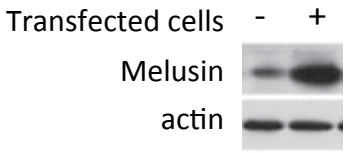
- GFP fluorescence microscopy
- Quantification (Cell counting)

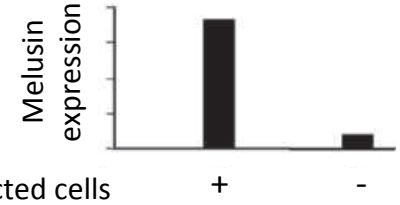




Is melusin overexpressed?

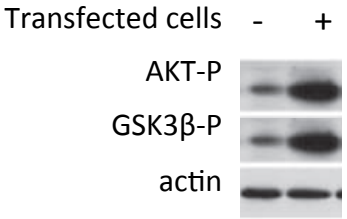
- Western blotting
- RT-PCR





Is melusin's pathway over activated?

- AKT-P and GSK3β-P Western Blotting



Are the cardiomyocytes protected?

- Mechanical stress test

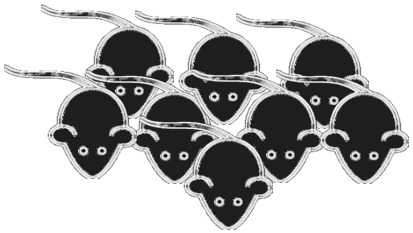
Response more or less similar from WT and treated mice

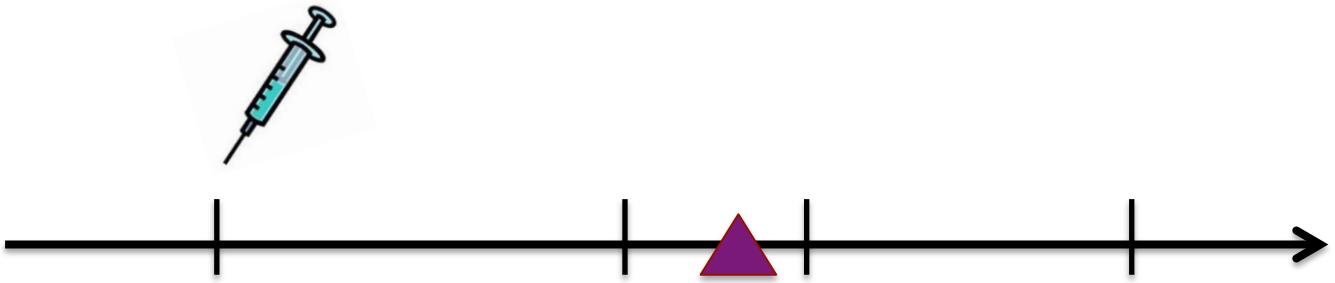
All experiments on: WT + $Lmna^{N195K/N195K,}$ non treated, treated with empty LV or LV-Melusin

In vivo experiments

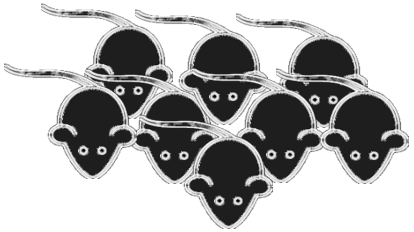
Monitoring line

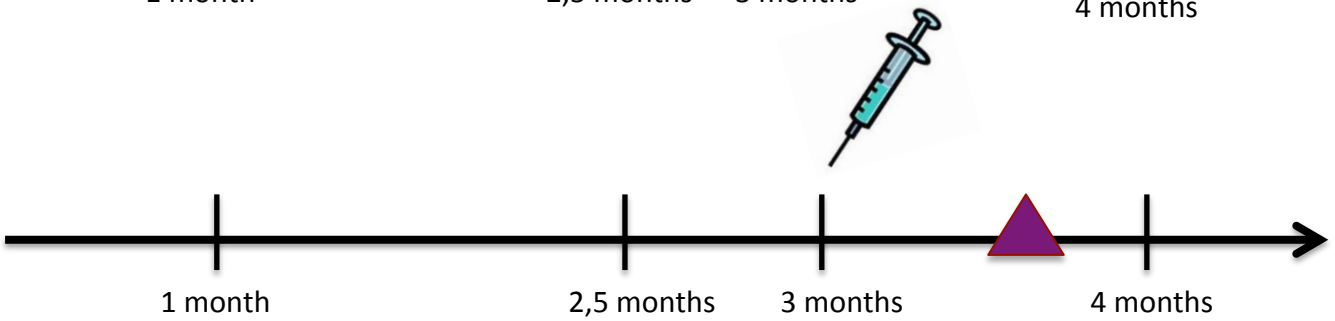
Prevention





Cure





Group of 8 mice per condition

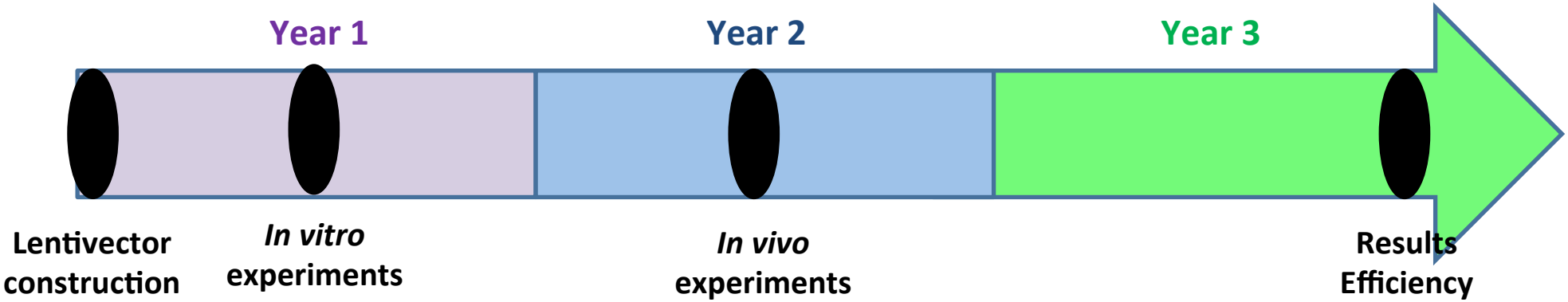
Legends:

- Electrocardiogram
- PIIINP and MMP2/8/9 serological monitoring
- Life span and mouse size
- Hemodynamic analyses

Sacrifice for (n=3 per condition):
Fibrosis Hystological Analyses
GFP fluorescence microscopy
Western blot on Melusin and Melusin pathway

All experiments on: WT + $Lmna^{N195K/N195K,}$ non treated, treated with empty LV or LV-Melusin

Timeline and costs



Costs:

6 *Lmna*$^{N195K/N195K}$ mice (2 males, 4 females) ≈ 1 200 €
6 C57BL6 mice (WT) (2 males, 4 females) ≈ 300 €
Animal facilities ≈ 50 000 €
Lentivector production ≈ 1 500 €
Western blotting kit ≈ 2 000 €
RT-PCR kit ≈ 500 €
Cell culture ≈ 1 500 €
Immunohistochimie ≈ 2 000 €
Molecular biology equiped laboratory (chemical compounds, plastic tools, glassware, surgery instrument) and electrocardiograph experiments ≈ 2 000 €

In total ≈ 70 000 € (without salary of researchers)

References

• Brancaccio et a. (2009). **Melusin a muscle specific protein, as a drug target for prevention and treatment of heart failure**. US 7,531,714 B2

• Chyan-Jang Lee, Xin Fan, Xiao Xin Guo, Jeffrey A. Medin. (2010). **Promoter-specific lentivectors for long-term, cardiac-directed therapy of Fabry disease.** Journal of Cardiology (2011) 57, 115—122

• Gustavo Tiscornia, Oded Singer & Inder M Verma (2006). **Production and purification of lentiviral vectors.** doi: 10.1038/nprot.2006.37.

• J. L. V. Broers , F. C. S. Ramaekers , G. Bonne *et al.* (2006). **Nuclear Lamins: Laminopathies and Their Role in Premature Ageing.** Physiological Reviews., 3: 967-1008

• Jing Zhao Gavin J. Pettigrew Joan Thomas Jamie I. Vandenberg Luc Delriviere Eleanor M. Bolton Andrew Carmichael Jody L. Martin Michael S. Marber Andrew M. L. Lever. (2002) . **Lentiviral vectors for delivery of genes into neonatal and adult ventricular cardiac myocytes in vitro and in vivo**. Basic Res Cardiol 97: 348 – 358

• Jonathan T. Lu, Antoine Muchir, Peter L. Nagy *et al.* (2011) ***LMNA* cardiomyopathy: cell biology and genetics meet clinical medicine. Disease Models & Mechanisms** 4: 562-568

• K Breckpot1, JL Aerts1 and K Thielemans (2007). **Lentiviral vectors for cancer immunotherapy: transforming infectious particles into therapeutics.** Gene Therapy 14, 847–862

• Leslie C. Mounkes, Serguei V. Kozlov, Jeffrey N. Rottman *et al.* (2005). **Expression of an LMNA-N195K variant of A-type lamins results in cardiac conduction defects and death in mice.** Human Molecular Genetics, 15: 2167-2180

References

• Mara Brancaccio, Luigi Fratta, Antonella Notte, Emilio Hirsch, Roberta Poulet, Simona Guazzone, Marika De Acetis, Carmine Vecchione, Gennaro Marino, Fiorella Altruda, Lorenzo Silengo, Guido Tarone & Giuseppe Lembo. (2003). **Melusin, a muscle-specific integrin β1–interacting protein, is required to prevent cardiac failure in response to chronic pressure overload.** doi:10.1038/nm805

• Mara Brancaccio, Simona Guazzone, Nadia Menini, Elena Sibona, Emilio Hirsch, Marco De Andrea, Mariano Rocchi, Fiorella Altruda, Guido Tarone and Lorenzo Silengo. (1999*)*. **Melusin Is a New Muscle-specific Interactor for** b**1Integrin Cytoplasmic Domain** *J. Biol. Chem., 274:29282-29288*

• Marika De Acetis, Antonella Notte, Federica Accornero, Giulio Selvetella, Mara Brancaccio, Carmine Vecchione, Mauro Sbroggiò, Federica Collino, Beniamina Pacchioni, Gerolamo Lanfranchi, Alessandra Aretini, Roberta Ferretti, Angelo Maffei, Fiorella Altruda, Lorenzo Silengo, Guido Tarone and Giuseppe Lembo. (2005). **Cardiac Overexpression of Melusin Protects From Dilated Cardiomyopathy Due to Long-Standing Pressure Overload**. Circ Res. 96:1087-1094

• PL Sinn, SL Sauter and PB McCray Jr. (2005). **Gene Therapy Progress and Prospects: Development of improved lentiviral and retroviral vectors – design, biosafety, and production.** Gene Therapy 12, 1089–1098

• Sebastian Brokat, Jenny Thomas, Lars R. Herda, Christoph Knosalla, Reinhard Pregla , Mara Brancaccio , Federica Accornero, Guido Tarone, Roland Hetzer , Vera Regitz-Zagrosek. (2007). **Altered melusin expression in the hearts of aortic stenosis patients**. European Journal of Heart Failure 9 568–573

References

• Sylvain Fleury, Eleonora Simeoni, Christian Zuppinger, Nicole Déglon, Ludwig K. Von Segesser, Lukas Kappenberger and Giuseppe Vassalli. (2003). **Multiply Attenuated, Self-Inactivating Lentiviral Vectors Efficiently Deliver and Express Genes for Extended Periods of Time in Adult Rat Cardiomyocytes In Vivo Genes for Extended Periods of Time in Adult Rat Cardiomyocytes In Vivo.** *Circulation; 107:2375-2382*