DUCHENNE MUSCULAR DYSTROPHY: CARDIAC PHENOTYPE AS A RESULT OF DNA DAMAGE INDUCED CHANGE IN STEM CELL FATE..

V. Rotrekl, M. Pešl, D. Beckerová, Š. Jelínková, A. Lacampagne, A. Meli

CZECH CARDIOVASCULAR RESEARCH AND INNOVATION DAYS 2022

Brno, November 28th, 2022

DUCHENNE MUSCULAR DYSTROPHY: CARDIAC PHENOTYPE AS A RESULT OF DNA DAMAGE INDUCED CHANGE IN STEM CELL FATE..

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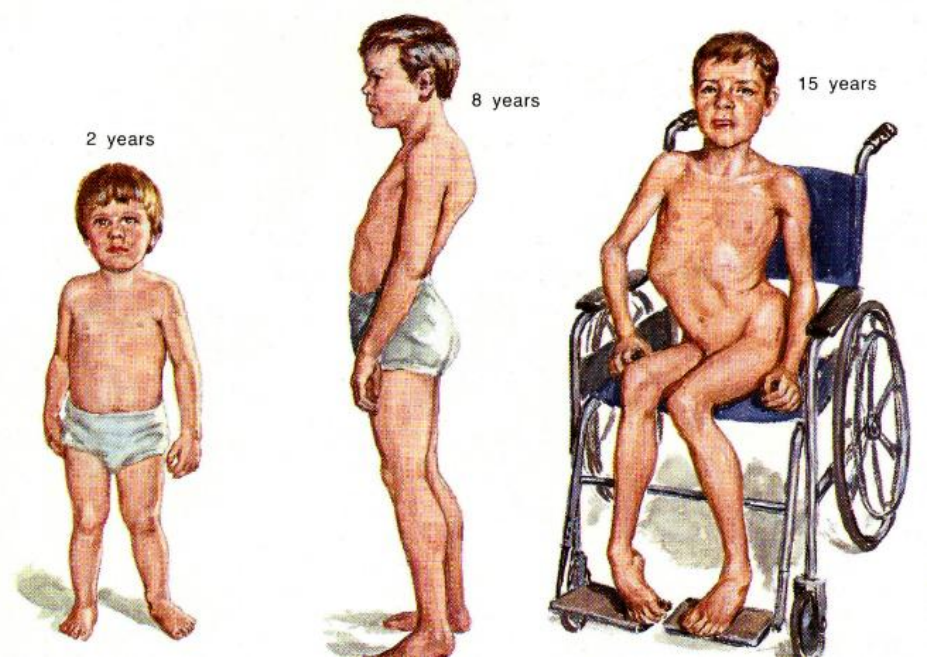


- Mutation in dystrophin
- Progresive skeletal muscle wasting
- Dialated kardiomyopathy, ventricular arrhytmia and heart failure

..leading cause of death

NO CURE but treatment: corticosteroids, ACE inhibitors, exon skipping (eteplirsen), microdystrophin (exp.gene therapy)

We need more targets to aim..

STEM CELLS DISEASE MODELING - DMD

Design of pluripotent stem cell lines carrying patient specific DMD mutation

4 DMD boys/patients

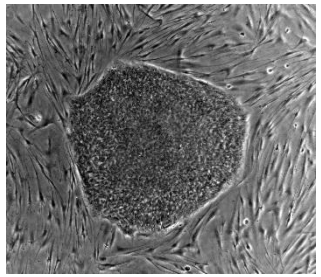
non isogenic control

reprogramming

DMD hiPSC

WT hiPSC

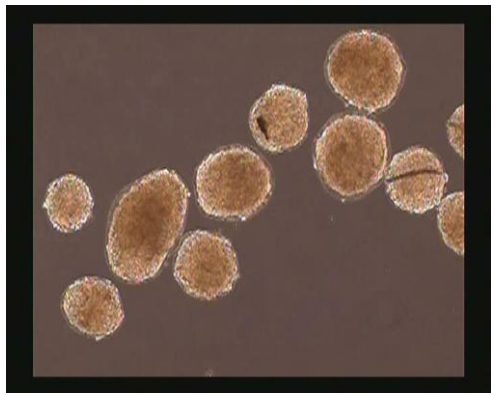
hESC



WT hESC

CRISPR/CAS dystrophin gene deletion

DMD hESC



Functional cardiomyocyte in the dish…

CARDIAC ORGANIOD

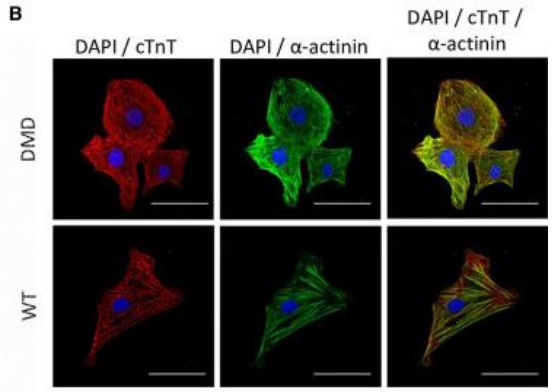
✓ **SPONTANEOUSLY BEATING**

✓ carrying DMD mutation

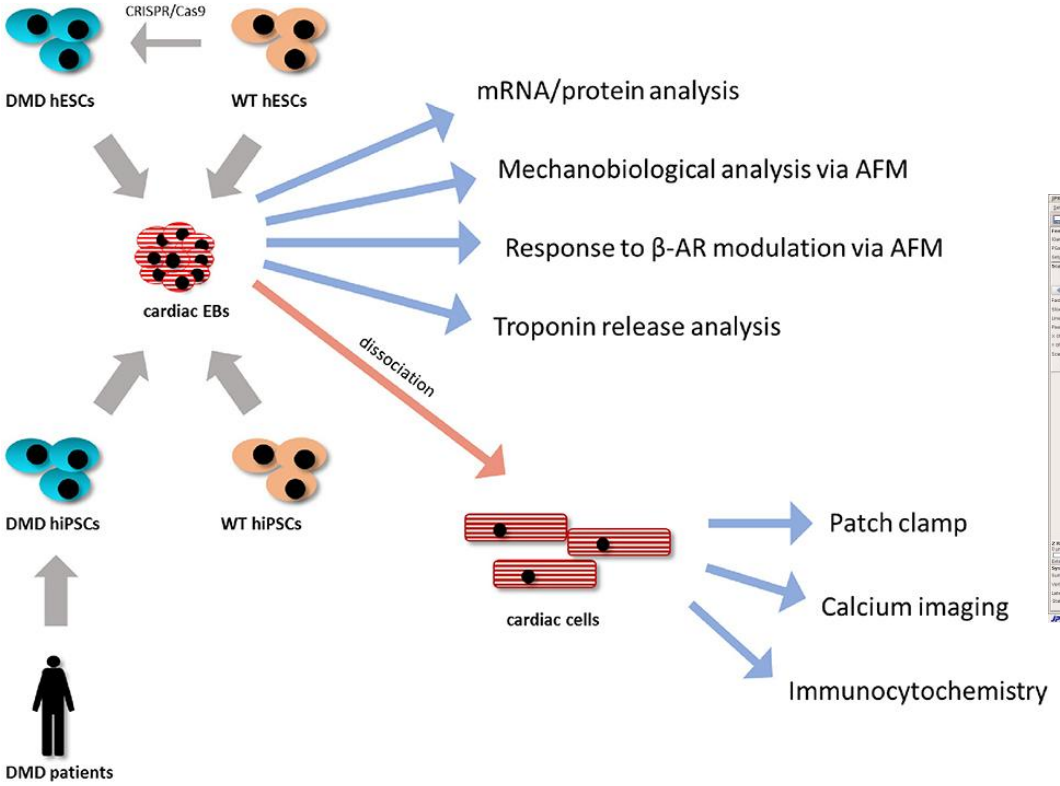
Stem Cell Res, 2019

STEM CELLS DISEASE MODELING - DMD

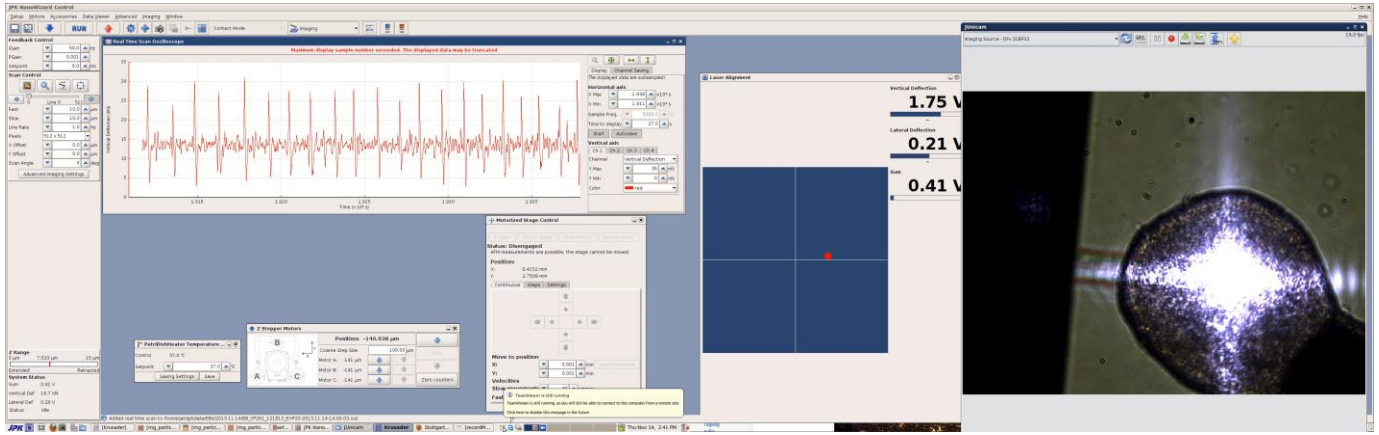
Striated pattern of cardiac troponin



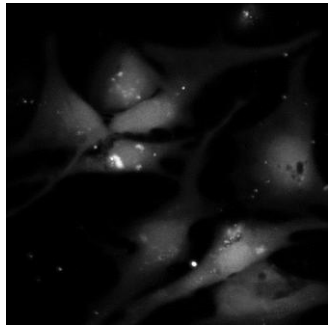
Do *in vitro* DMD cardiomyocytes recapitulate human cardiac pathology?



Mechanical properties in response to β-adrenergic stimulation



Sarcomeric Ca2+ release



Front Bioeng Biotechnol 2020
Front Physiol 2018
IEEE Nanobio2018
BiosensBioelectronics 2019
Stem Cell Res, 2019

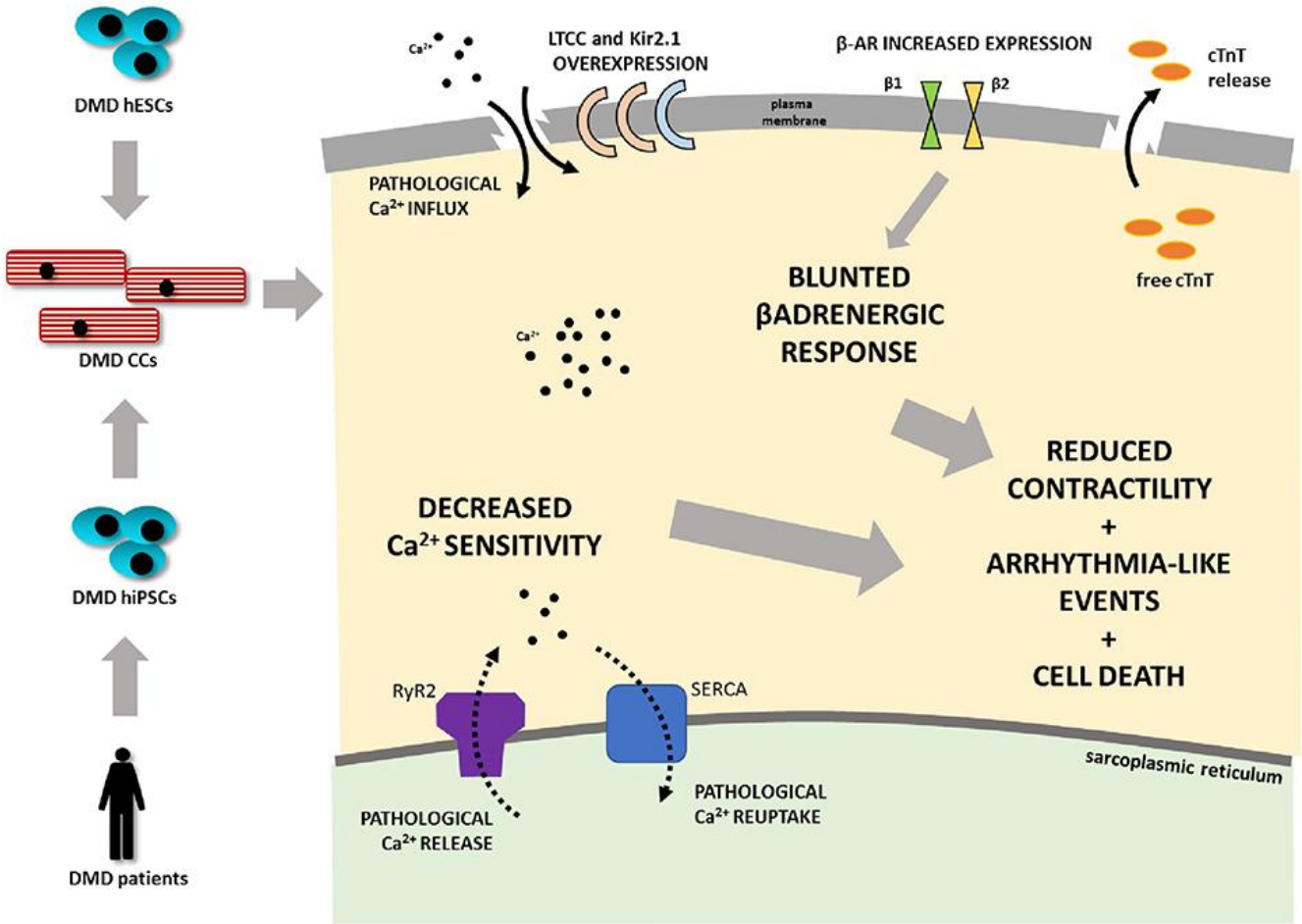
STEM CELLS DISEASE MODELING - DMD

Do *in vitro* DMD cardiomyocytes recapitulate human cardiac pathology? ✓ **YES**

DMD cardiomyocytes:

- Blunted adrenergic response
- Patological Ca2+ handeling
- Reduced contractility
- Troponin release

Arrhytmia
Cardiomyocyte death



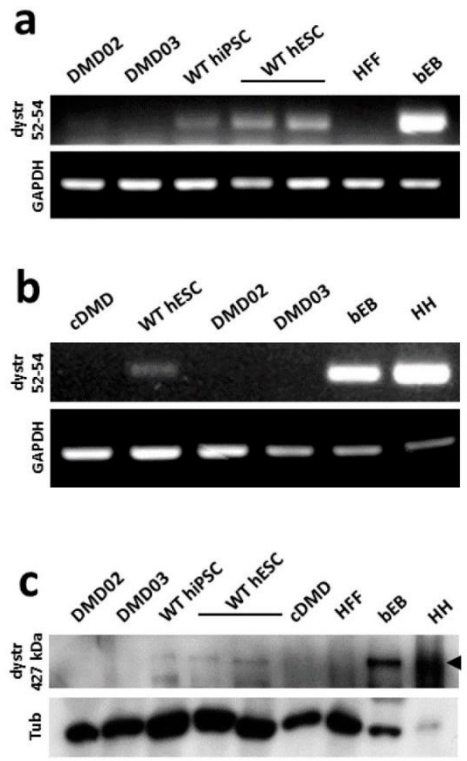
Front Bioeng Biotechnol 2020

STEM CELLS DISEASE MODELING - DMD

DEFFECTIVE STEM CELLS *in vitro* IN DMD..?

- **Already Stem Cells express dystrophin**

DP427 dystrophin expression
in WT/DMD hPSC



Cells 2019

STEM CELLS DISEASE MODELING - DMD

DEFFECTIVE STEM CELLS *in vitro* IN DMD..?

- **Already Stem Cells express dystrophin**
- **DMD stem cells have limited cardiac differentiation capacity**

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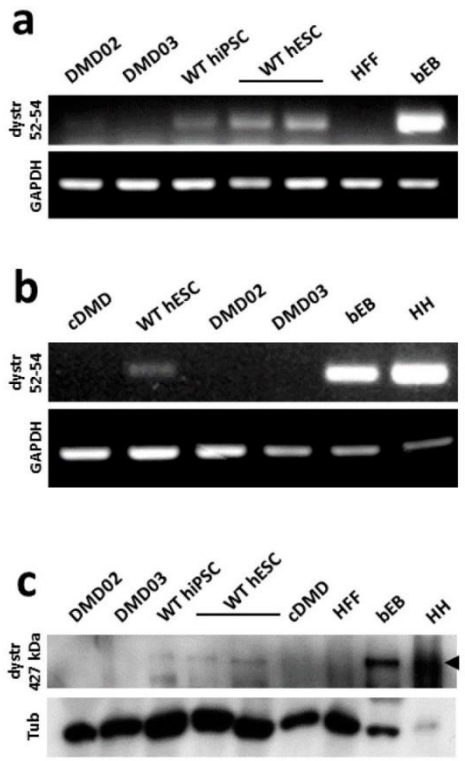
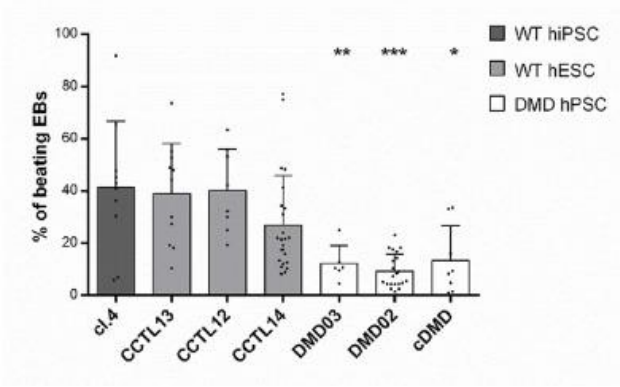


Impaired cardiac differentiation capacity of DMD hPSC



Cells 2019

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DEFFECTIVE STEM CELLS *in vitro* IN DMD..?

- **Already Stem Cells express dystrophin**
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- **DMD have impaired cardiac mesoderm maturation**

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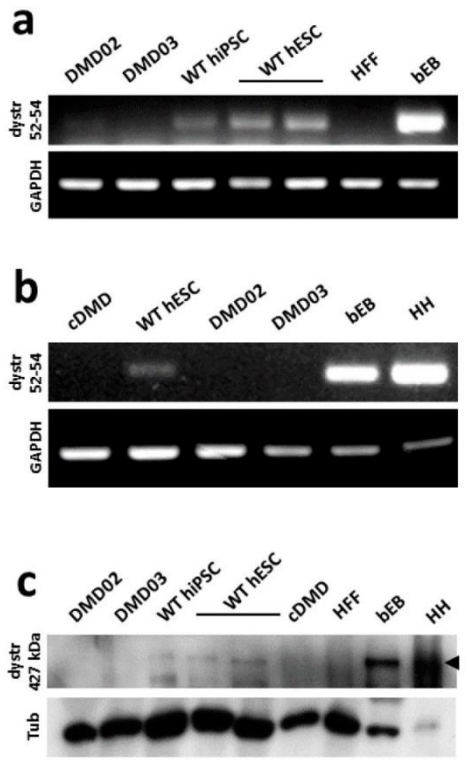

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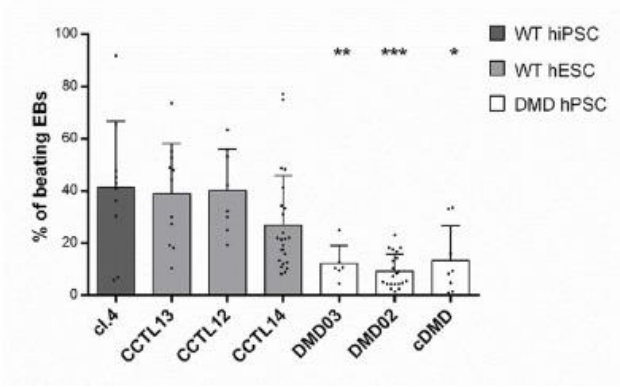
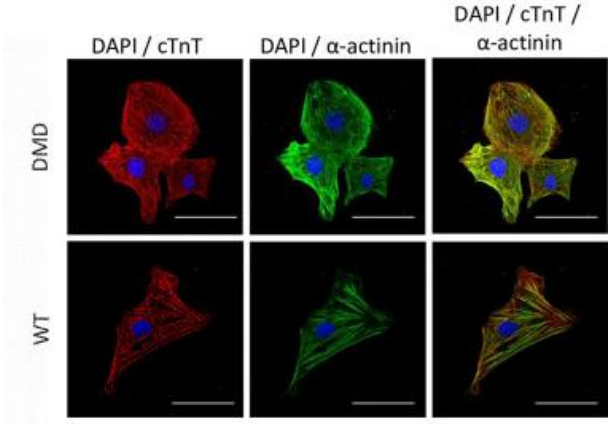
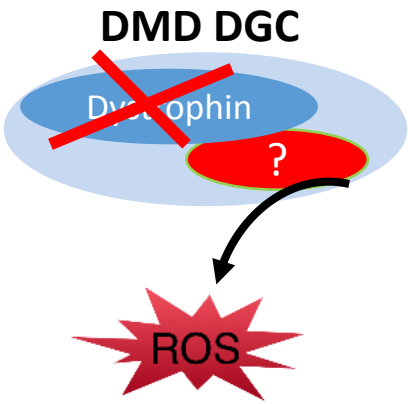


Impaired maturation of DMD cardiomycytes



Cells 2019

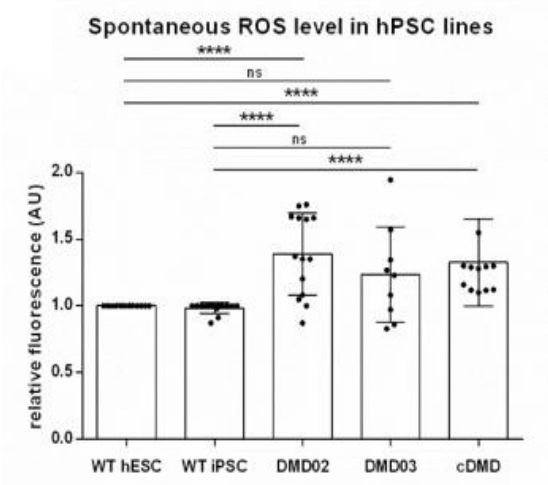
STEM CELLS DISEASE MODELING - DMD



DEFFECTIVE STEM CELLS *in vitro* IN DMD..?

.. ELEVATED REACTIVE OXYGEN SPECIES

- **Already Stem Cells express dystrophin**
- **DMD stem cells have limited cardiac differentiation capacity**
- **DMD have impaired cardiac mesoderm maturation**
- **DMD stem cells suffer from elevated ROS**



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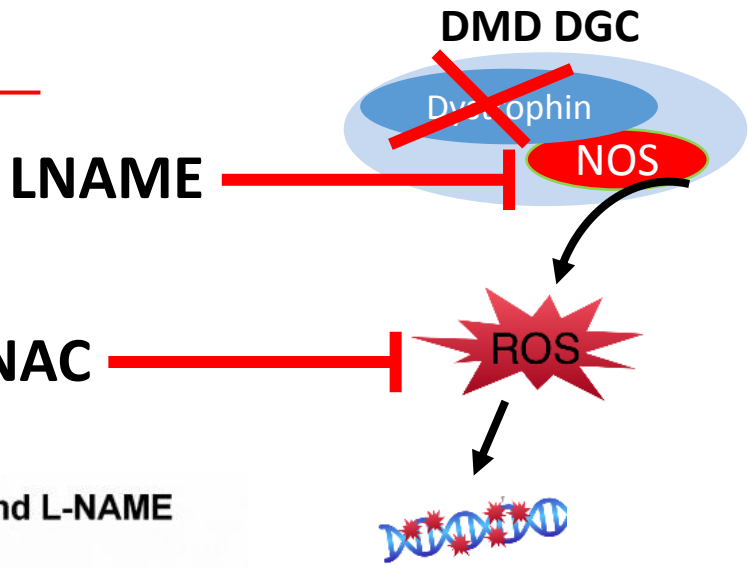
STEM CELLS DISEASE MODELING - DMD

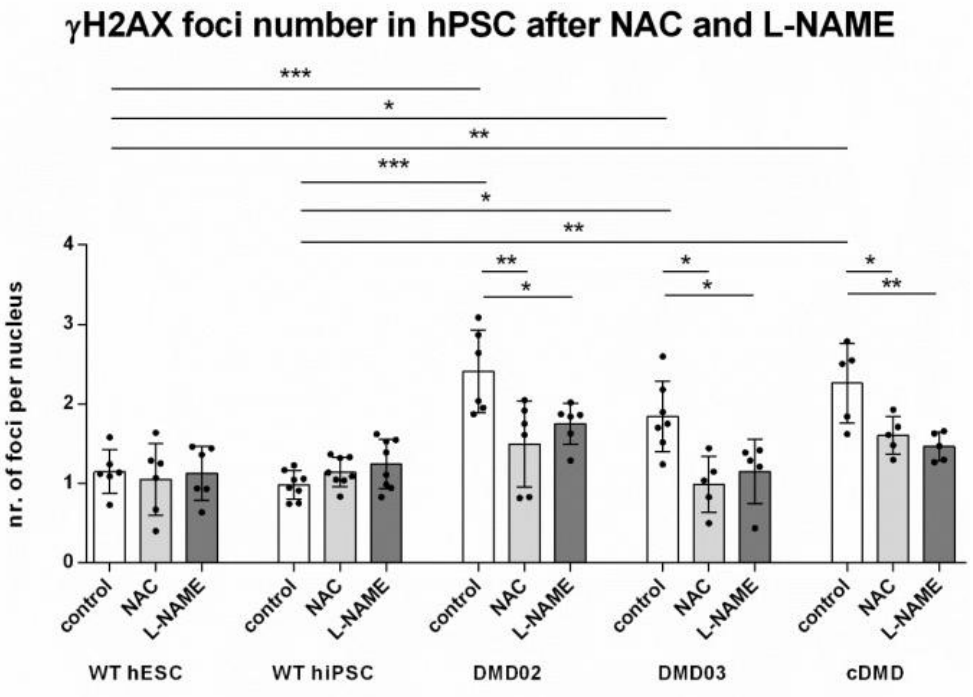


DEFFECTIVE STEM CELLS *in vitro* IN DMD..?

.. ELEVATED REACTIVE OXYGEN SPECIES

.. NOS induces ROS mediated DNA damage

- **Already Stem Cells express dystrophin**
- **DMD stem cells have limited cardiac differentiation capacity**
- **DMD have impaired cardiac mesoderm maturation**
- **DMD stem cells suffer from elevated ROS**
- **NOS inhibition ameliorates ROS and DNA abrasion**



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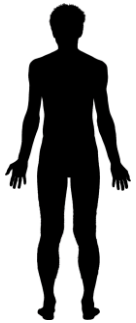
Cells 2020

STEM CELLS DISEASE MODELING - DMD

HUMAN HEART C-V PROGENITORS *EX VIVO*



Becker muscular dystrophy (BMD)
same genetics as DMD, less phenotype

DMD Stem Cells pathology

Cardiac resident stem cell-like cells in human BMD heart:

ORPHANET J Rare Dis 2020

STEM CELLS DISEASE MODELING - DMD

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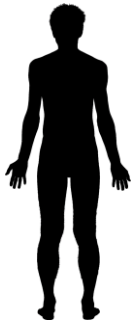

Becker muscular dystrophy (BMD)
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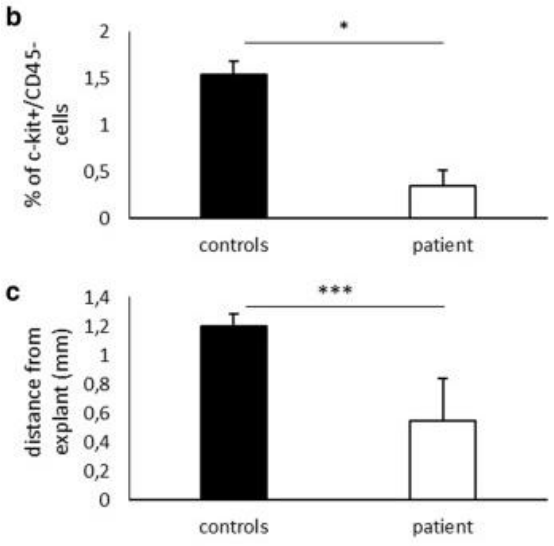
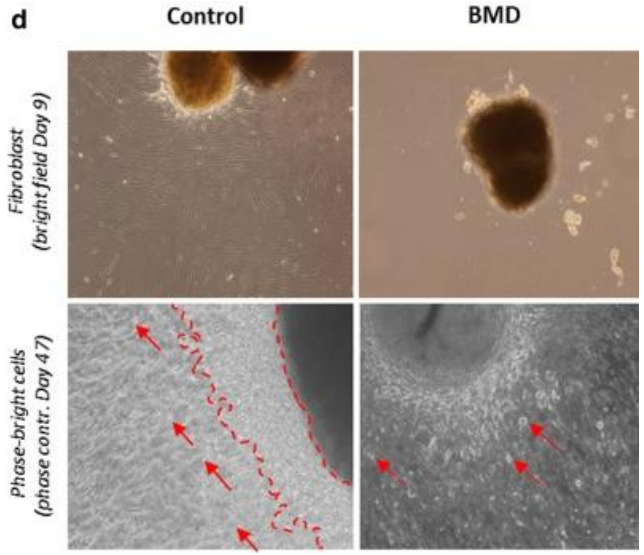
DMD Stem Cells pathology

Cardiac resident stem cell-like cells in human BMD heart: .. ARE IMPAIRED!!!!

- Less CRSLS in BMD human myocard, lower plasticity, lower resiliance





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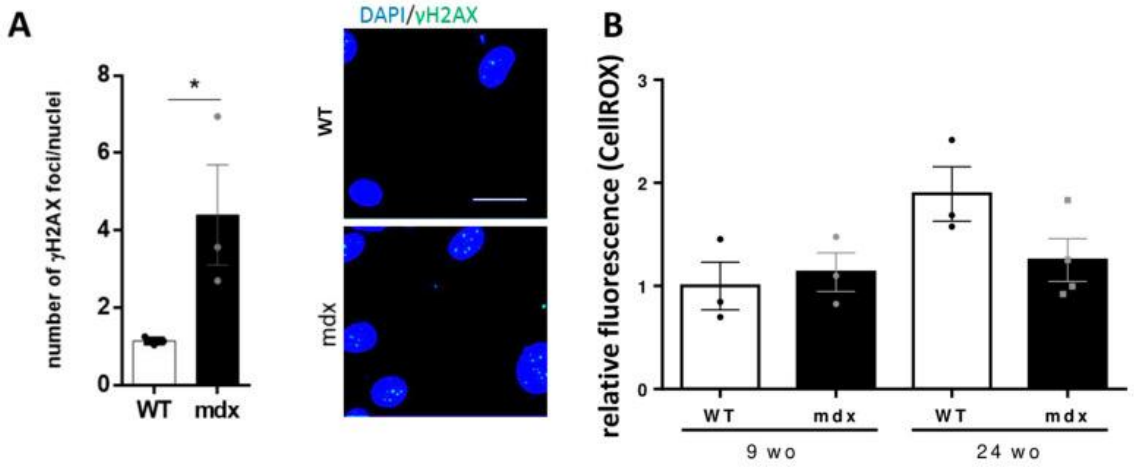
STEM CELLS DISEASE MODELING - DMD

MOUSE HEART C-V PROGENITORS *EX VIVO*

mdx MOUSE – MODEL OF DMD

DMD Stem Cells pathology

Cardiac resident stem cell-like cells in mouse *mdx* heart:



Cardiac resident stem cell-like cells:

- Elevated prolipheration
- Dramatic decrease with age
- Elevated DNA damage
- Elevated ROS

Int J Mol Sci 2021

STEM CELLS DISEASE MODELING - DMD

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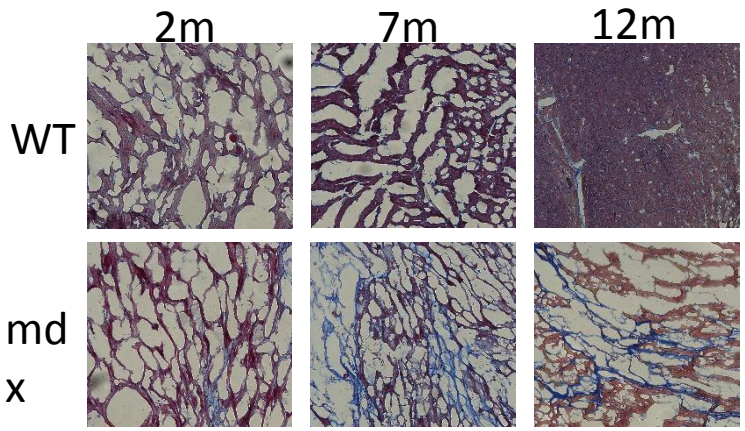

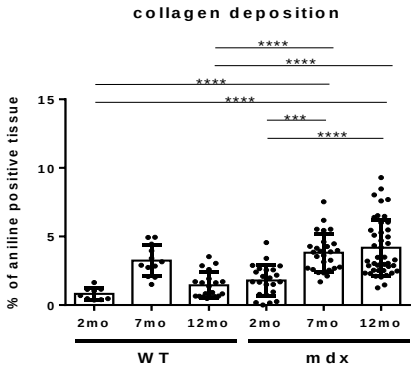

Cardiac resident stem cell-like cells:

- Elevated prolipheration
- Dramatic decrease with age
- Elevated DNA damage
- Elevated ROS
- Association with fibrosis

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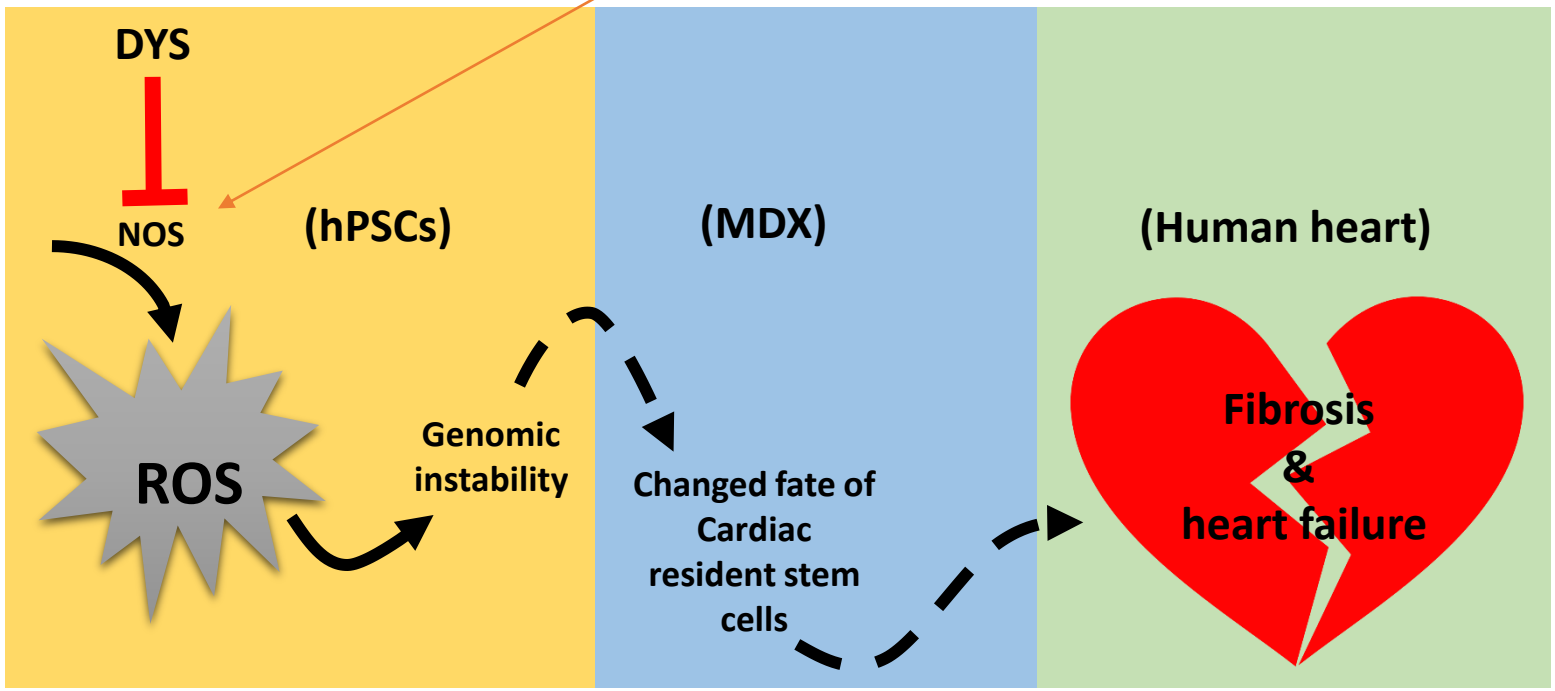
STEM CELLS DISEASE MODELING - DMD

… and we have putative drug LNAME (to be tested on mice)



STEM CELLS DISEASE MODELING - DMD

Many thanks..

FNUSA ICRC

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Vladimír Rotrekl

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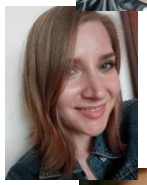

Martin Pešl

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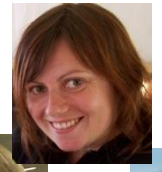

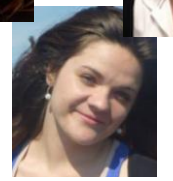

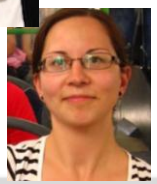

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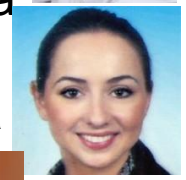
Jan Krejčí

Petr Němec

Maria Holická



Vita Žampachová

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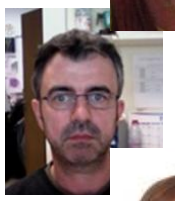


Albano Meli

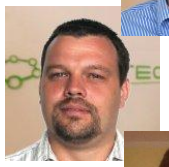
Franck Aimond

Gilles Carnac

Amanda Finan-Marchi

Valerie Scheuermann

Alain Lacampagne



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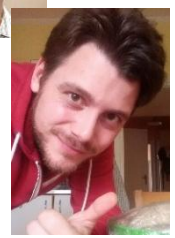

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