

**STEM CELLS AS THE MAIN STRATAGEM OF BIOLOGICAL  
REGENERATIVE MEDICINE:**

**FROM ANTI-AGING AND PREVENTIVE MEDICINE TO  
TREATMENT OF CHRONIC DEGENERATIVE DISEASES**

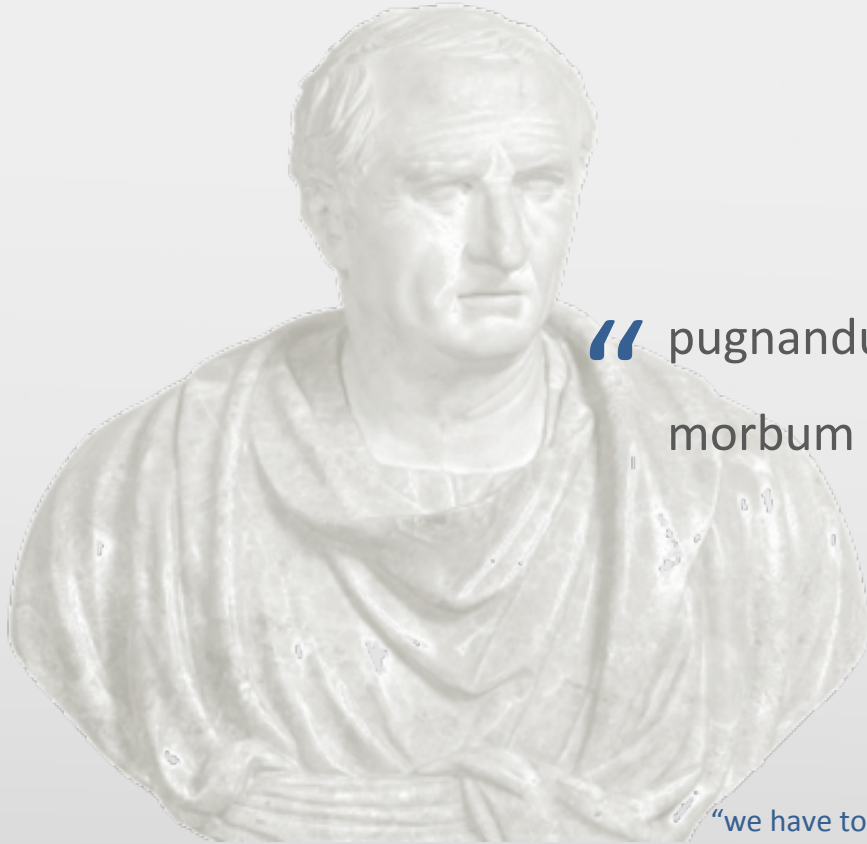
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“ pugnandum, tamquam contra  
morbum sic contra senectutem ”

“we have to fight against aging, as we do against a disease”

“ In principle, if you understood the mechanisms of keeping things repaired, you could keep things going indefinitely ”

Cynthia Kenyon

# MAMMALIAN AGING CAN BE DELAYED WITH

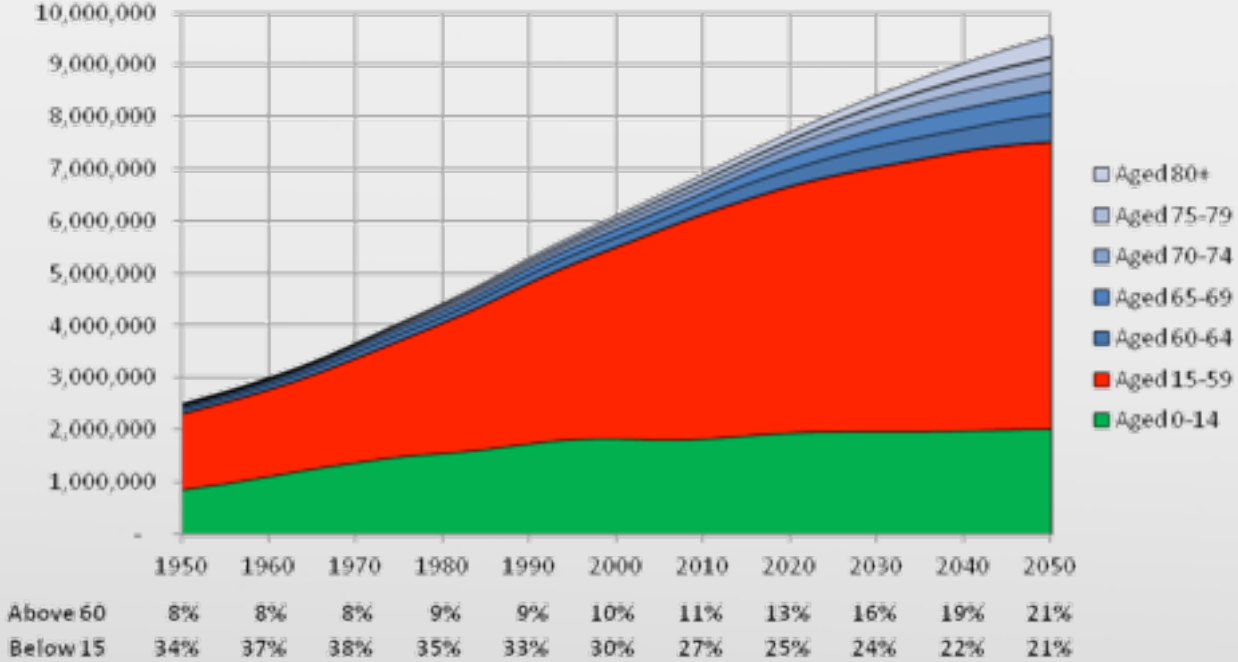
Genetic

Dietary

Pharmacological

Biological & biotechnological

# WORLD POPULATION AGEING 2015.



United Nations, Department of Economic and Social Affairs, Population Division.

Cardiovascular diseases

Cerebrovascular diseases

Hypertension

DM and metabolic syndrome

Cancer

Parkinson's disease

Dementia, Incl. Alzheimer's

Muscular dystrophy

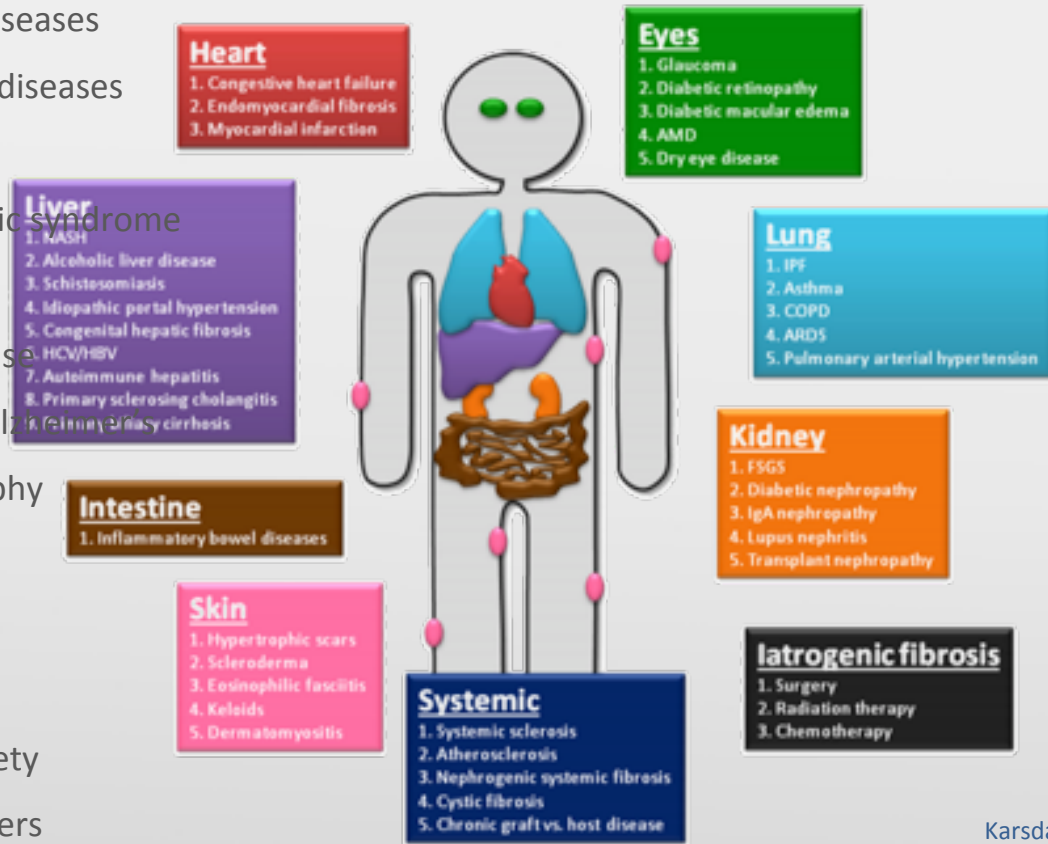
Osteoarthritis

Cataract

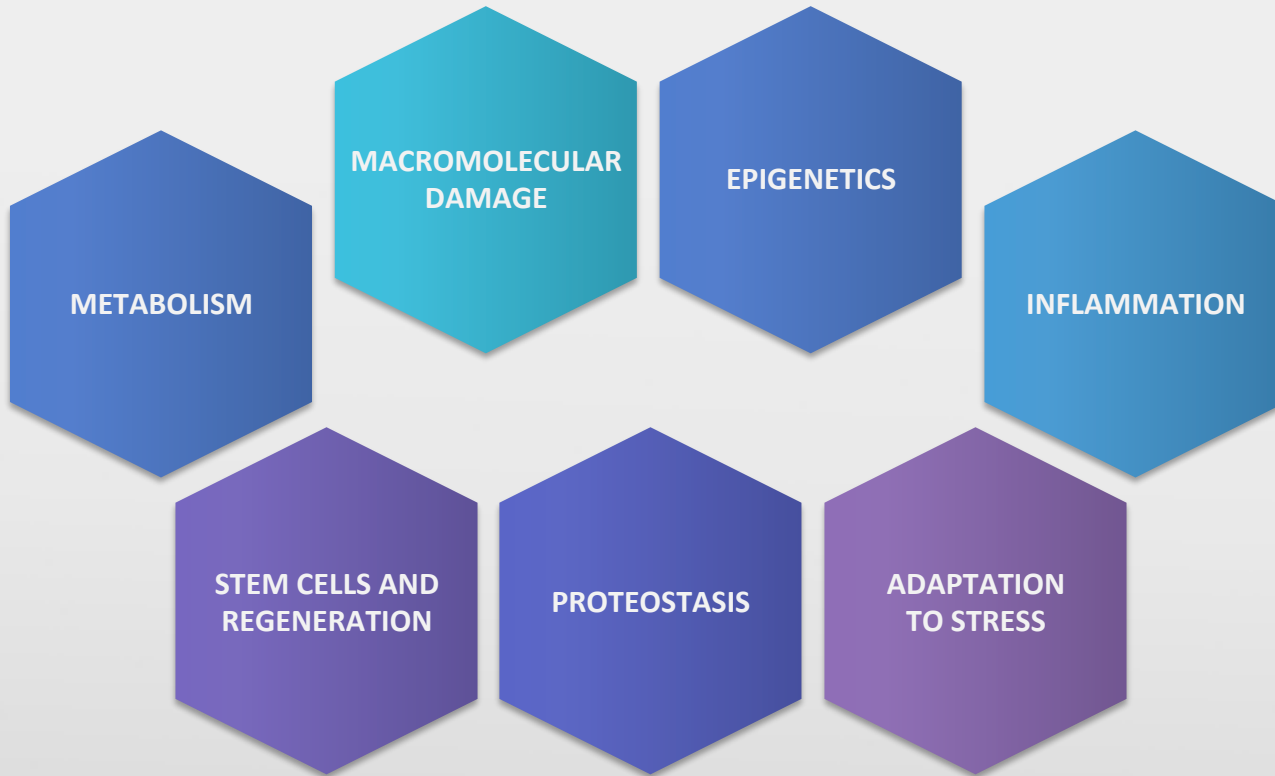
Hearing loss

Depression, anxiety

Hormonal disorders







Kennedy, Berger, Brunet and Tony Wyss-Coray  
“Aging: a common driver of chronic diseases and a target for novel interventions”, Cell, 2016.





- Metformin
- Quercetin
- Resveratrol
- Dasatinib
- etc...

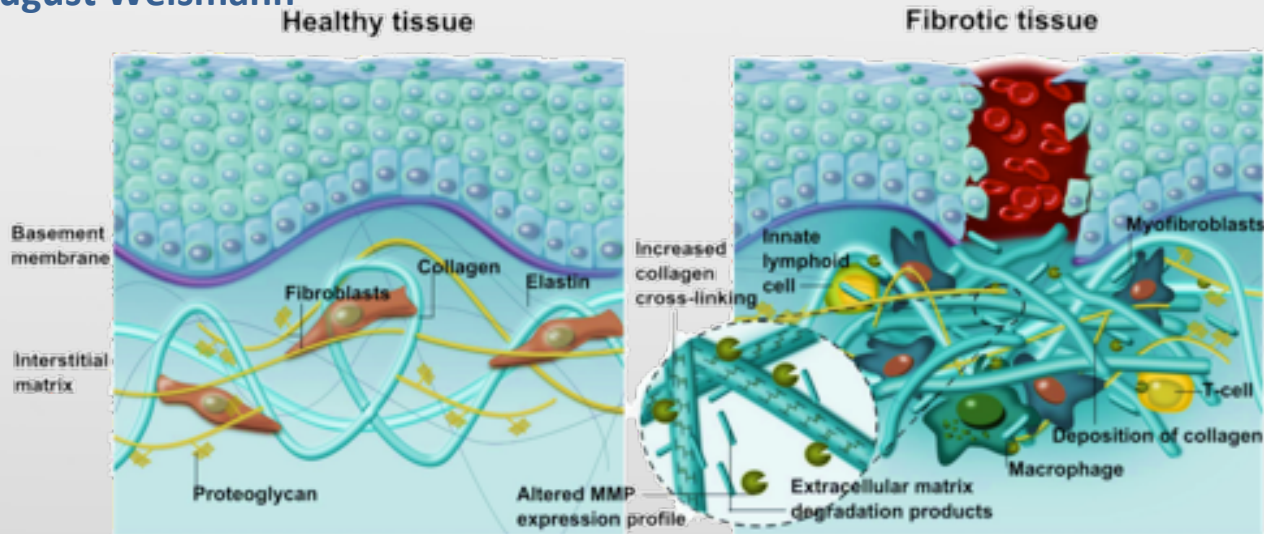


- Tumor suppressor p53
- SIRT<sub>1-3</sub> genes
- IGF-1
- Toll-like receptor family
- mTOR



“ Death takes place because a worn-out tissue cannot forever renew itself, and because a capacity for increase by means of cell division is not everlasting but finite. ”

### August Weismann



## FRESH CELL THERAPY STUDY

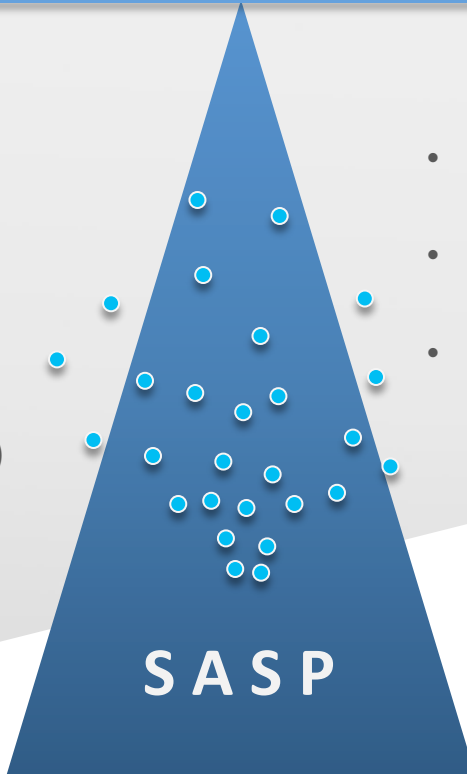
- Risks and benefits assessment and statistical evaluation
- Done by Independent Consultants in Medical Statistics and Biometry in Medicine
- Main investigator DR. Volker W. Rahlfs, CStat (RSS/UK), Biometry in Medicine (GMDS)
- 186 patients from 22 countries
- Allergic reactions in a mild form 1.07%
- Clinical improvement 98.4%
- Desire to repeat therapy 97.8%

## BENEFICIAL

- Embryonic development
- Tissue regeneration
- Wound healing
- Tumor suppression (p53 intact)

## DETRIMENTAL

- Tissue degeneration
- Chronic inflammation
- Tumor promotion (p53 deficient)



SASP

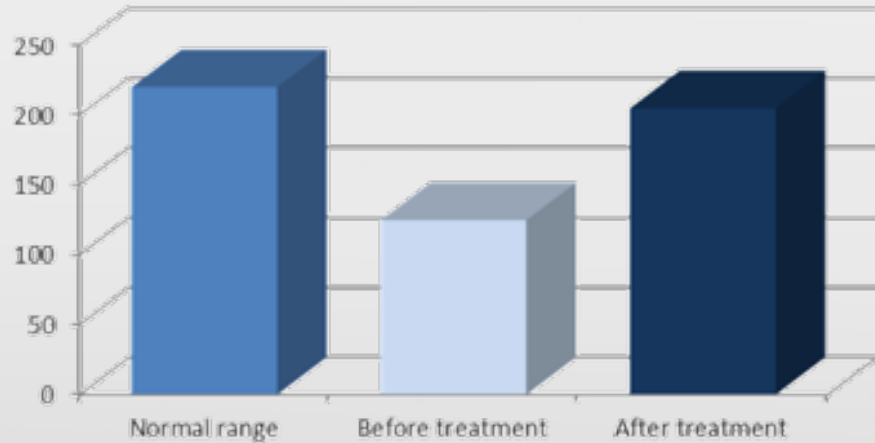
AGEING

# MITOCHONDRIAL FUNCTION AND ANTI-OXIDANT SYSTEM ASSAY

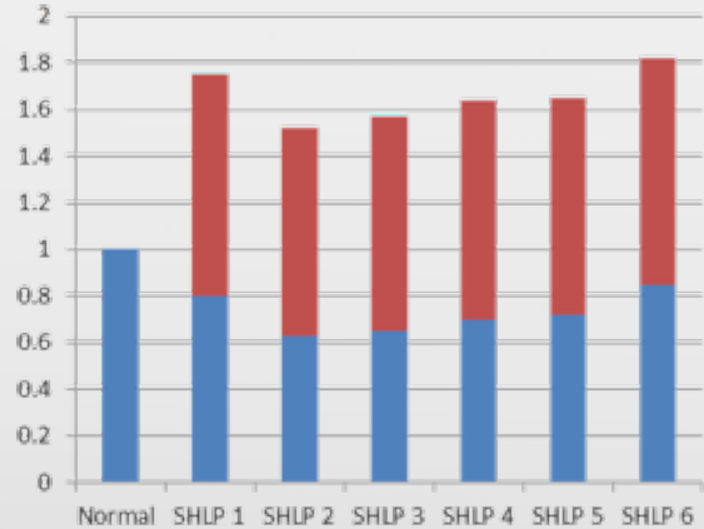
- Serum Humanin and SHLP 1-6 levels measured with standard Commercial Humanin enzyme-linked immunosorbent assay (ELISA) Kit
- Superoxyde dismutase (SOD2) assay – Commercial SOD ELISA kit
- Catalase assay – Aebi method (1984) in modification of Mittal and Flora, 2007
- Reduced and Total serum Glutathione measured with Glatathione Assay Kit (Sigma-Aldrich)

# MITOCHONDRIAL FUNCTION ASSAY

## Humanin pg/ml



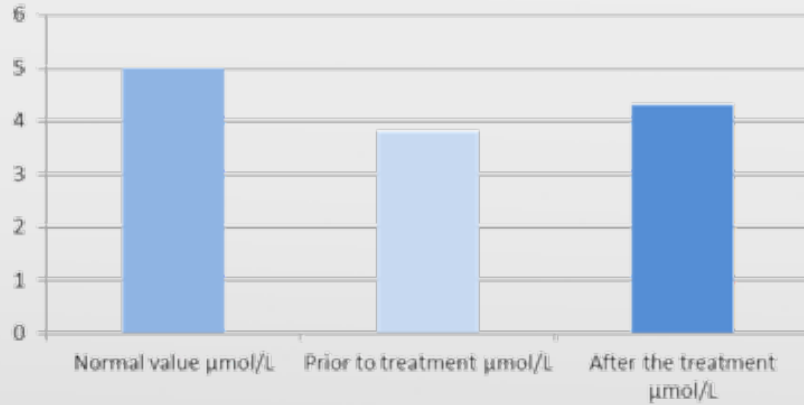
Mean serum HN increase from  $123 \pm 1.2$  to  $206 \pm 1.3$  pg/ml



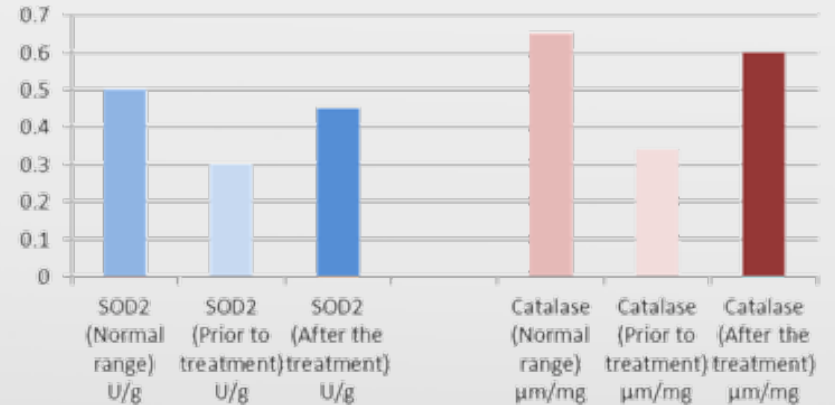
■ After 8 weeks  
■ Day 0

# ANTI-OXIDANT SYSTEM ASSAY

## Reduced Glutathione assay



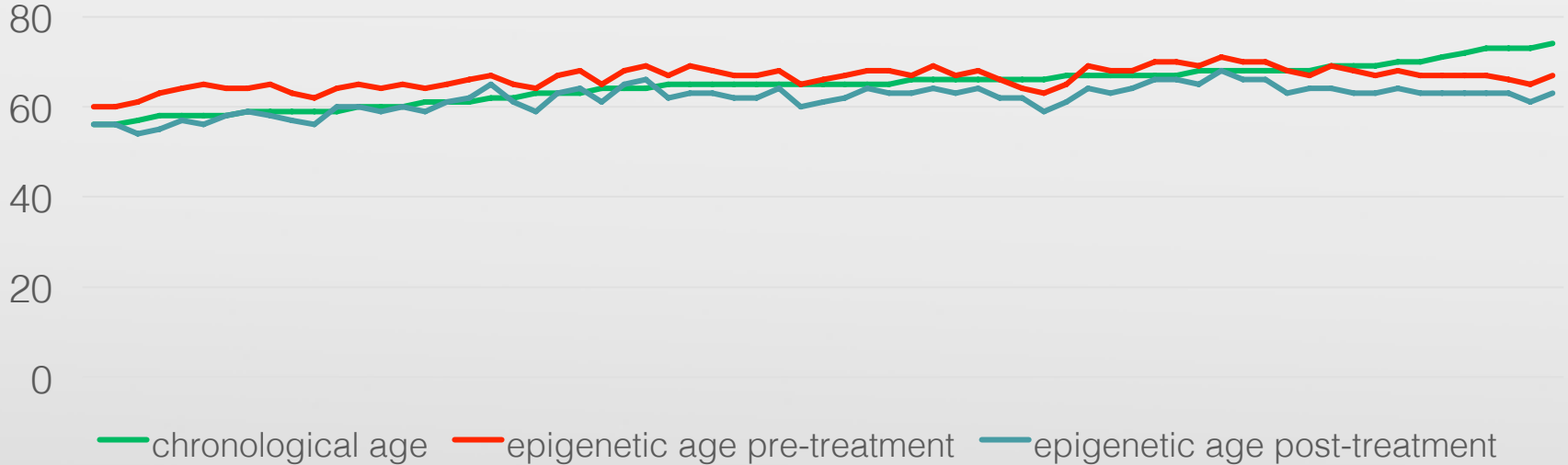
## SOD2 and catalase assay



Mean RGx increase from  $2.9 \pm 0.03$  to  $4.3 \pm 0.04$   $\mu\text{mol/L}$

# PROOF OF ANTI-AGING PROPERTIES AND BIOLOGICAL ACTIVITY

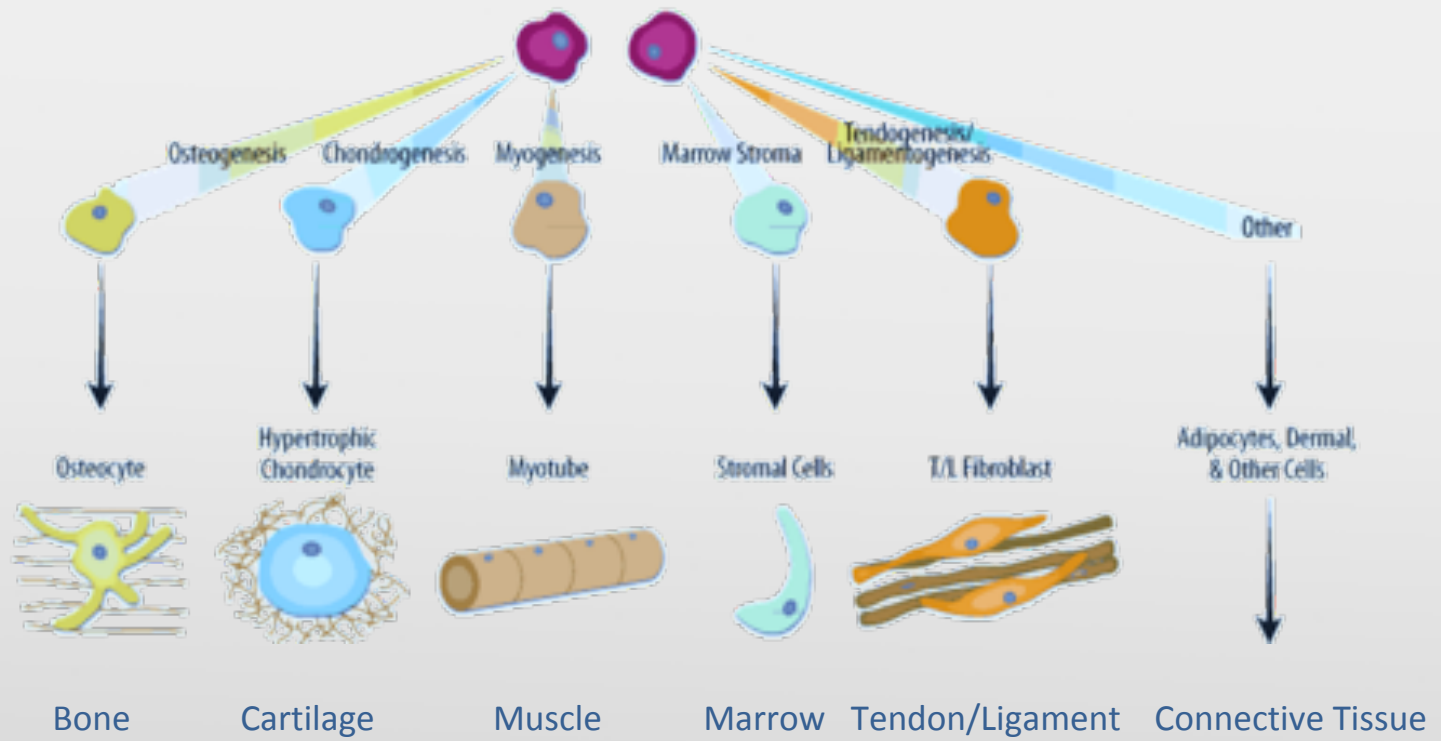
Biological clock shift (DNAm age test)

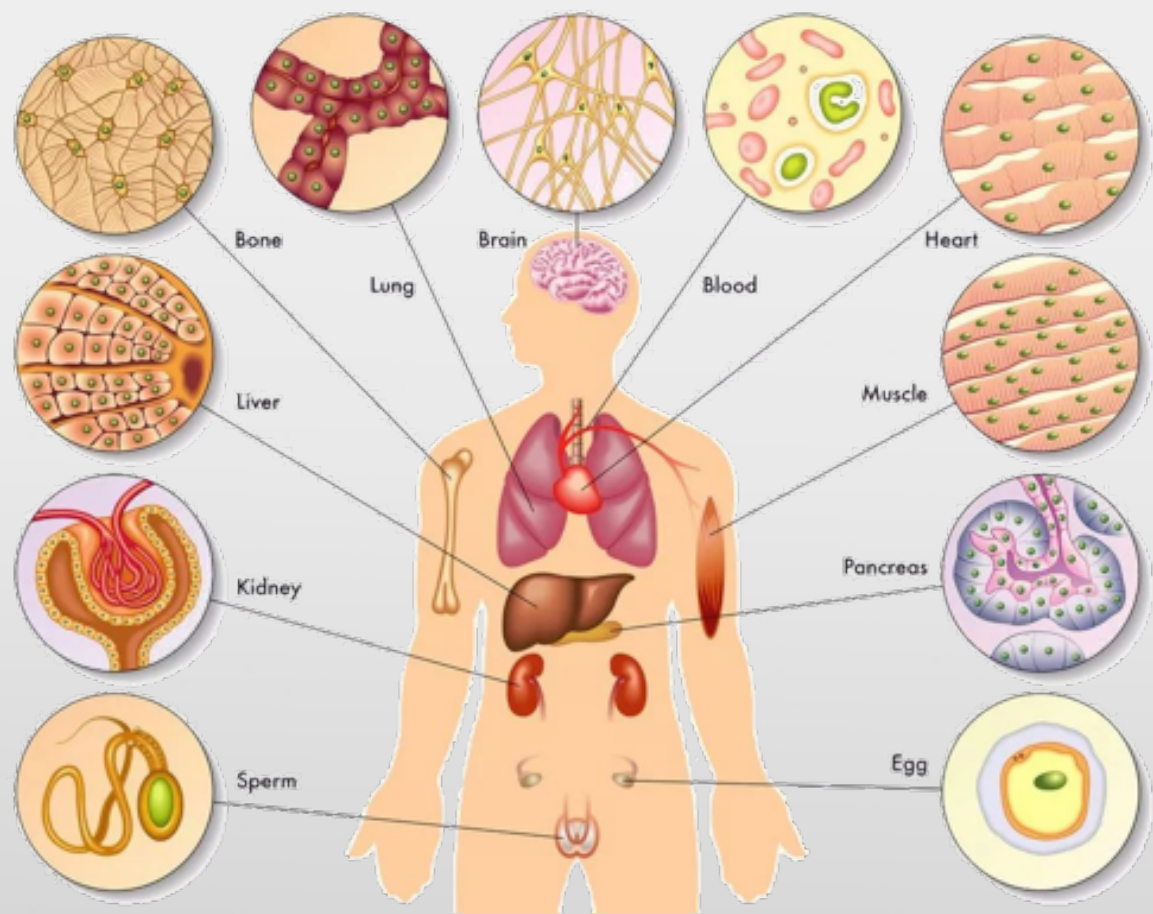


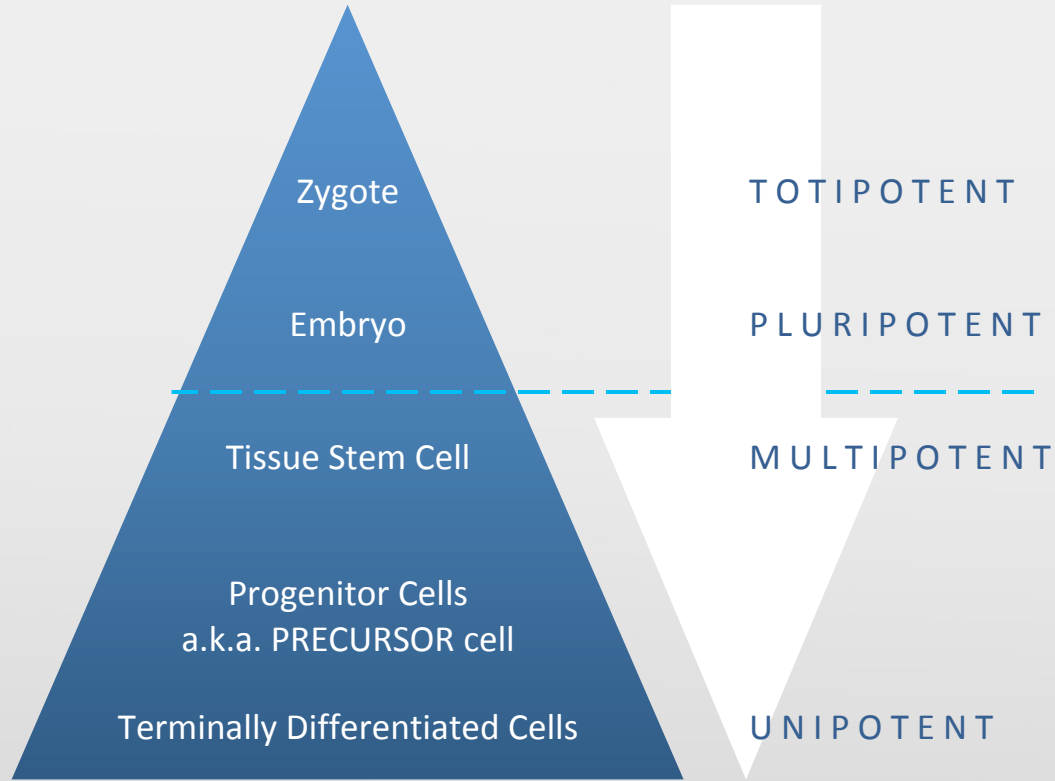
**4-9 years rejuvenating effect, Mean rejuvenation by 6-8 years**



# MSCs







# Neuron

NucBlue stain

10x

2x magnification

Characteristics: Suspension

50  $\mu$ m

# Neuron

NucBlue stain

10x

2x magnification

Characteristics: Suspension

A white horizontal scale bar with a black border is located in the bottom right corner. It contains the text '50 μm' in a black, sans-serif font.

50  $\mu$ m

# Pancreas

NucBlue stain

10x

1.6x magnification

Characteristics: Adherent, elongated

100  $\mu$ m

A fluorescence micrograph showing a field of fetal pancreatic cells. The cells are stained with NucBlue, which highlights their nuclei in a bright cyan color. The cells are elongated and spindle-shaped, with some showing thin, radiating processes. They are distributed across the field of view, appearing to be adherent to a surface. The background is dark, making the stained cells stand out.

# Pancreas

Mitotracker stain

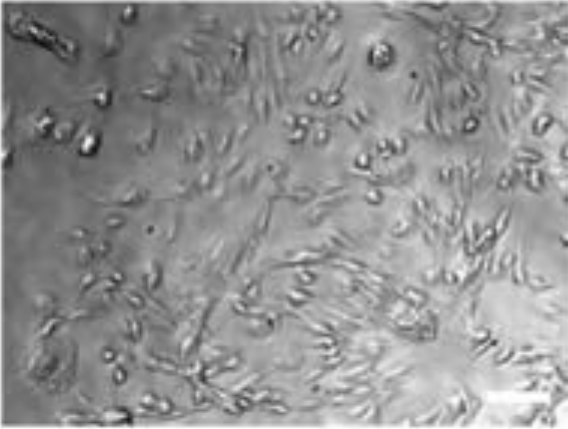
10x

1.6x magnification

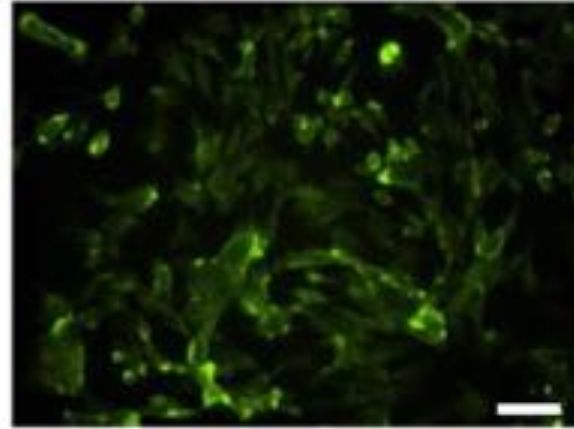
Characteristics: Adherent, elongated

100  $\mu$ m

## 2. MICROSCOPIC IDENTIFICATION – FLUORESCENCE MICROSCOPY (SPECIFIC MARKERS)



**Bright Field microscopy (10x)**



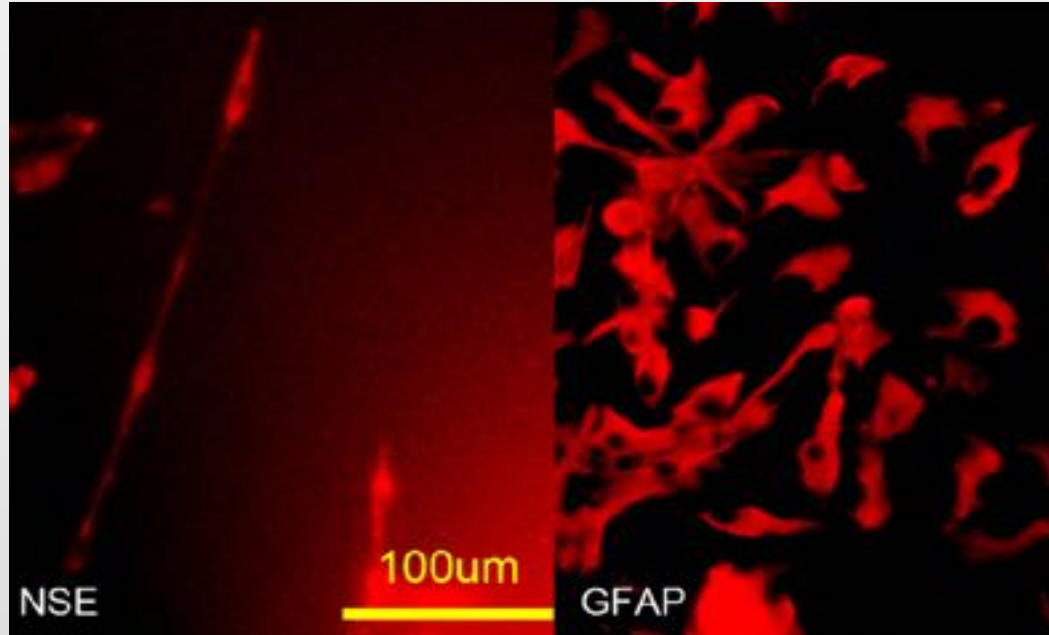
**Fluorescence microscopy (10x)**

Microscopy images of xenogeneic (rabbit) mesenchymal cells immunostained with CD44 surface markers. (AbD Serotec, Inc., Raleigh, NC, USA)

(Bakhtina et al., 2013)



## 2. MICROSCOPIC IDENTIFICATION – FLUORESCENCE MICROSCOPY (SPECIFIC MARKERS)



Neural stem cells: Cy3-NSE and Cy3-GFAP immunofluorescent staining

(Shen et al., 2010)

# FATTY LIVER DISEASE AND LIVER FAILURE



PROGRESSION OF LIVER DAMAGE			
HEALTHY LIVER	FIBROTIC LIVER	CIRRHOTIC LIVER	LIVER CANCER
			
A healthy liver is able to perform its normal functions effectively, e.g. aiding digestion and breaking down harmful drugs and poisons.	Continuous inflammation of the liver caused by hepatitis C can lead to fibrosis – the formation of scar tissue within the liver.	Extensive scarring can block the flow of blood through the liver and cause liver function to deteriorate over time - this is called cirrhosis.	Hepatitis C is a leading cause of liver cancer – the formation of a malignant tumour in the liver.

## CELL THERAPY IN LIVER DISEASE

- Implanted cells begin to secrete albumin within first 48 hrs post implantation
- Various routes of implantation available
- Applicable in either acute or chronic liver failure as well as metabolic syndrome (Crigler-Najjar, Gilbert syndrome, etc)
- Results in improvement of liver function test, reduction of ammonia, reduction of encephalopathy, and cardiovascular instability

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<http://www.modernrespub.org/jsrs/index.htm>



*Full Length Research Paper*

## **Efficacy of renal precursor stem cells in management of chronic kidney disease: a cohort study**

**Mike Chan<sup>1</sup>, Michelle Wong<sup>2</sup>, Mikhail Teppone<sup>1</sup>, Dina Tukhvatullina<sup>1</sup> and Dmitry Klokol<sup>1\*</sup>**

<sup>1</sup>Stellar Biomolecular Research, Germany.

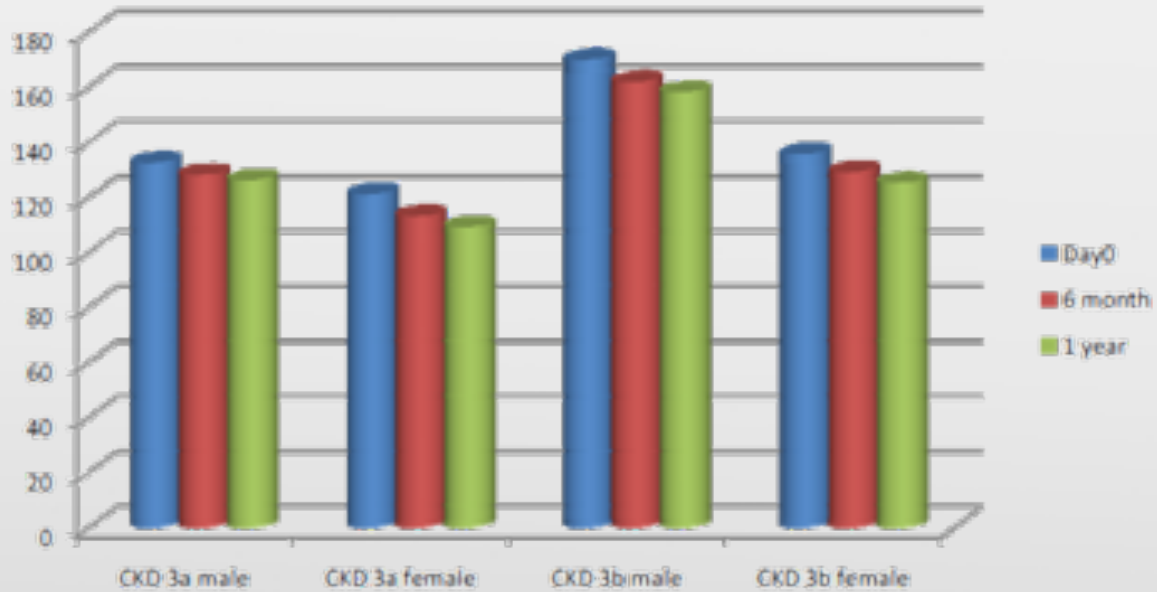
<sup>2</sup>Frontier Cytobiological Therapies International, EU.

\*Corresponding author. E-mail: [Dr.dmytro@sbi-europe.com](mailto:Dr.dmytro@sbi-europe.com)

Accepted 24 June, 2016

**In spite of tremendous achievements of modern medical science, chronic kidney disease (CKD) is still**

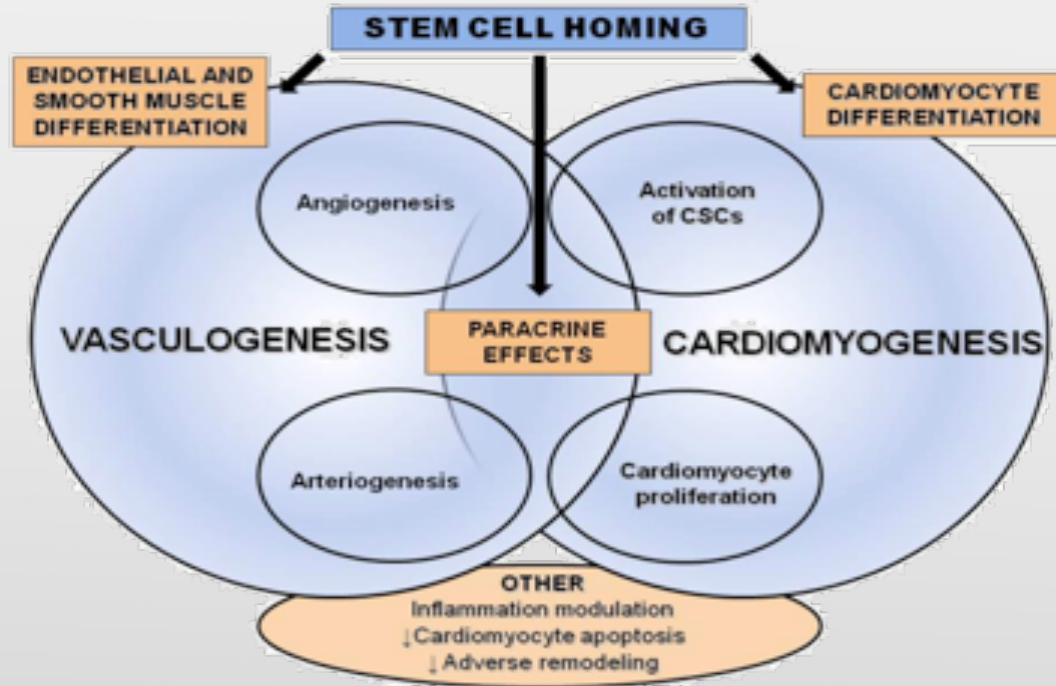
## OBTAINED RESULTS



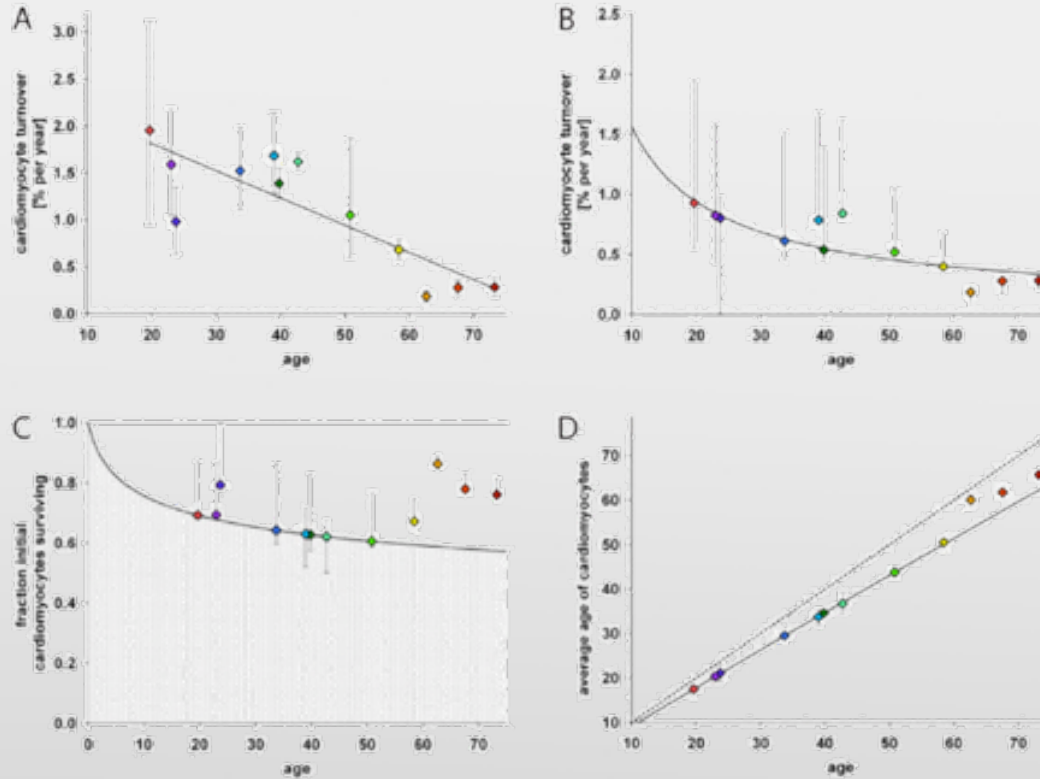
CKD stage 3a – down-staging of CKD in 78.5%

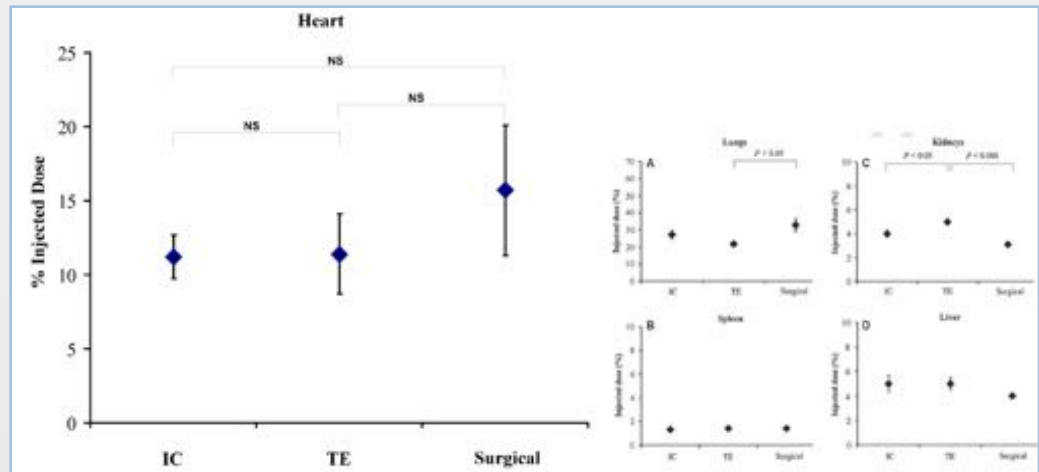
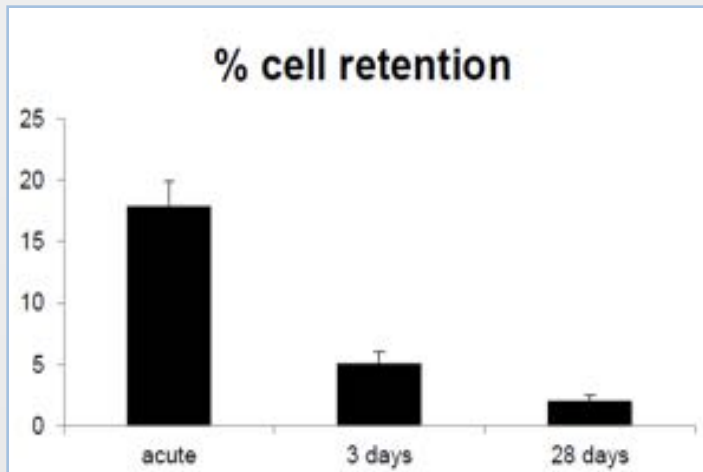
CKD stage 3b – down-staging of CKD in 66.7%

# PSC IN CARDIOMYOPATHY

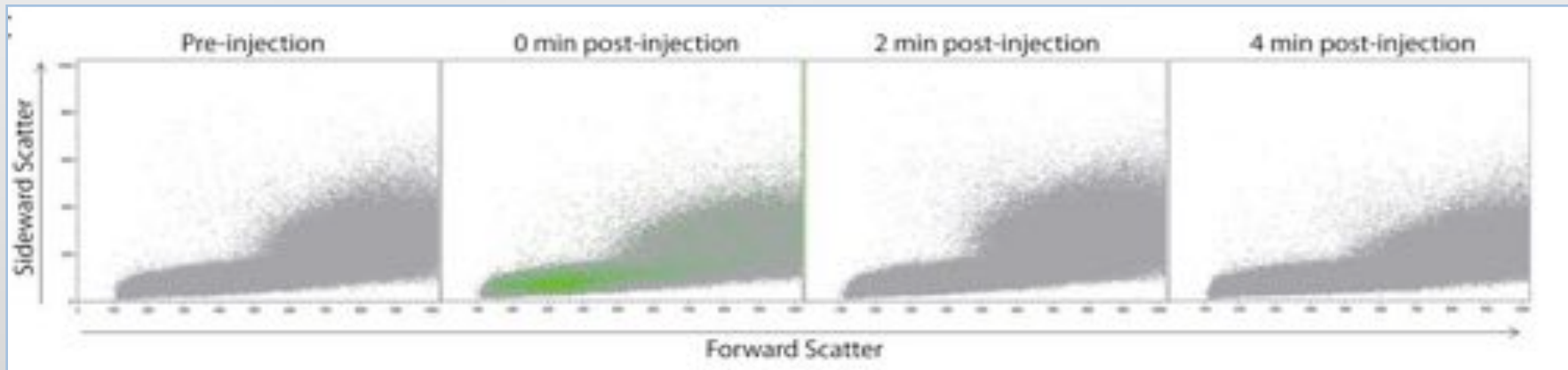


# CARDIAC CELL TURNOVER





Van der Spoel, 2011



Van den Akker, 2016



## CELL THERAPY IN CARDIOMYOPATHY

- Effect observed after 3 months or more and may last up to 2 years
- EF increase from 13.5% to 20% depending on the case
- Downstaging of NYHA FC observed in practically all cases within 6 months of treatment
- Re-implantation of PSC within 3-6 months significantly improves long-term outcomes
- Prolonged administration of cardiac-specific peptides and growth factors is pivotal for successful treatment



Contents lists available at ScienceDirect

## International Journal of Surgery

journal homepage: [www.journal-surgery.net](http://www.journal-surgery.net)



### Review

## Current status of neuronal cell xenotransplantation



Marta Vadori<sup>a</sup>, Romina Aron Badin<sup>b</sup>, Philippe Hantraye<sup>b</sup>, Emanuele Cozzi<sup>a,c,\*</sup>

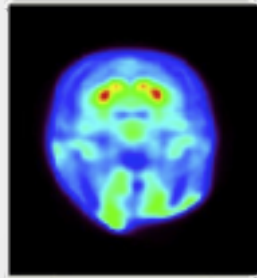
<sup>a</sup> COGIT (Consorzio for Research in Organ Transplantation), Via dell'Università 30, 35020 Legnaro, Padova, Italy

<sup>b</sup> MIRCen, CEA UMR 9198, 18 Route du Panorama, Fontenay-aux-Roses 92265, France

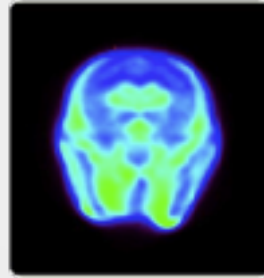
<sup>c</sup> Transplant Immunology Unit, Department of Transfusion Medicine, Padova University Hospital, Via Giustiniani, 2, 35128 Padova, Italy

This study provides evidence that neural stem cells can safely survive in brain and have the potential to be used as a replacement therapy after a neurological injury such as a brain stroke. It has been demonstrated that undifferentiated fetal cells can support a functional recovery from parkinsonian symptoms, survive and migrate to lesioned brain areas for up to 7 months. They differentiate into dopaminergic neural fibers in close association with host neurons and express the glial cell line-derived neurotrophic cell factor (GDNF), that may provide protective support to dopaminergic neurons.

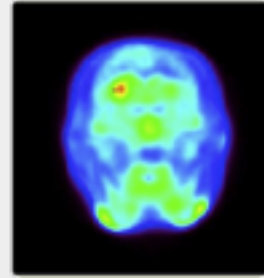
PET  
IMAGING  
([<sup>18</sup>F]F-L-DOPA)



Pre-MPTP lesion

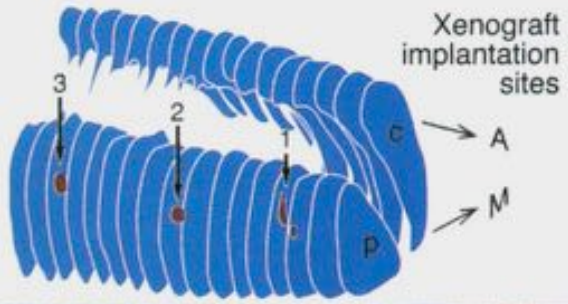


Post-MPTP lesion

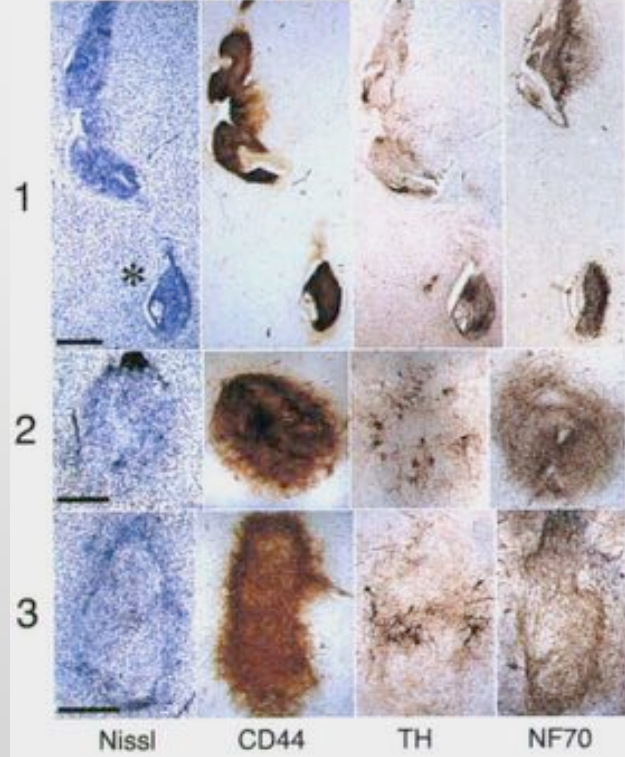


Post-transplantation

- Initial data have shown improvement in 30% of the patients with Parkinson or Huntington disease after xenotransplantation with fetal neuronal cells (*Fink JS, Schumacher JM et al*).
- 14 weeks after transplantation of fetal neural progenitor cells transplants, only mild infiltration of CD8 T cells and macrophages was noticed
- Improved prescriptions allowed increase efficacy to 70-75% in Alzheimer's and Parkinson's
- Ongoing Xenome project UE LSHB-CT-2006-037377 currently continues evaluation of fetal precursor stem cell application efficacy in Parkinson's disease.



69 y.o. patient with 15 years history of Parkinson's treated with xenogeneic stem cells, demonstrated dramatic recovery but passed away due to a different cause (acute pulmonary embolism).



**Cresyl violet stain for xenogeneic CD44 antigen**

**Tyrosine hydroxylase – dopamine cell marker**

**NF70 xenogeneic neurofilament axon marker**

From Deacon et al. Natur med, 1997

# ALZHEIMER'S DISEASE AND DEMENTIA

**Name:** DBR (Male)

**Age:** 82 years old as of 04/11/2009

**Symptoms:** Insomnia, lethargy, memory loss, slow and slur speech, quick temper, lost muscle strength and libido, chronic fatigue and disorientation.

## Attending physician's comments:

- More energetic as he is able to walk longer distance and faster than before without complication
- Less frequent after lunch nap
- More enthusiastic e.g in gardening, activities at home, qigong workshop
- Great improvement in mood
- Ability in thinking and planning ahead, improvement in heart ejection fraction.

## Wife's feedback:

- Infrequent mood swings, emotionally stable.
- Blood pressure and cholesterol normal range.
- Speech expressivity is good.
- ECG good, Rhythm good.
- Cardiologist commented that he is doing well and will only see him after 12 months.

## Patient's Feedback:

- Better memory.
- Better mobility, can walk further than before, can do mild exercises without resting and has more energy.
- Short of breath if he's rushing, anxious & worried. Sleep apnea has improved.

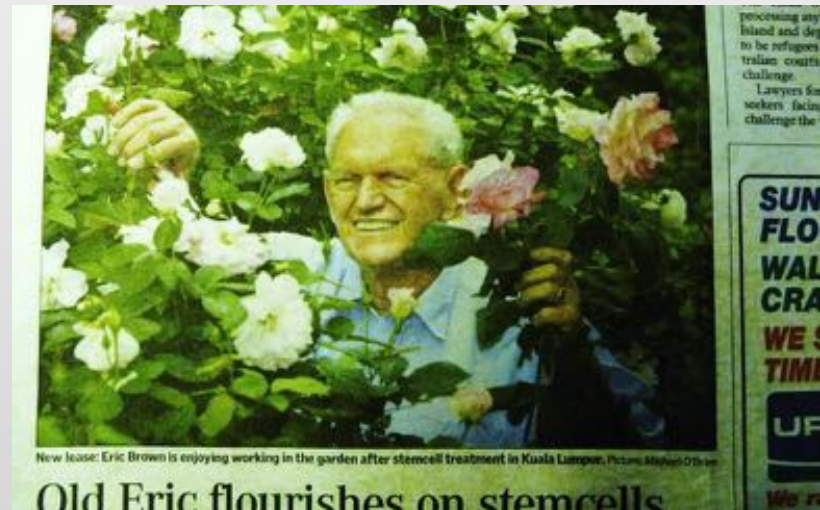


Photo dated September 2013 – Buddhist temple in China

Courtesy of [www.fetal-cells.com](http://www.fetal-cells.com)



Photo dated October 2013



New lease: Eric Brown is enjoying working in the garden after stemcell treatment in Kuala Lumpur. Picture: Michael O'Brien

Old Eric flourishes on stemcells



**MELATONIN, PINEAL GLAND AND SLEEP DISORDERS: FROM THEORY TO PRACTICE**

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**ABSTRACT**

The article discusses the physiology of sleep and mechanisms of sleep disorders. Possible therapeutic solutions to treat sleep disorders can be provided through functional and holistic biological medicine, which employs a number of device-based treatment, stem cells and therapy with pineal gland cell extracts.

**KEYWORDS:** Pineal gland, sleep, sleep disorders, insomnia, stem cells.

The conundrum of sleep has always drawn special attention not only as a biological process but also due to

suffer from some form of sleep disorders<sup>[1-4]</sup> In some severe cases, which are relatively uncommon, substantial

## HOW TO SUCCEED

1. Profound analysis of patient's state of health:  
screening of biological markers of aging i.e.  
complete hormonal profiling including precursor of  
hormones DHEA, ferritin, IGF, epigenetic test,  
parameters of the oxidative stress, mitochondrial  
function etc
2. Don't neglect "minor things", as there are  
no "minor things".
3. More trials, research and experience.



**THANK YOU FOR YOUR KIND ATTENTION**

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