ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI IRCCS

La terapia rigenerativa in nefrologia: le prospettive future

Giuseppe Remuzzi

Prendiamoci a cuore il rene

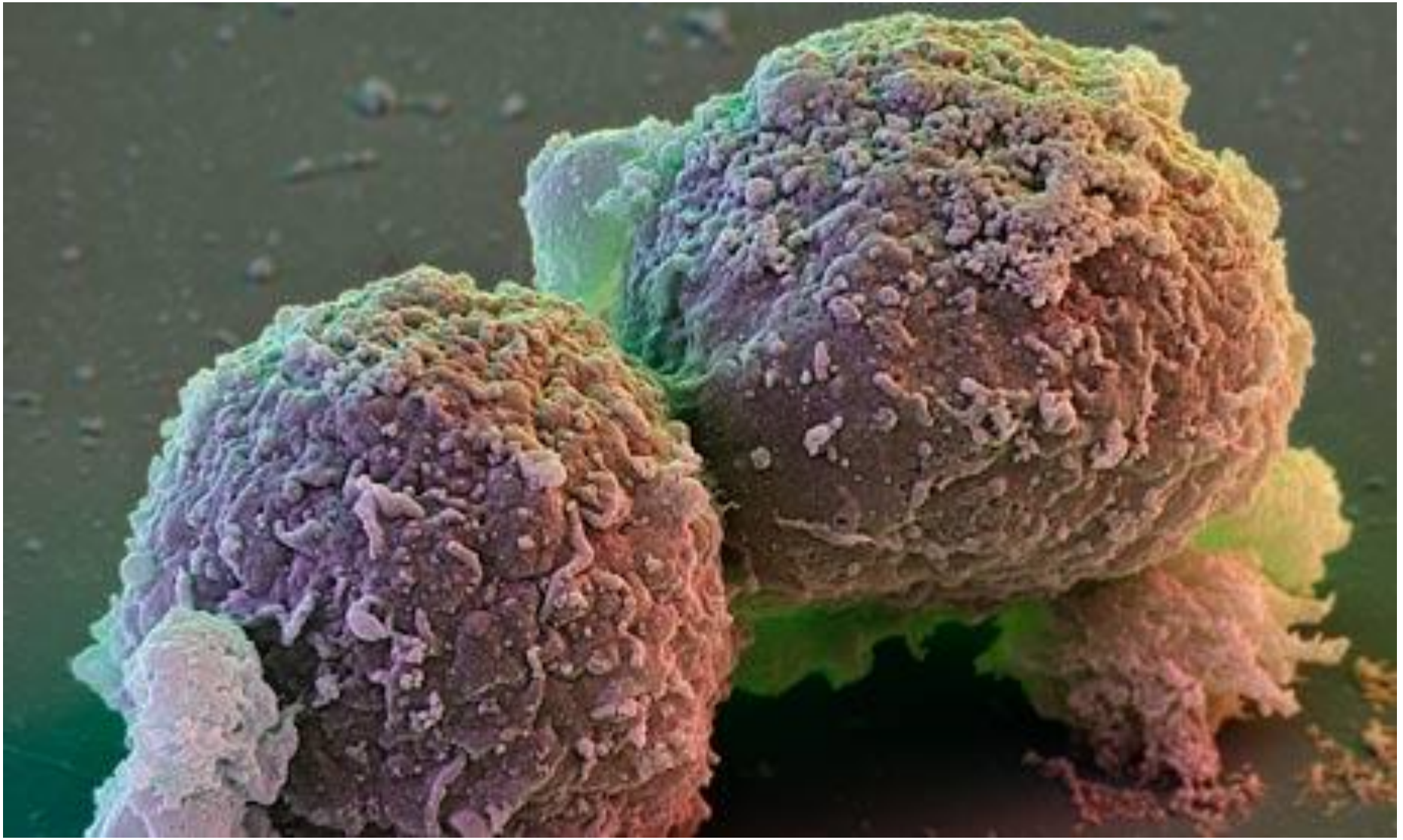
Nuove prospettive basate su attuali certezze

Milano, 2 dicembre 2016

- *A 56-year-old man had progressive renal insufficiency and proteinuria of unknown origin*
- *A renal biopsy showed that the renal tissue was extensively damaged*
- *He has been told that the disease will progress to the need of dialysis and that he will have a diminished quality of life*
- *He is a modern individual, well informed about alternatives*

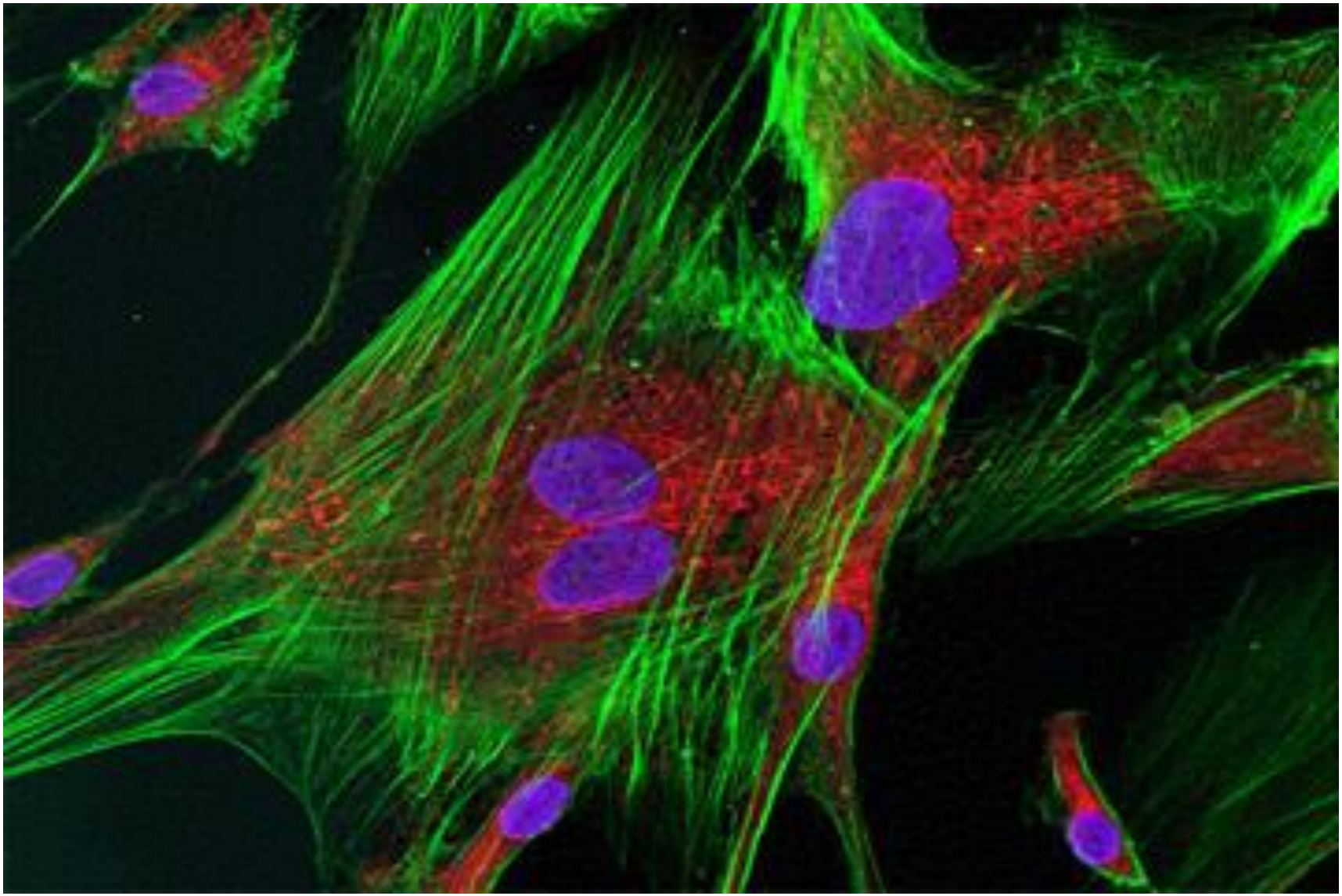
What options?

David Scharf/ Science Faction/Corbis

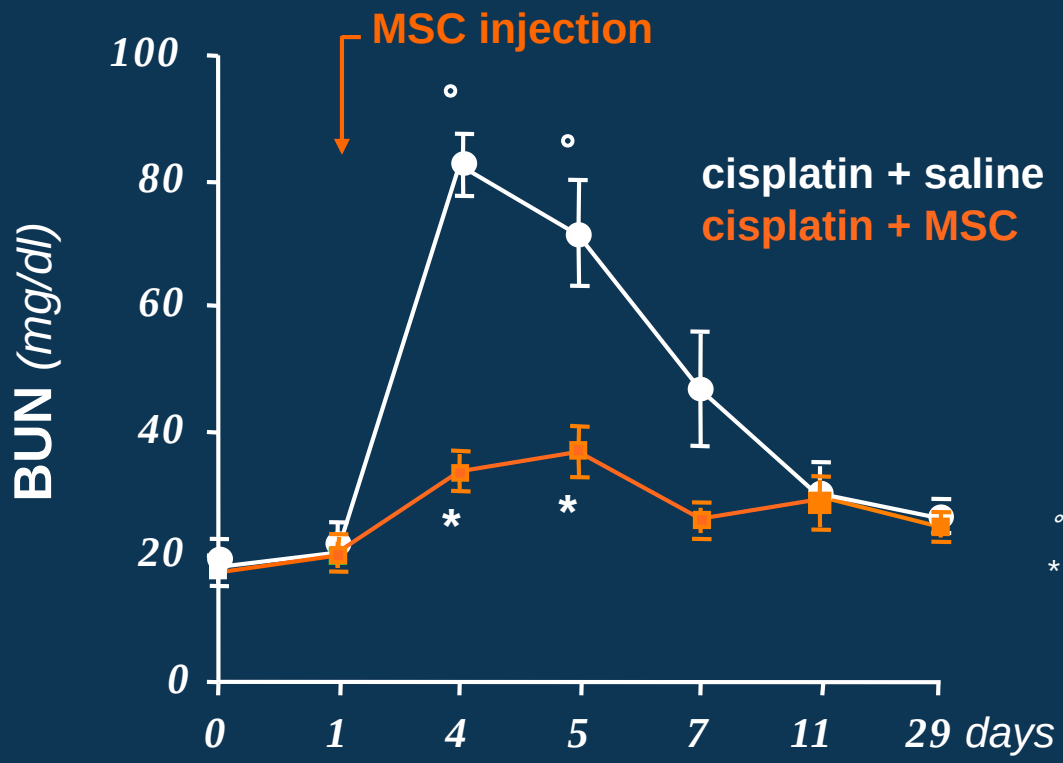



17 December 1999
Science
Vol. 286 No. 5448
Pages 2221–2416 $8
Breakthrough of the Year
Stem Cells Show Their Potential
American Association for the Advancement of Science

Mesenchymal stem/stromal cells



MESENCHYMAL STEM CELLS ARE RENOTROPIC, HELPING TO REPAIR THE KIDNEY AND IMPROVE FUNCTION IN ACUTE RENAL FAILURE

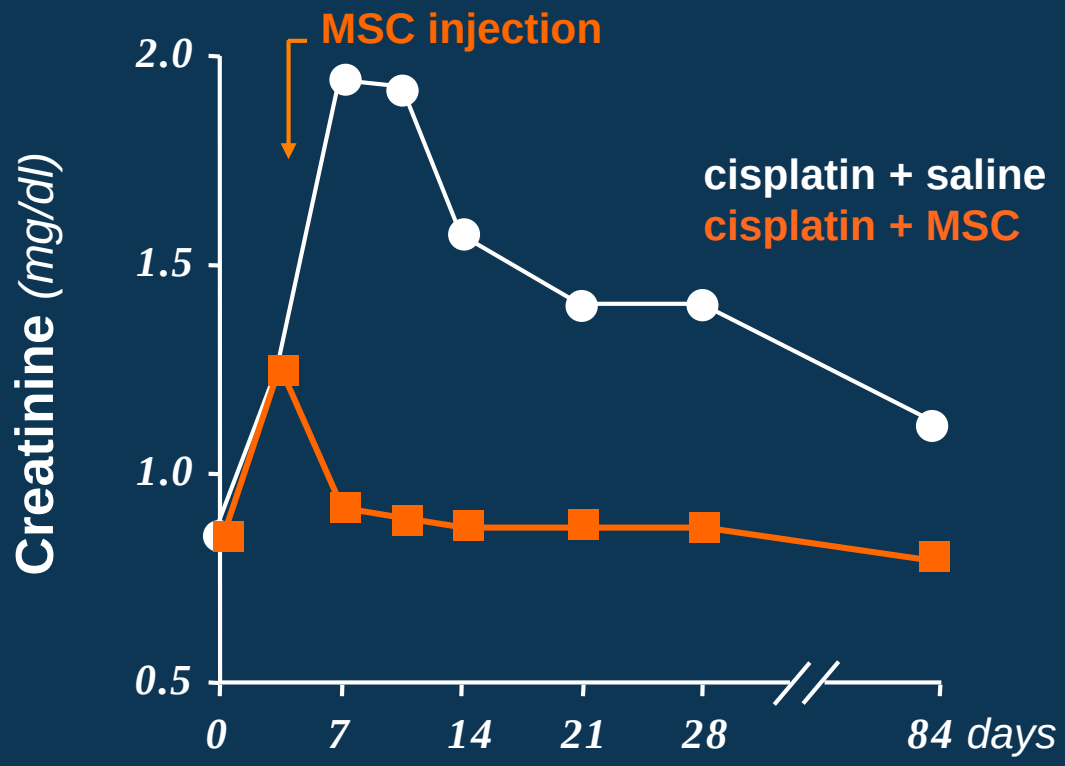


° p<0.01 vs day 0, 1, 7, 11 and 29;
* p<0.01 vs cisplatin + saline

Murine MSC** (2x10^5 cells) **when i.v. injected in cisplatin mice exert a protective effect on renal function and tubular injury

Morigi et al.,** J Am Soc Nephrol, **2004

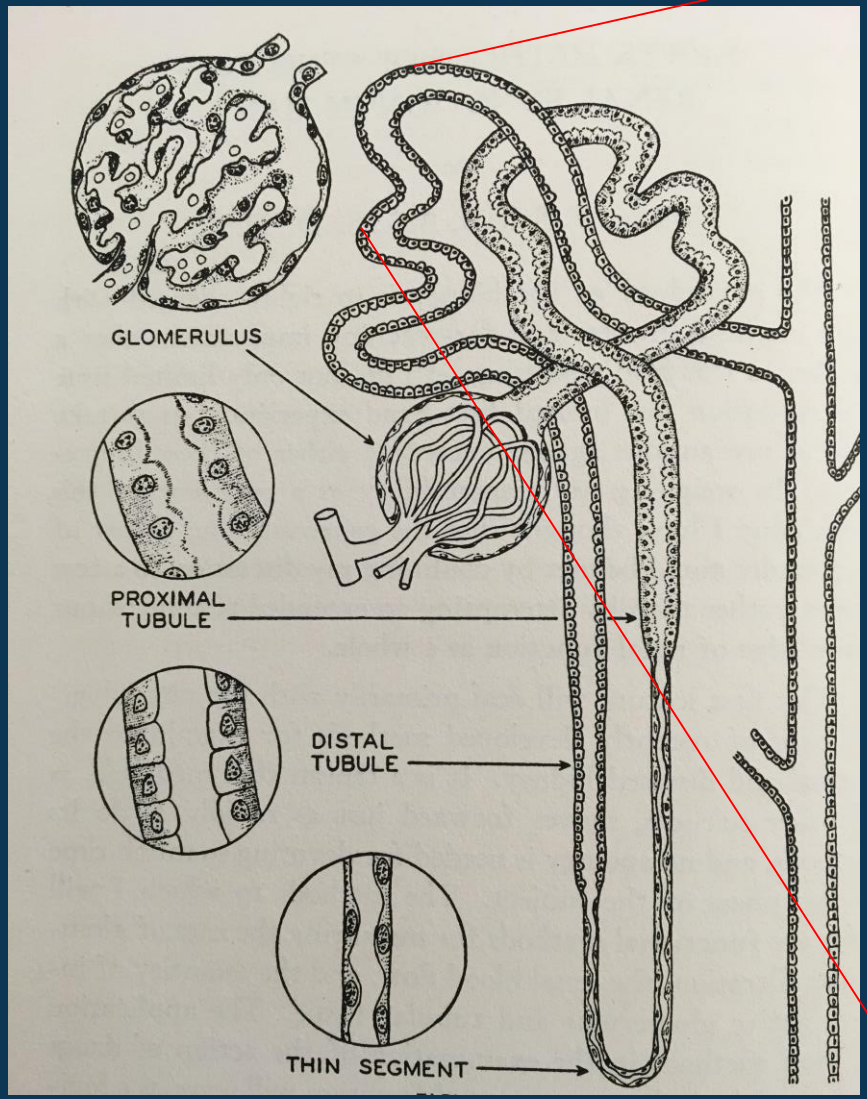
INTRA-RENAL ARTERIAL INJECTION OF AUTOLOGOUS BONE MARROW MSC AMELIORATES CISPLATIN-INDUCED AKI IN A RHESUS MACAQUE MULATTA MONKEY MODEL

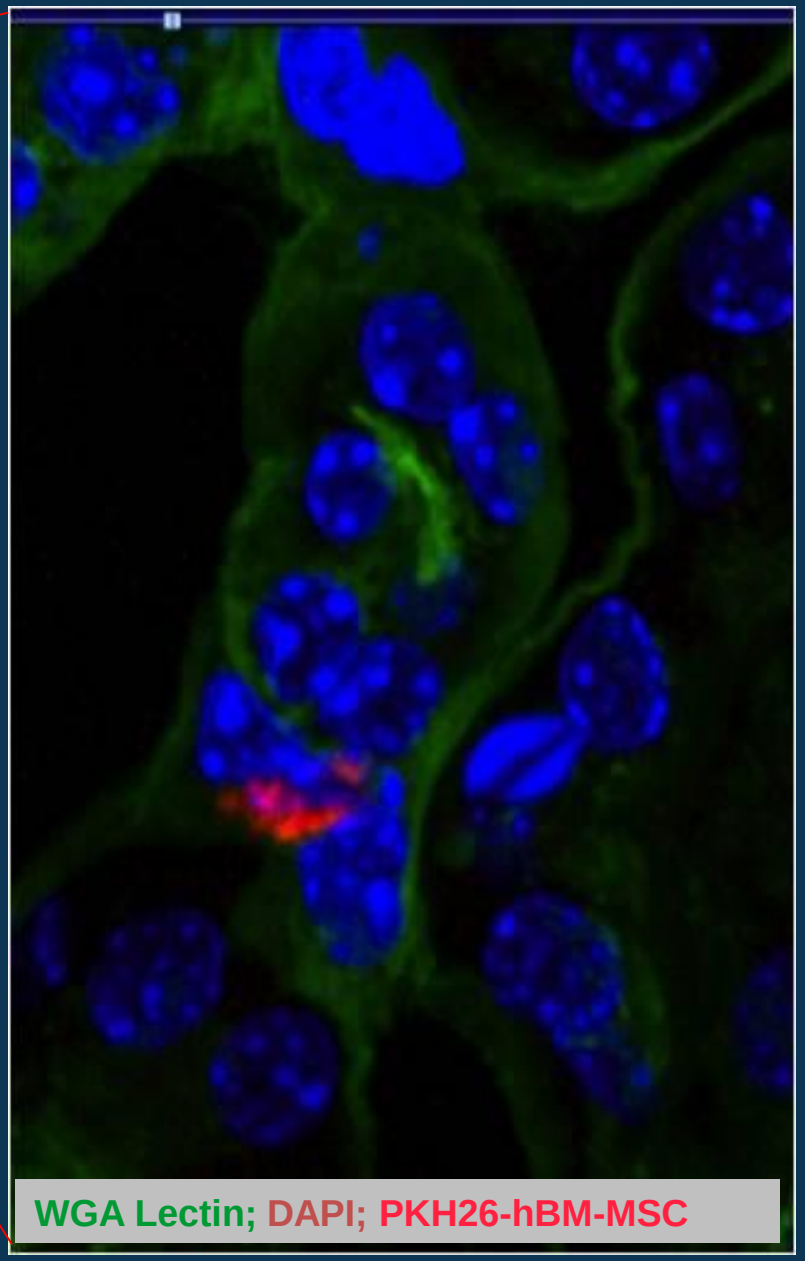


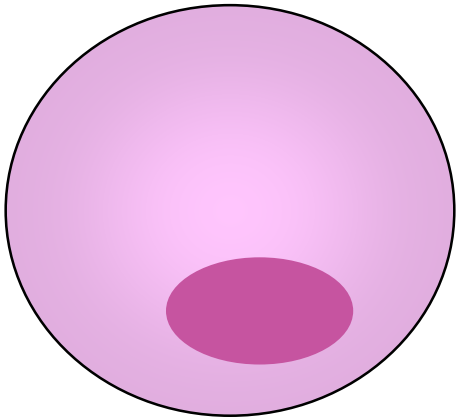
Monkey MSC** (5x10⁶ cells/kg) **when i.v. injected in cisplatin monkey exert a protective effect on renal function and tubular injury

Moghadasali et al., Cytotherapy, 2014

MESENCHYMAL STEM CELLS ENGRAFT THE KIDNEY AT LOW LEVEL AND DO NOT DIFFERENTIATE INTO TUBULAR EPITHELIAL CELLS



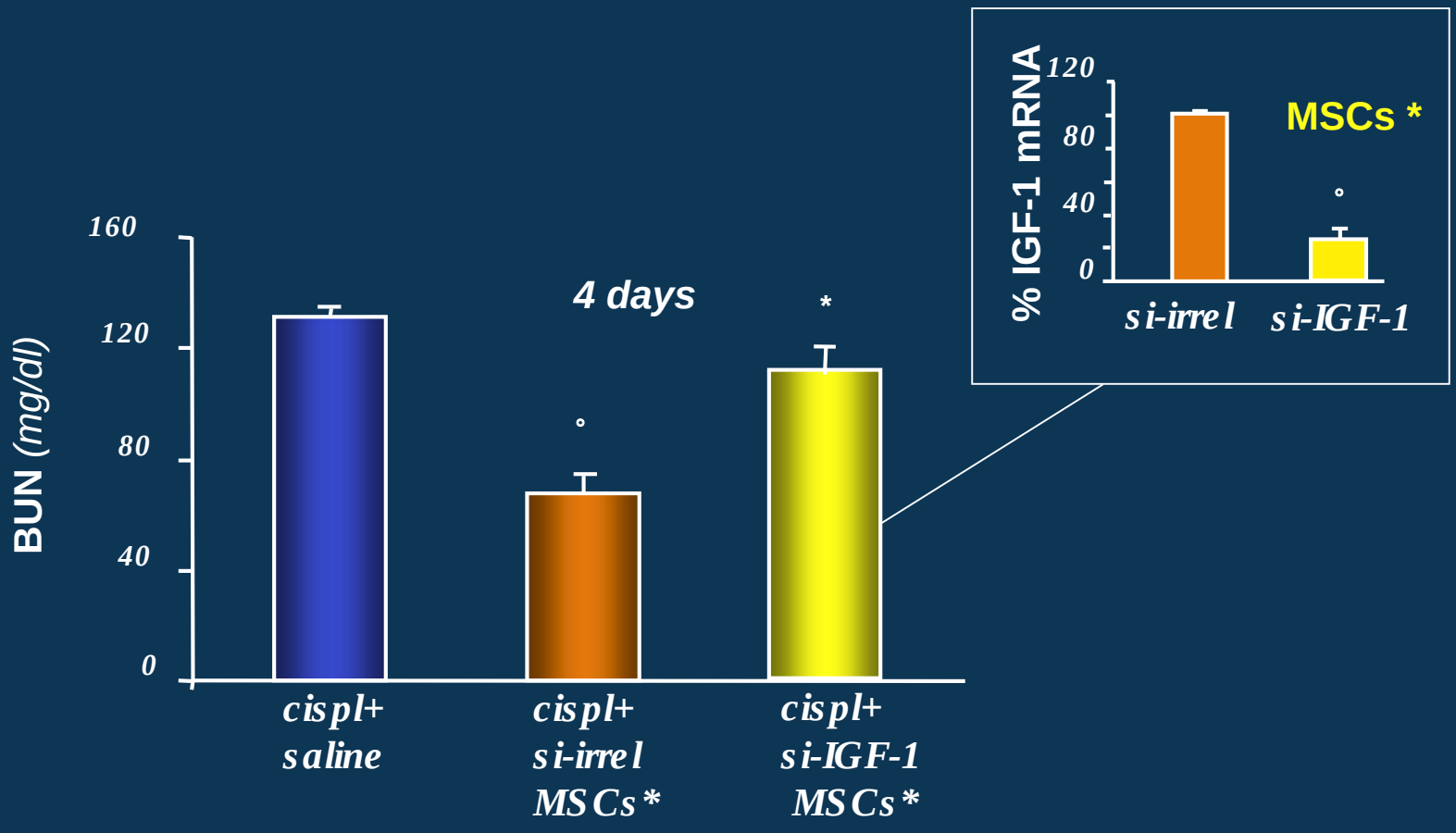




Cultured MSC

- ***Antioxidant***
- ***Anti-apoptosis***
- ***Chemoattraction***
- ***Angiogenesis***
- ***Support of growth and differentiation of stem and progenitor cells***
- ***Anti-scarring (anti-fibrosis)***
- ***Immunomodulation***

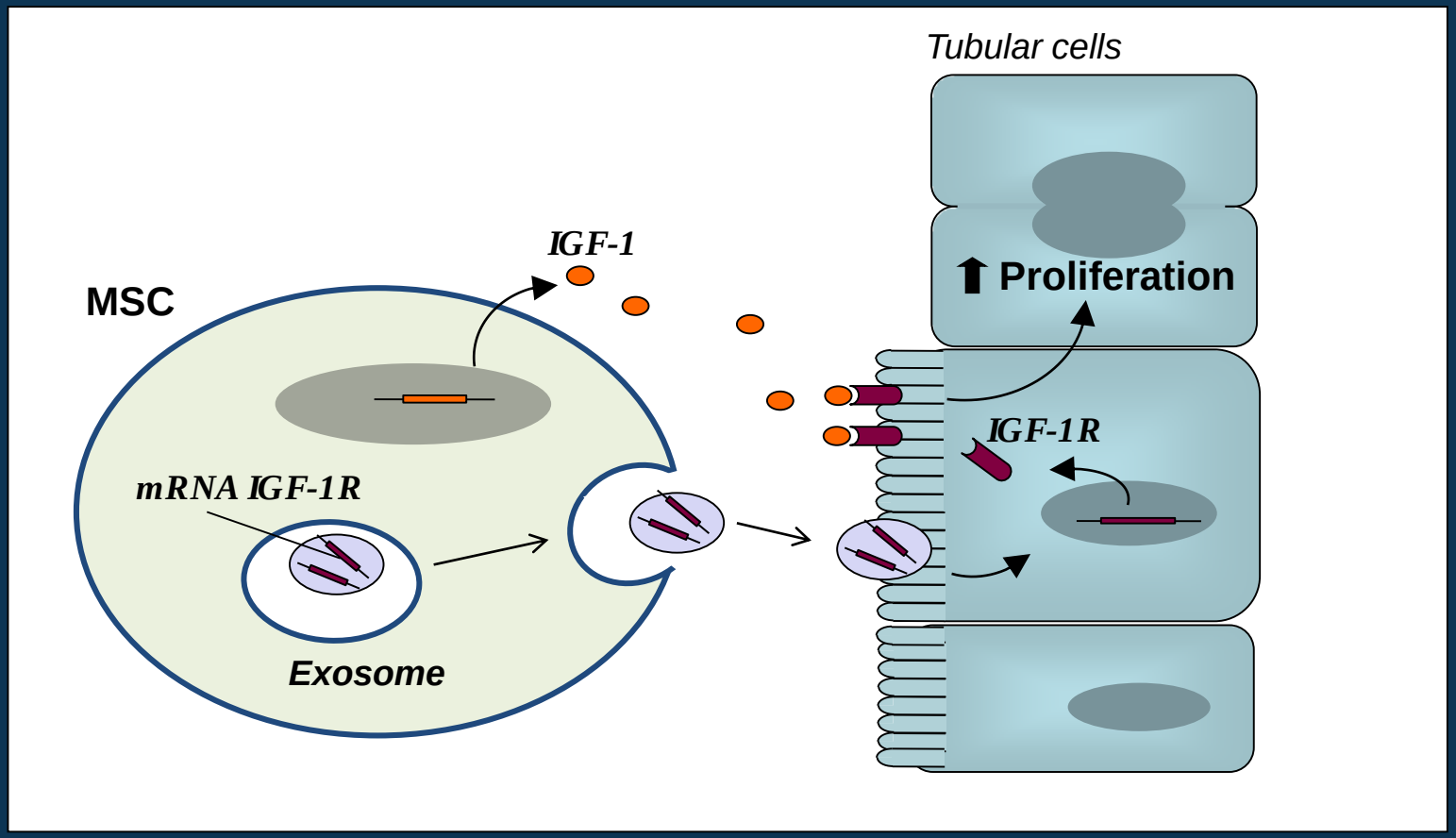
MSCs EXERT THEIR RENOPROTECTIVE EFFECT VIA THE LOCAL RELEASE OF IGF-1



Infusion of si-IGF-1 MSCs resulted in less protective effect on tubular injury*

Imberti, Morigi et al., J Am Soc Nephrol, 2007

12



IGF-1 released by MSC can be further amplified by horizontal transfer of mRNA of the corresponding receptor to tubular cells by exosomes which explains the ability of a low amount of MSC engrafting the kidney to promote prompt recovery from AKI

Tomasoni et al.,Stem Cell Dev 2012

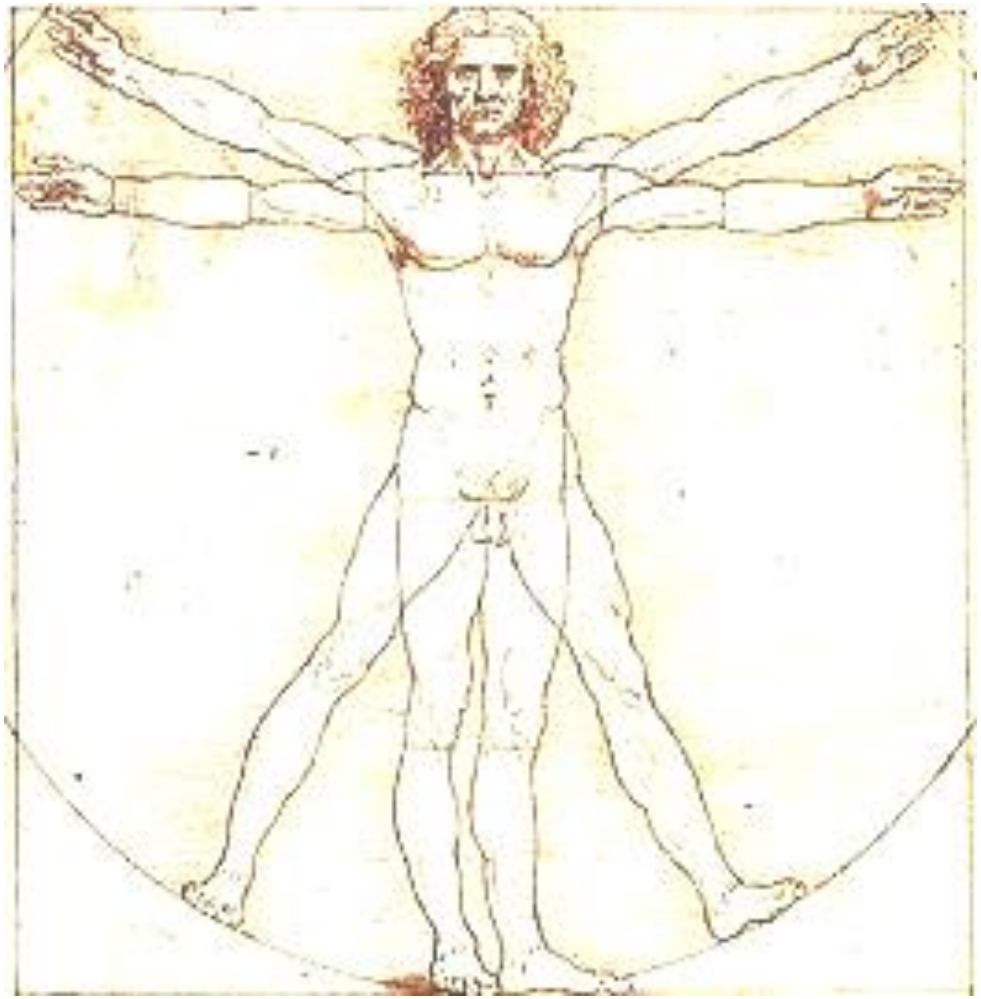
Stem Cell and Rep, 2012

Multipotent Mesenchymal Stromal Cell Therapy and Risk of Malignancies

Federica Casiraghi · Giuseppe Remuzzi · Mauro Abbate · Norberto Perico

Subjects given autologous or third party MSC > 700 MSC
Follow-up up to 7 years

Disease	Number of patients	MSC source		Follow up	Tumor development
Hematologic malignancies					
Graft-versus-Host-Disease	3	BM	Allogeneic	Not indicated	None
Graft-versus-Host-Disease	55	BM	Allogeneic	60 mo	None
Graft-versus-Host-Disease	8	BM	Allogeneic	3 yr	None
Graft-versus-Host-Disease	13	BM	Allogeneic	250 d	None
Graft-versus-Host-Disease	1	BM	Allogeneic	1 yr	None
Graft-versus-Host-Disease	18	BM	Allogeneic	1 yr	None
Graft-versus-Host-Disease	12	BM*	Allogeneic	730 d	None
Graft-versus-Host-Disease	31	BM*	Allogeneic	28 d	None
Graft-versus-Host-Disease	4	BM	Allogeneic	Not indicated	None
Hematopoietic stem cell Tx	6	BM	Allogeneic	4.8 yr	None
Umbilical Cord Blood Tx	15	BM	Allogeneic	6.8 yr	None
Hematopoietic stem cell Tx	7	BM	Allogeneic	29 mo	None
Hematopoietic stem cell Tx	14	BM	Allogeneic	28 mo	None
Aplastic anemia/HSC Tx	2	BM	Allogeneic	2 yr	None
Breast cancer	28	BM	Autologous	2 yr	None
Hematologic malignancies	10	BM	Allogeneic	3 yr	None
Hematologic malignancies	46	BM	Allogeneic	688 d	None
Hematologic malignancies	15	BM	Autologous	Not indicated	None

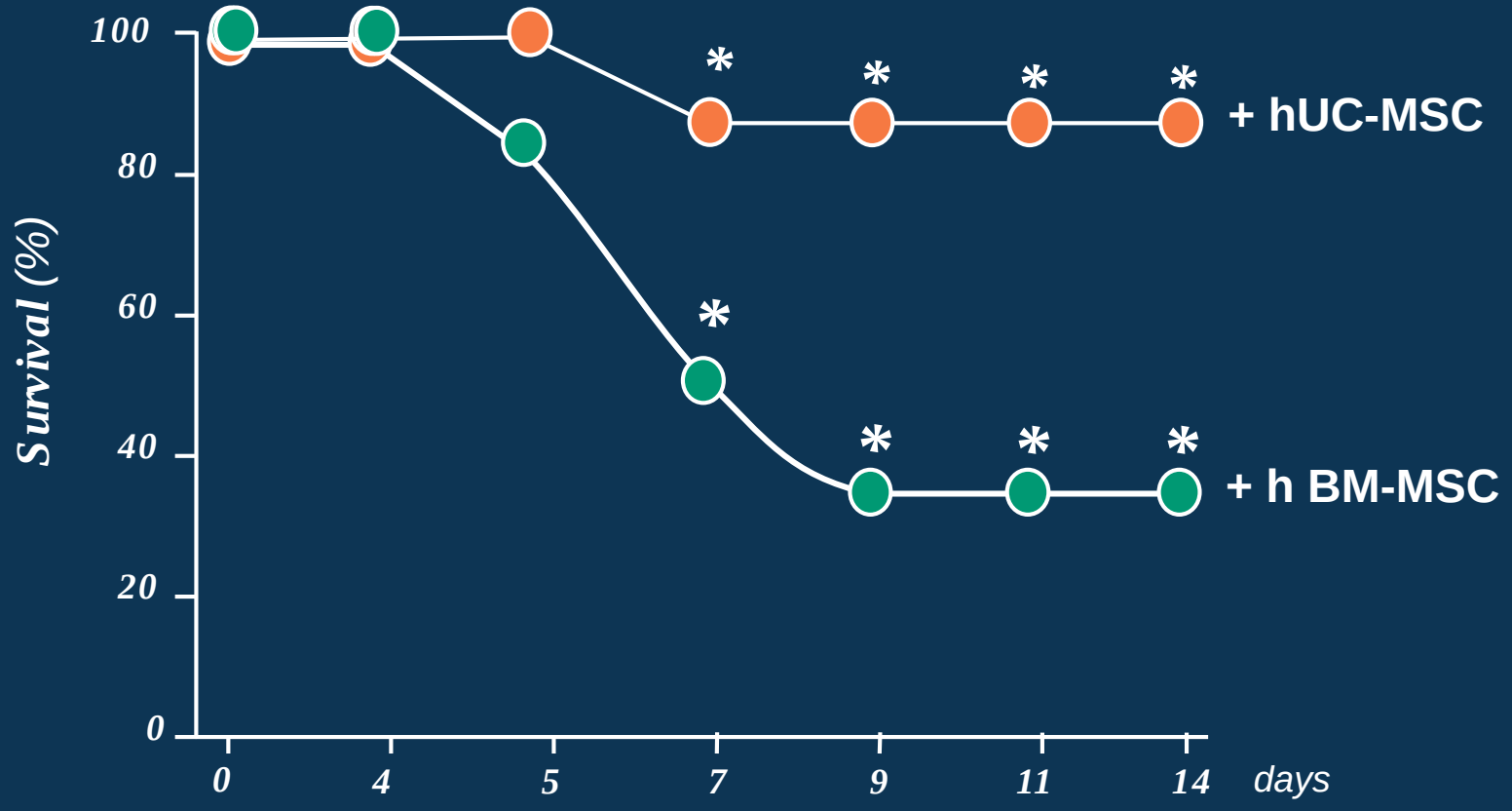


Mesenchymal stem cells

- ***Adipose tissue***
- ***Bone marrow***
- ***Umbilical cord***
- ***Amniotic fluid***

HUMAN UMBILICAL CORD-MESENCHYMAL STEM CELLS PROLONG SURVIVAL OF CISPLATIN MICE

Infusion of hUC-MSC (CD44+, CD105+ CD90+, CD73, HLA class I) in cisplatin -treated NOD/SCID mice improved renal function and tubular injury

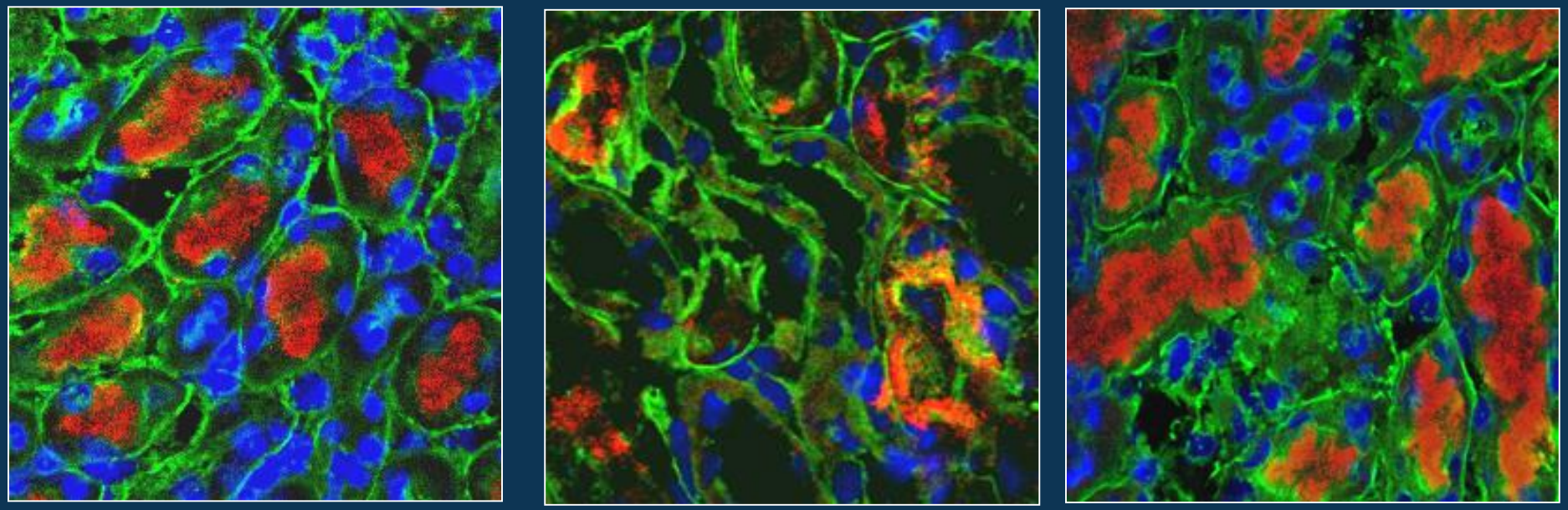


*p<0.01 vs saline

Morigi, Rota et al., Stem Cells, 2010

HUMAN UMBILICAL CORD-MSC INFUSION ACTIVATES PROSURVIVAL AND MITOGENIC PATHWAYS Akt*-DEPENDENT IN AKI MICE

Control | *Cisplatin+saline* | *Cisplatin+hUC-MSCs*



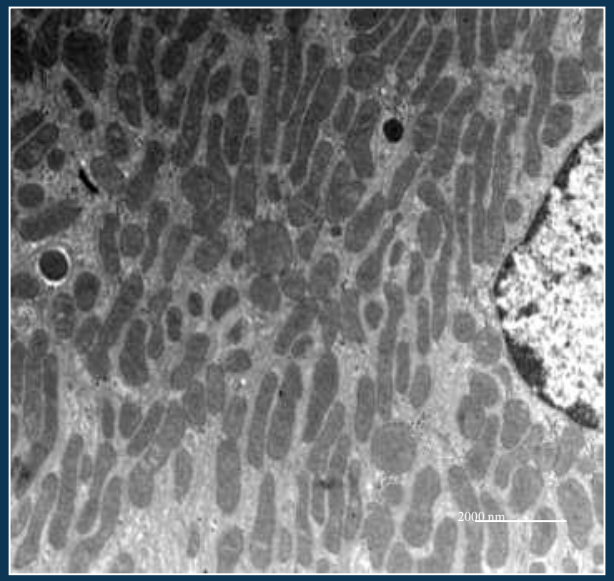
Green: Lectin
Red: activated Akt
Blue: DAPI

**Akt: Serine/threonine specific kinase*

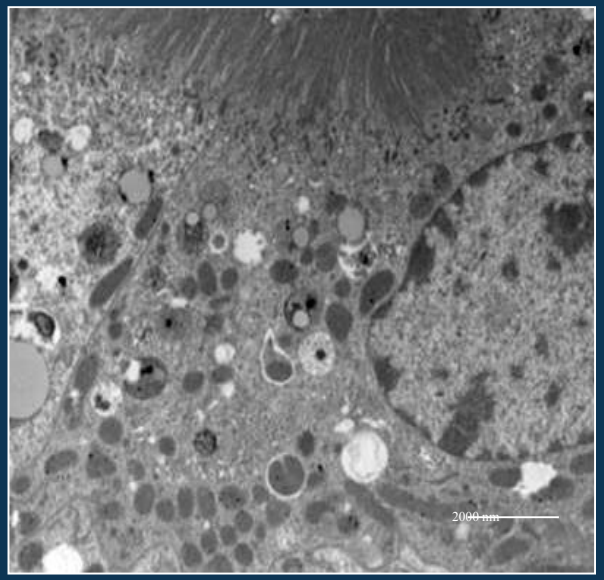
Morigi, Rota et al., Stem Cells, 2010

MSC THERAPY COUNTERACTS MITOCHONDRIAL DYSFUNCTION IN RENAL TUBULI IN MICE WITH CISPLATIN INDUCED-AKI

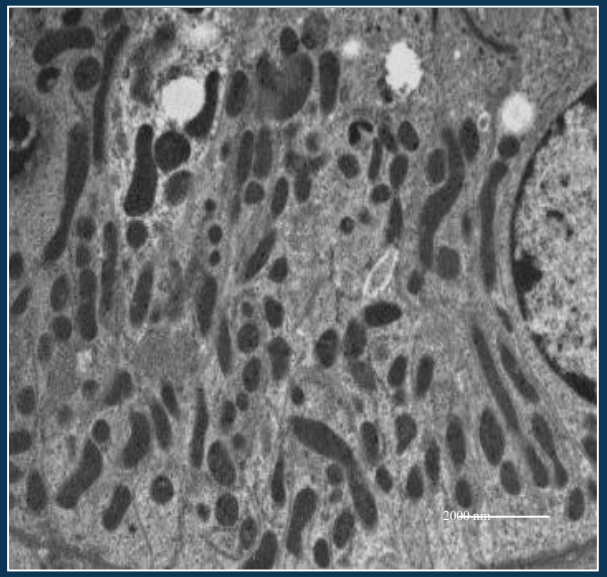
Control



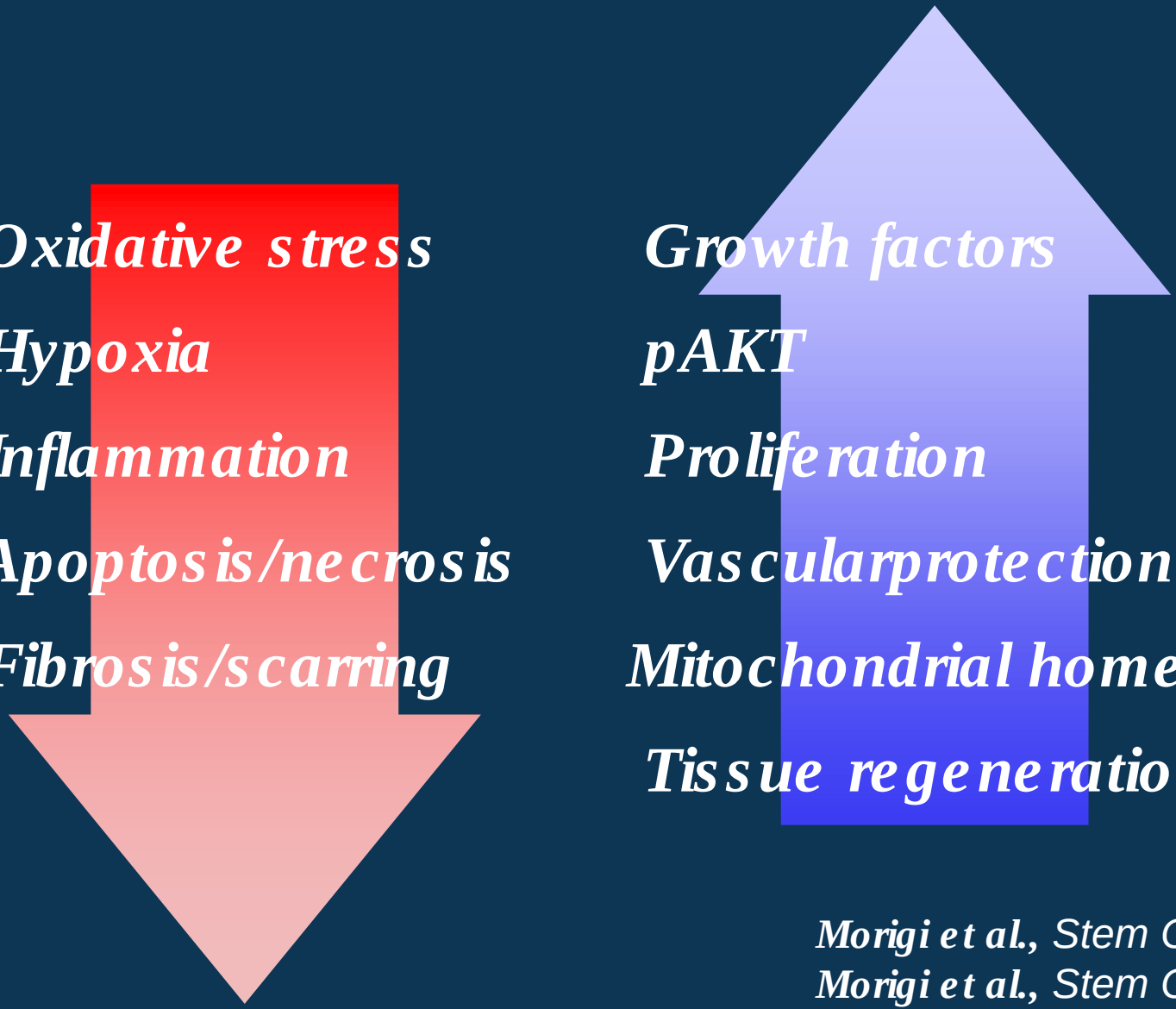
Cispl



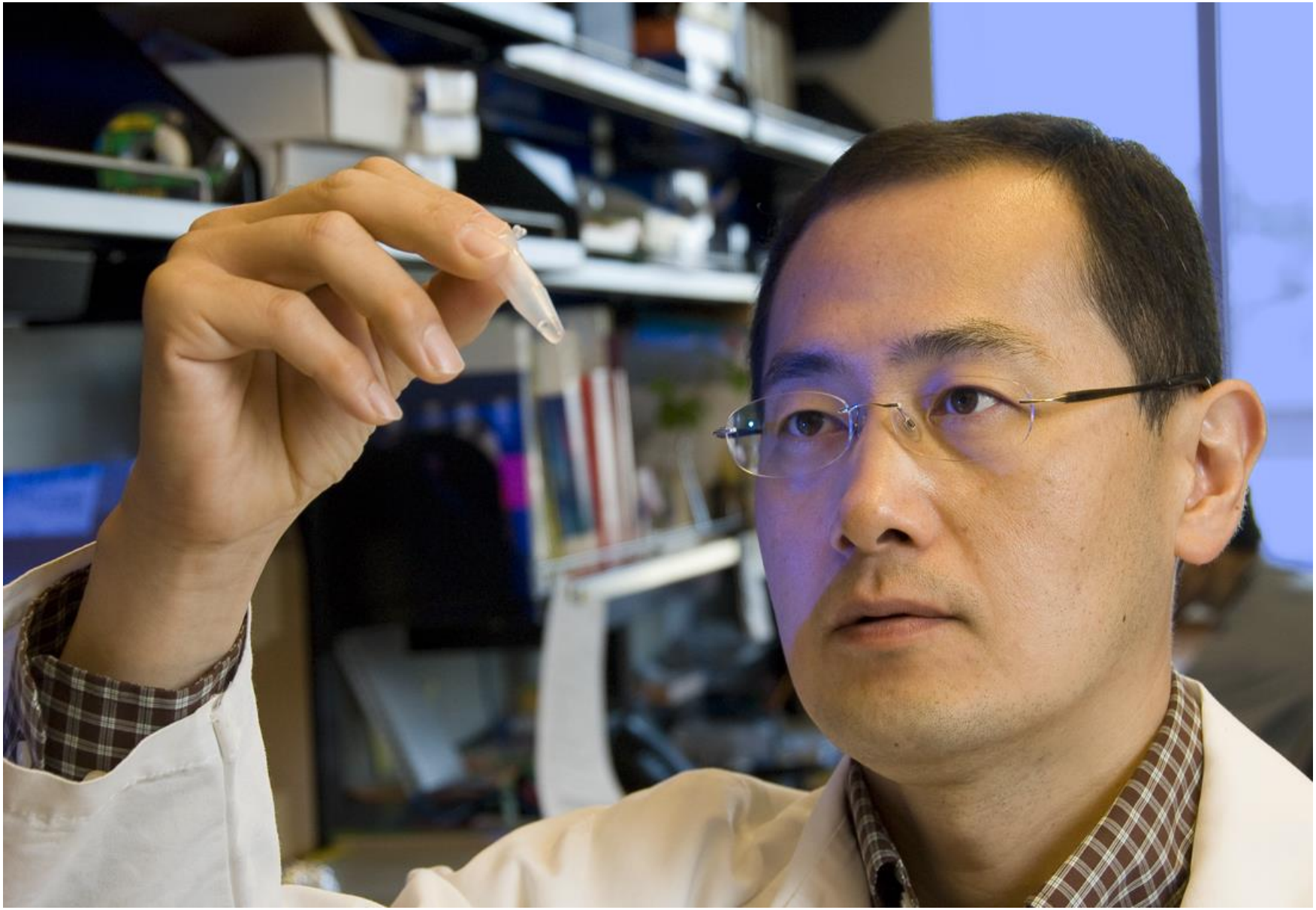
Cispl + hUC-MSCs



PARACRINE ACTION OF MSC IN KIDNEY REPAIR



Morigi et al., Stem Cells, 2008
Morigi et al., Stem Cells, 2010
Perico et al., Submitted



Shinya Yamanaka

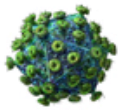
Cell, November 30, 2007

Derivation of Induced Pluripotent Stem (iPS) Cells

Fibroblasts or PBMC

Reprogramming by 4 factors:

- *Oct4*
- *Sox2*
- *Klf4*
- *cMyc*



iPS cells

Specific nephrogenic factors

- *Retinoic Acid + Activin A*
- *FGF2, BMP7 and GDNF*

Renal committment

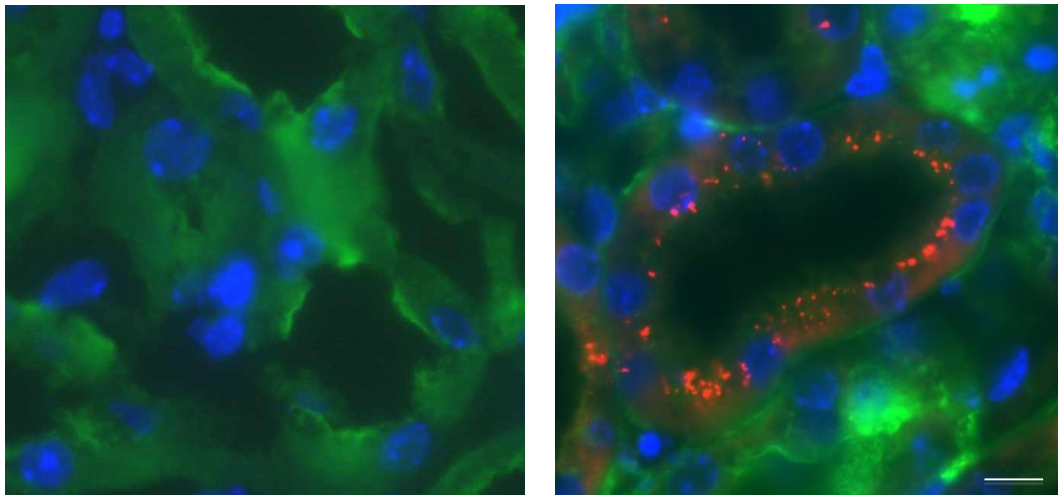
- *Sall1*
- *Pax2*
- *Six2*
- *NCAM*
- *Claudin1*
- *Aquaporin1*

Imberti et al., Scientific Report, 2015

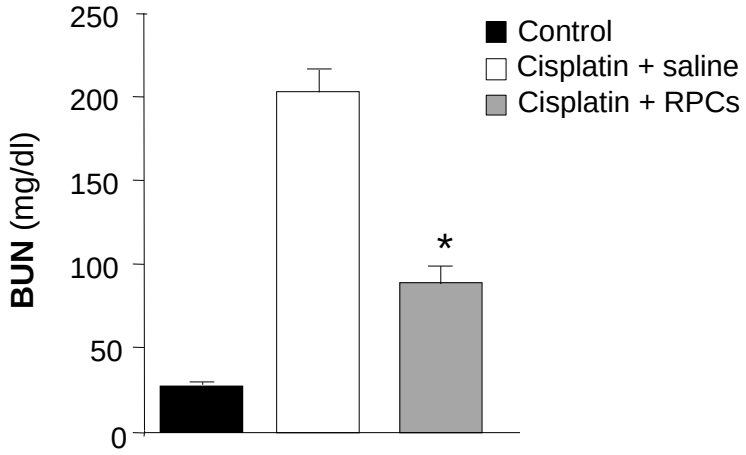
IPS-DERIVED RENAL PROGENITOR CELLS ENGRAFT THE KIDNEY AND PRESERVE RENAL FUNCTION AND STRUCTURE

Cisplatin + saline

Cisplatin + RPCs



h-Mito/Lectin/ DAPI



Imberti et al., Scientific Report, 2015

iPS CELLS PROGRESSING TO THE CLINIC

Age-related macular degeneration	***iPSCs/ESC***	⟶ *Retinal pigment epithelium*	***Clinical Phase I-II***
Parkinson disease	***iPSCs/ESC***	⟶ *A9 dopaminergic neuron*	***Clinical Phase I***
Spinal cord injury	***iPSCs/ESC***	⟶ *Oligodendrocyte Progenitor*	***Clinical Phase I***
Diabetes	***iPSCs/ESC***	⟶ *Pancreatic islet β-cell progenitor*	***Clinical Phase I-II***
Myocardial infarction	***iPSCs/ESC***	⟶ *Cardiomyocytes*	***Clinical Phase I***

Trounson et al., *Nature Review* ***2016***

Significant demographic changes in the transition between the 20th an 21st centuries

CHRONIC KIDNEY DISEASE: AN IMPORTANT CONTRIBUTOR TO THE NCD BURDEN

CKD attributable deaths - 2015

- ***Kidney disease is not entirely contained within the cardiovascular risk envelope***
- ***In the low/middle income countries up to 40 % of those identified with CKD do not have diabetes or any cardiovascular disease***

GBD 2015 Mortality and Causes of Death Collaborators, Lancet, 2016

Disease Models and Mechanisms, **2015**

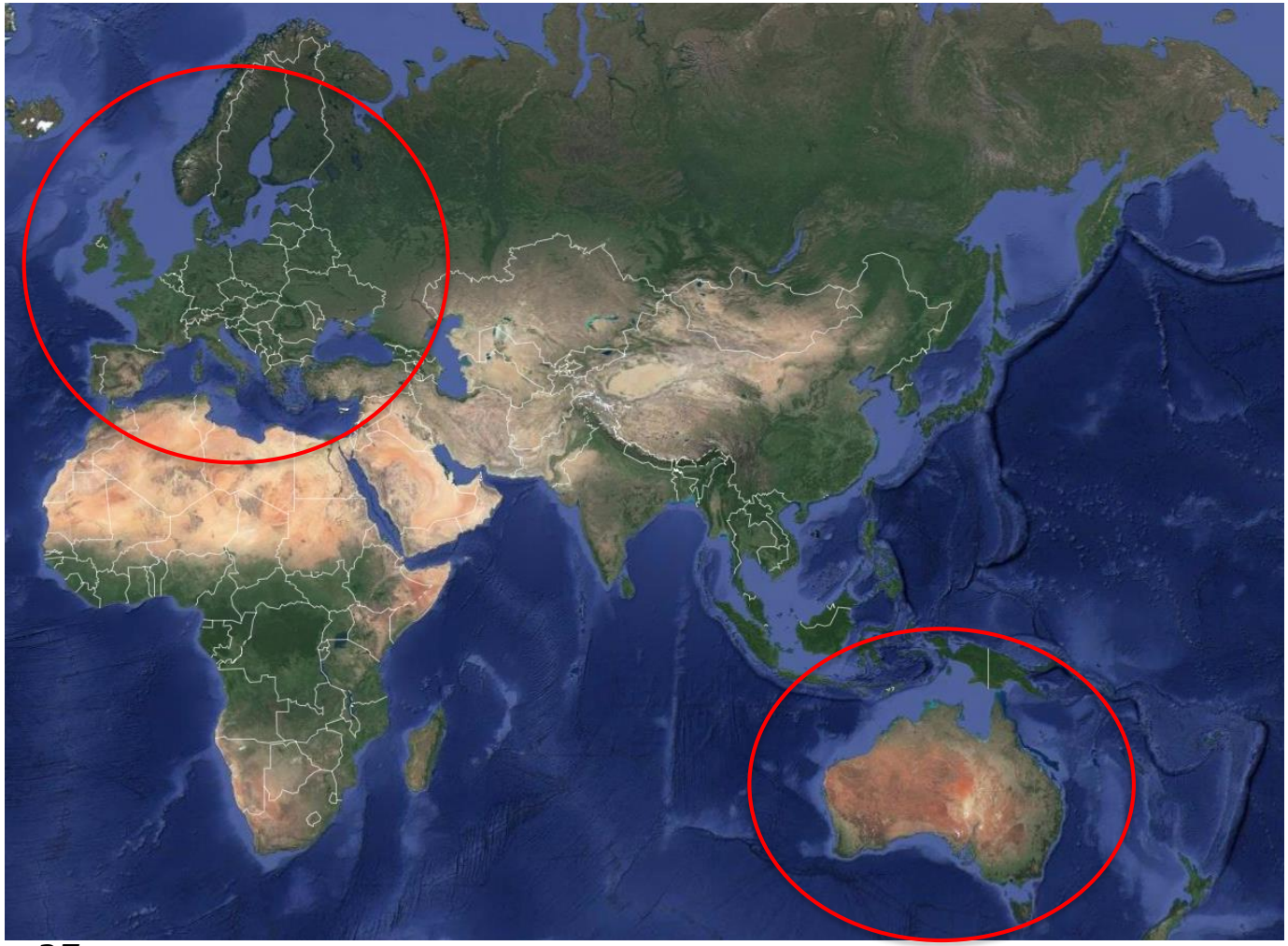
Cell-based therapies for experimental chronic kidney disease: a systematic review and meta-analysis

Diana A. Papazova*, Nynke R. Oosterhuis*, Hendrik Gremmels*, Arianne van Koppen, Jaap A. Joles and Marianne C. Verhaar‡

Our systematic review and meta-analysis showed that cell-based therapy reduced the development and progression of experimental CKD, as measured by several commonly and clinically used measures of renal function (creatinine, urea, GFR, BP and urinary protein) and for common experimentally used measures of renal damage

This finding proved to be consistent despite considerable differences between studies in the selection and preparation of cells, administration route and choice of disease model and model species

STELLAR *(Stem cell based therapy for kidney repair)*



An EU financed research consortium interested in developing an alternative to renal replacement therapy making use of newly discovered kidney mesenchymal stem cells

European Union-Australia Cooperation

Cells?

Number?

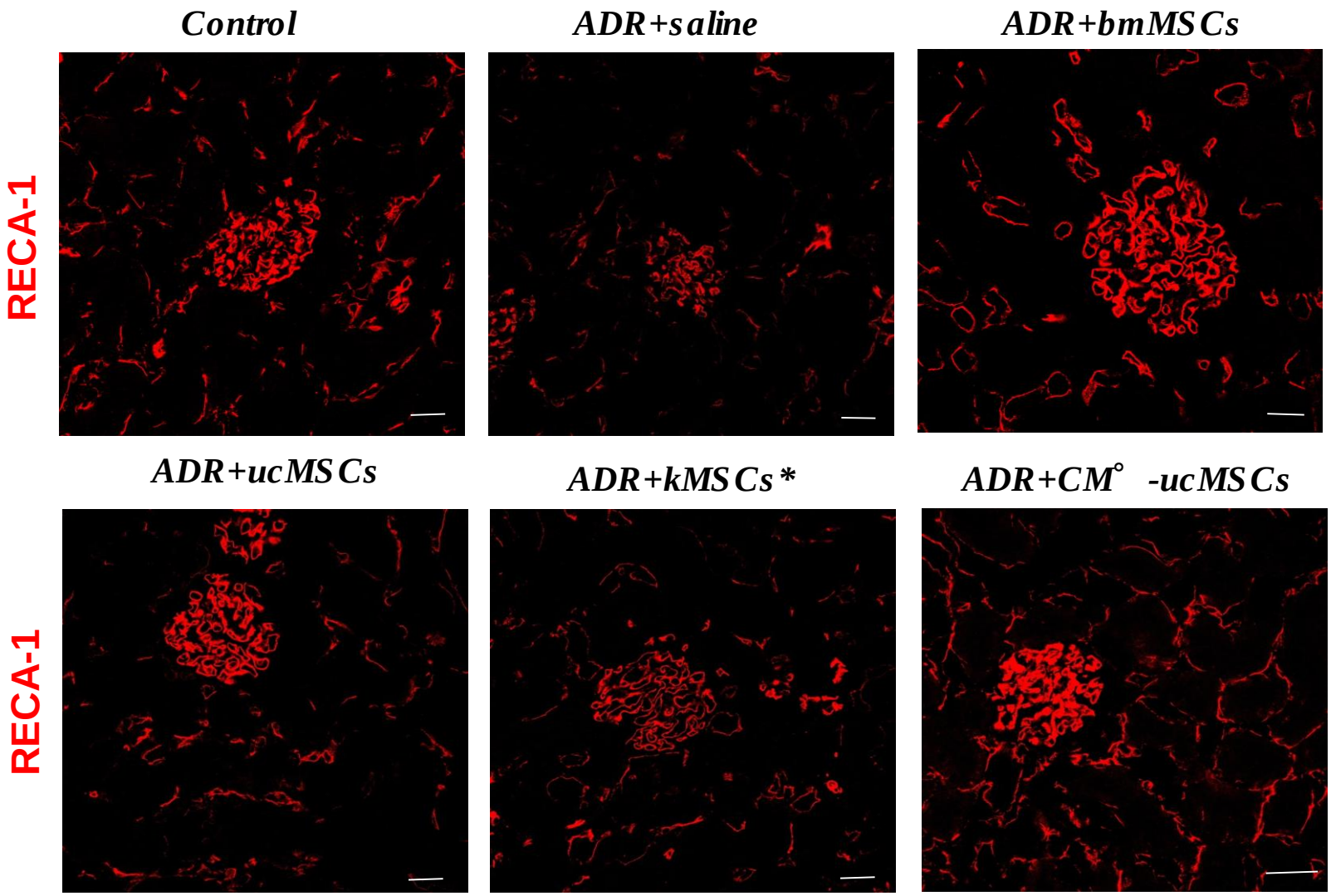
Whether they have to be differentiated into renal cells before transplantation?

Is this procedure safe in the long term?

To which extent these cells are retained into myocardial tissue?

Maldifferentiation or tumor formation?

EFFECT OF HUMAN MSC OF DIFFERENT ORIGINS OR CONDITIONED MEDIUM ON ENDOTHELIAL CELL DAMAGE IN RATS WITH CKD



** kMSCs: kidney-derived MSCs*

° CM: conditioned medium

NEPHSTROM clinical trial

novel stromal cell therapy for diabetic kidney disease

Leader: ***IRFMN, Bergamo, Italy***

Norberto Perico,
Federica Casiraghi
Giuseppe Remuzzi

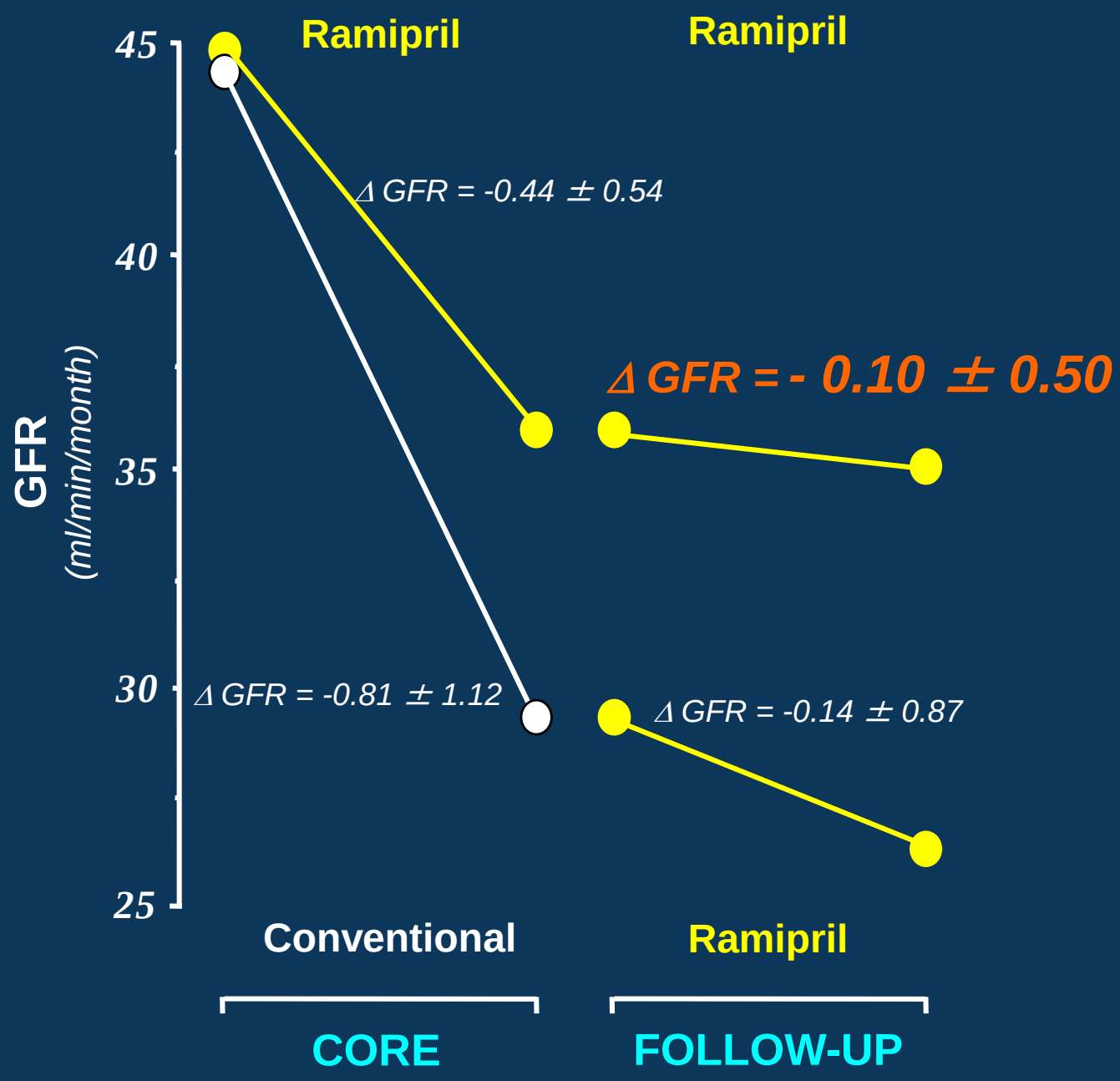
Coordination: ***IRFMN, Bergamo, Italy***

Nadia Rubis

Partners

- ***National University of Ireland, Galway, Ireland*** *(M. Griffin)*
- ***University Hospital Birmingham NHS Foundation Trust, Birmingham, UK*** *(P. Cockwell)*
- ***Belfast Health and Social Care Trust, Belfast, UK*** *(P. Maxwell)*
- ***IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy***

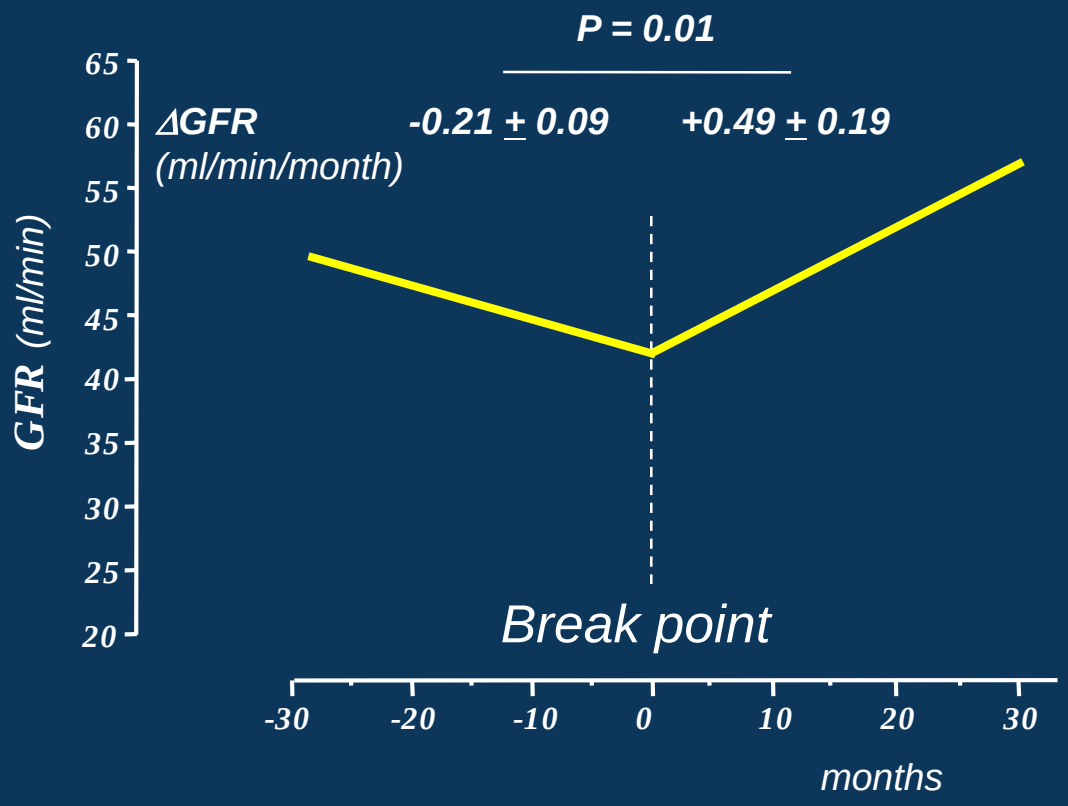
The case of kidney self repair



Ruggenenti et al., Lancet, 1998

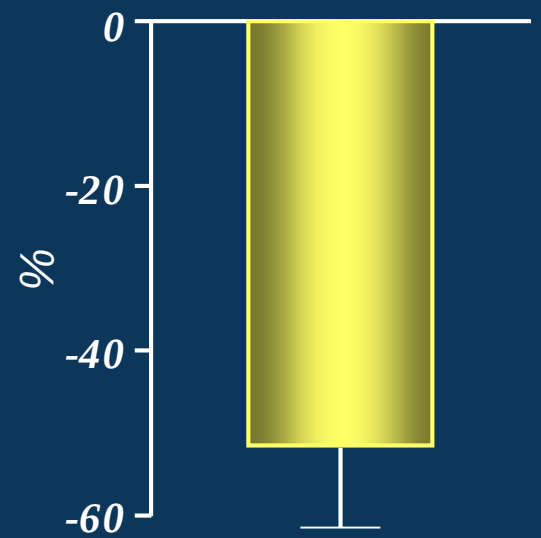
REGRESSION

10 patients with increasing GFR

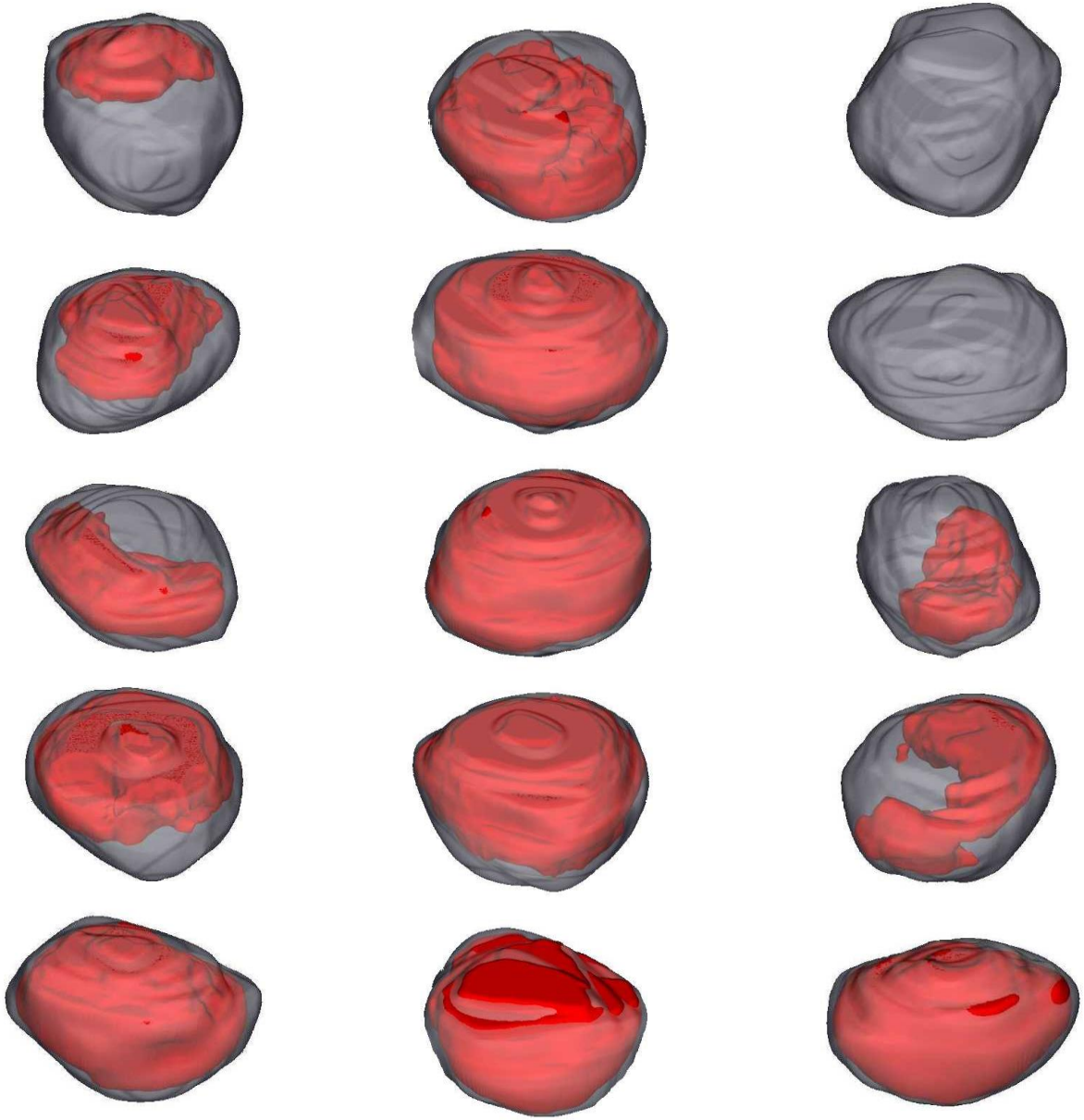


Change in proteinuria

(post vs pre break point)

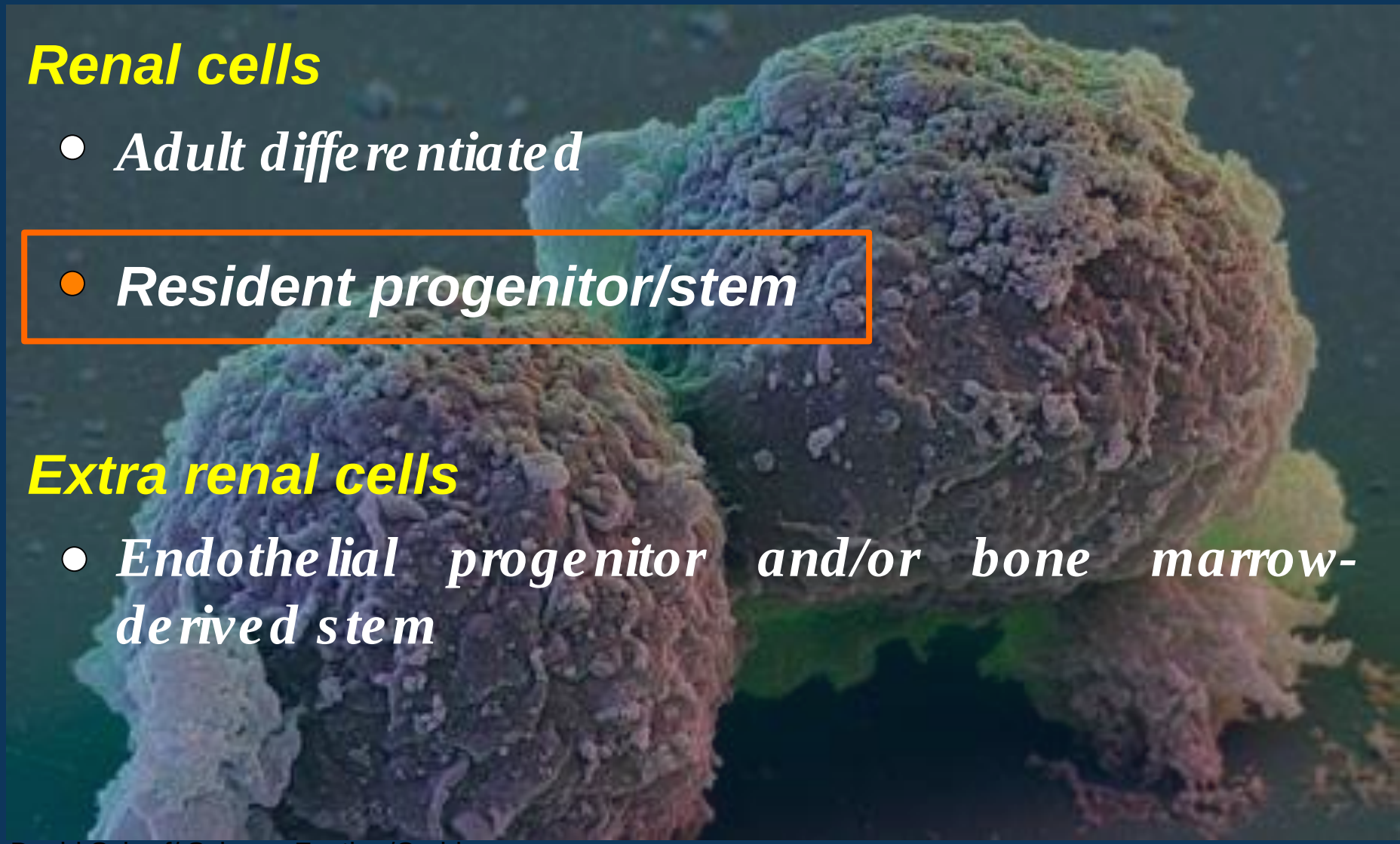


Ruggenenti et al., *J Am Soc Nephrol, 1999*



Remuzzi A. et al., *Kidney Int,* 2006

INSIGHT INTO ACE-INDUCED RENAL REPAIR/ANGIOGENESIS



Renal cells

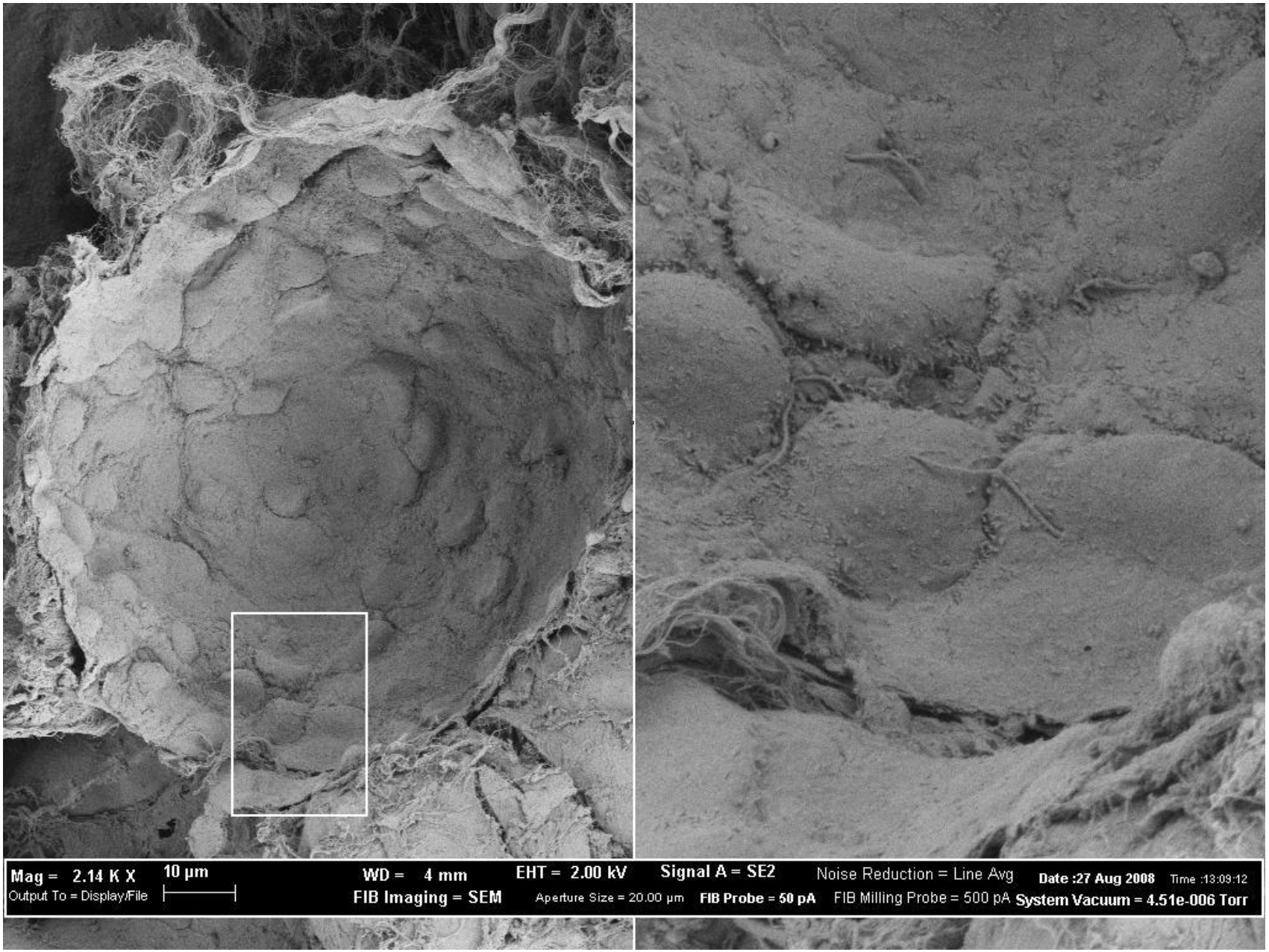
- *Adult differentiated*
- ***Resident progenitor/stem***

Extra renal cells

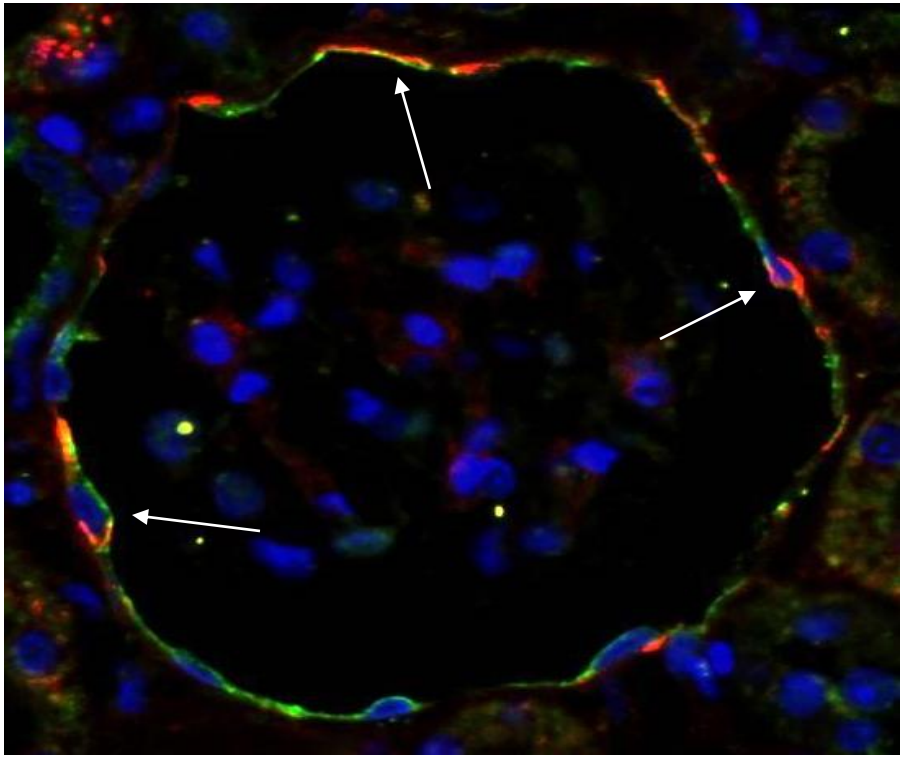
- *Endothelial progenitor and/or bone marrow-derived stem*

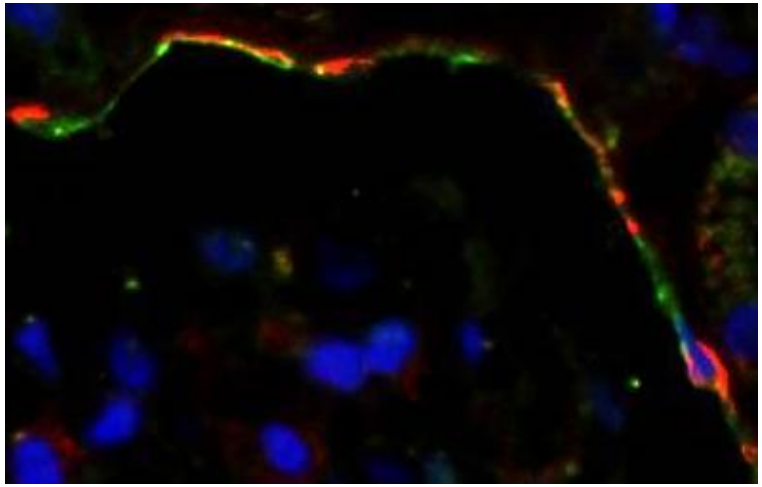
David Scharf/ Science Faction/Corbis

Bowman's capsule at SEM



NCAM* co-expresses progenitor cell marker CD24

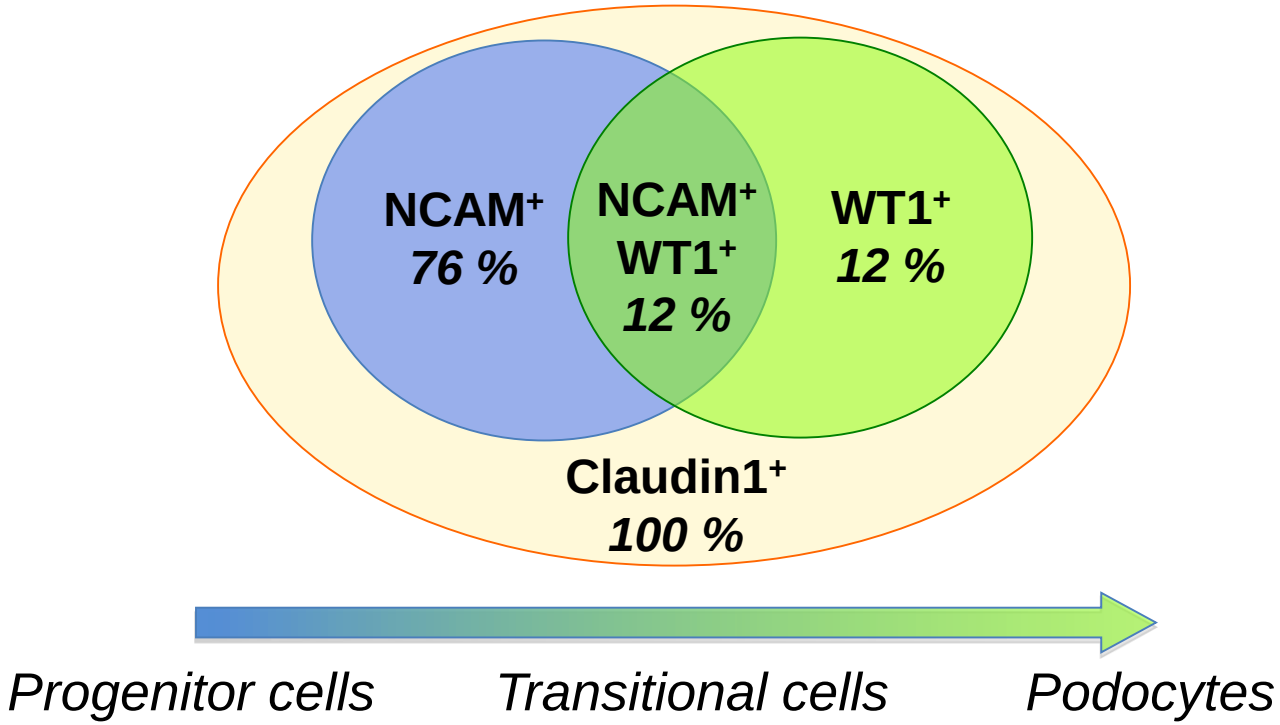




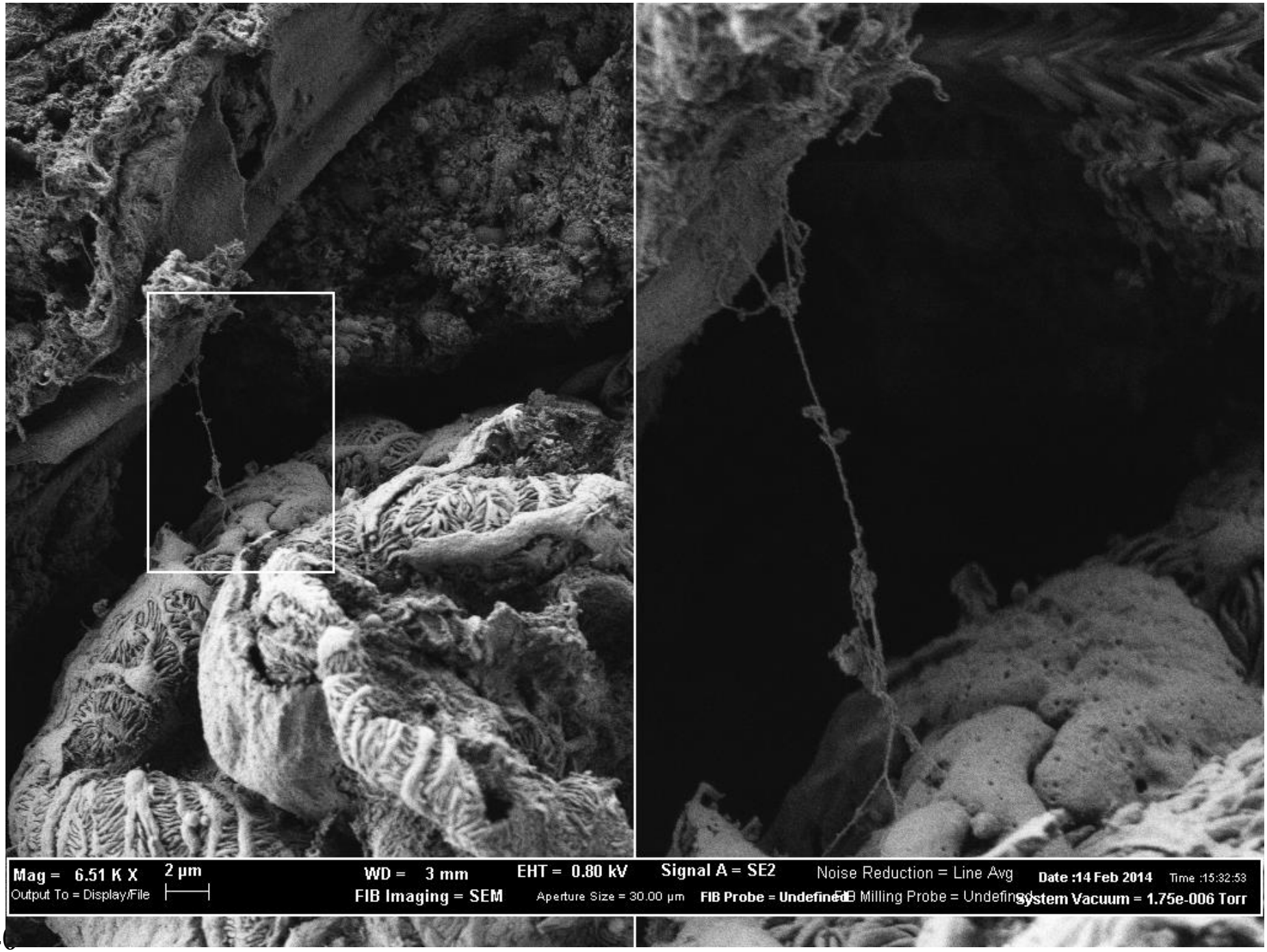
NCAM **CD24**

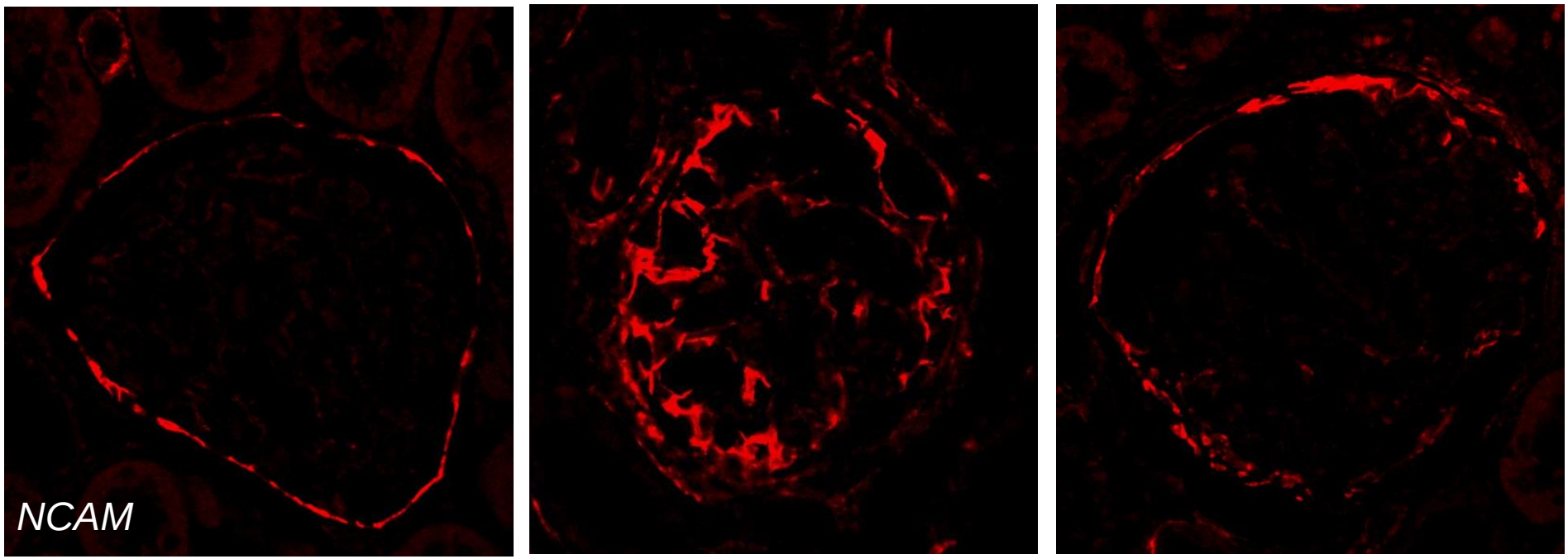
** Neural Cell Adhesion Molecule: a protein expressed in metanephric mesenchyme*

Benigni et al., *Am J Pathol,* ***2011***



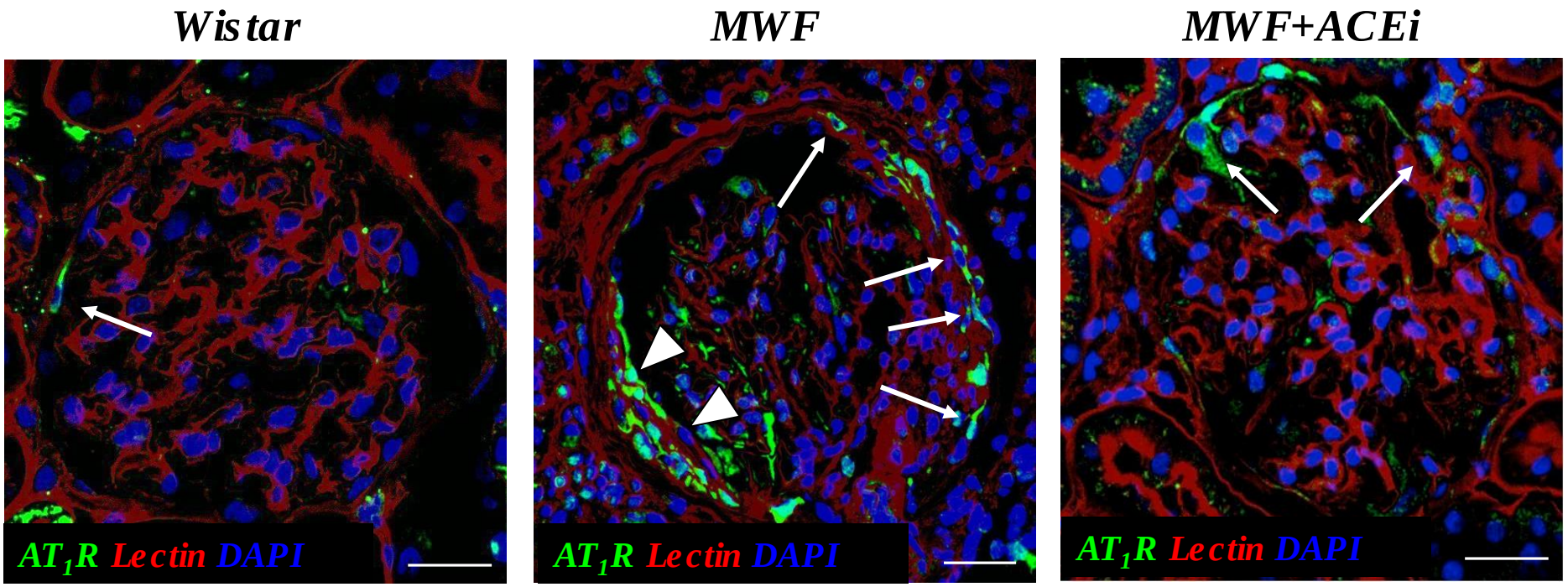
Benigni et al., *Am J Pathol,* ***2011***


Mag = 6.51 K X
2 µm
Output To = Display/File
WD = 3 mm
FIB Imaging = SEM
EHT = 0.80 kV
Aperture Size = 30.00 µm
Signal A = SE2
FIB Probe = Undefined
Noise Reduction = Line Avg
FIB Milling Probe = Undefined
Date :14 Feb 2014
Time :15:32:53
System Vacuum = 1.75e-006 Torr



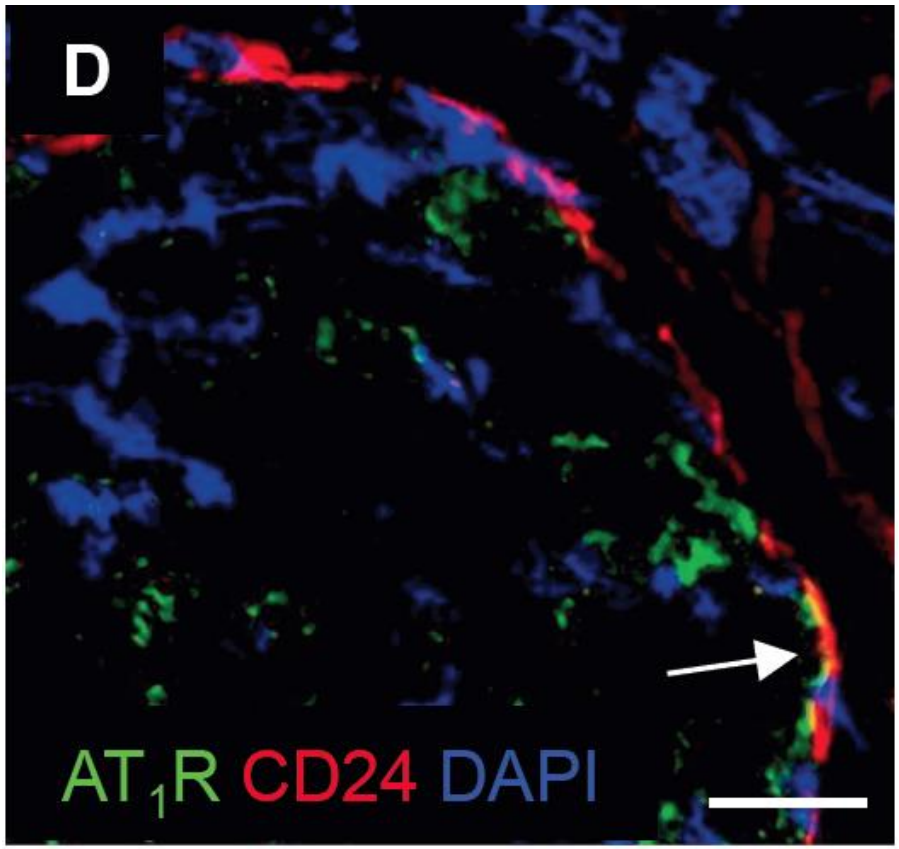
Benigni et al., Am J Pathol, 2011

ACE INHIBITORS LIMIT AT1R OVEREXPRESSION IN RENAL PROGENITOR CELLS



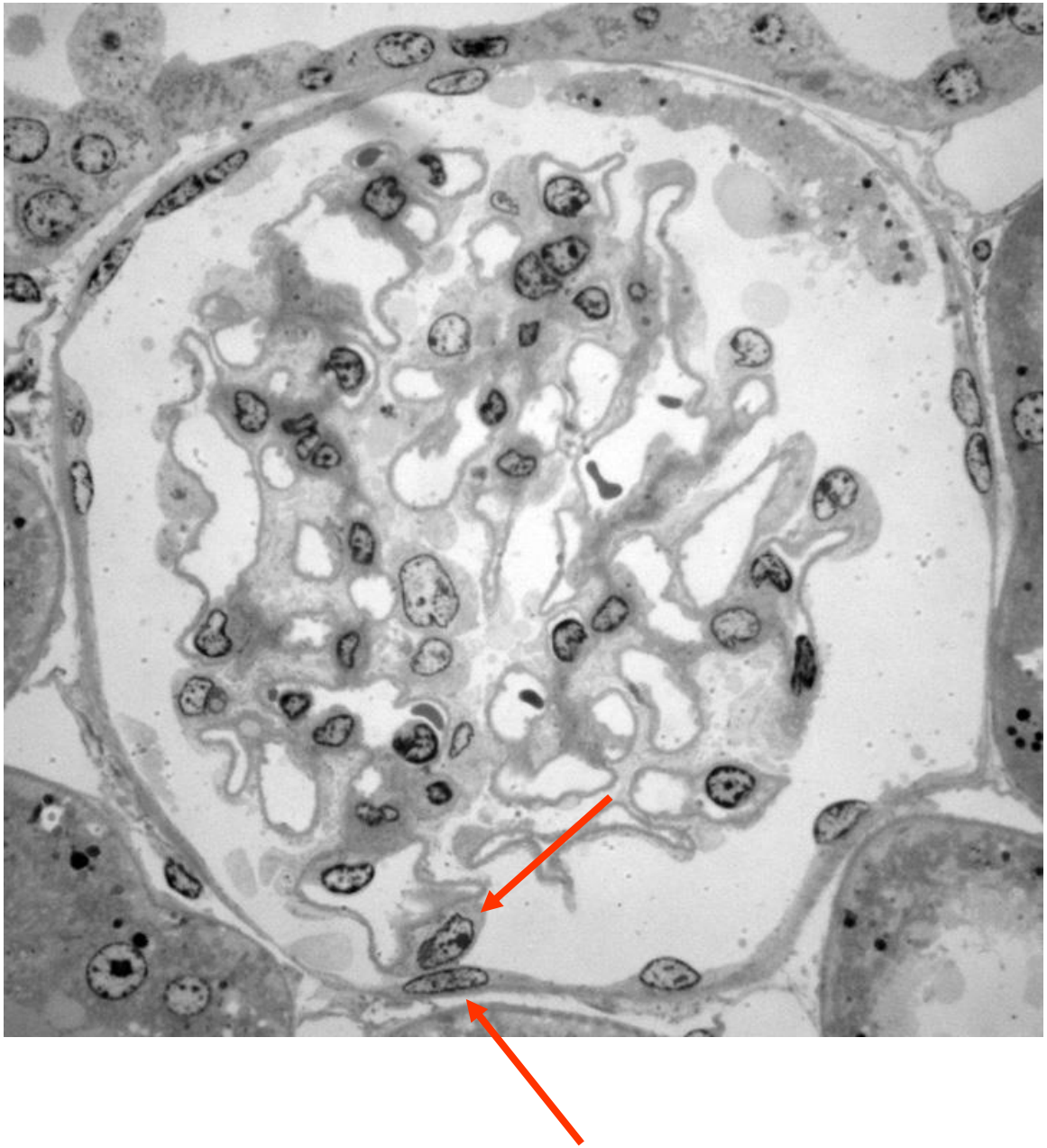
Rizzo et al., Am J Pathol, 2013

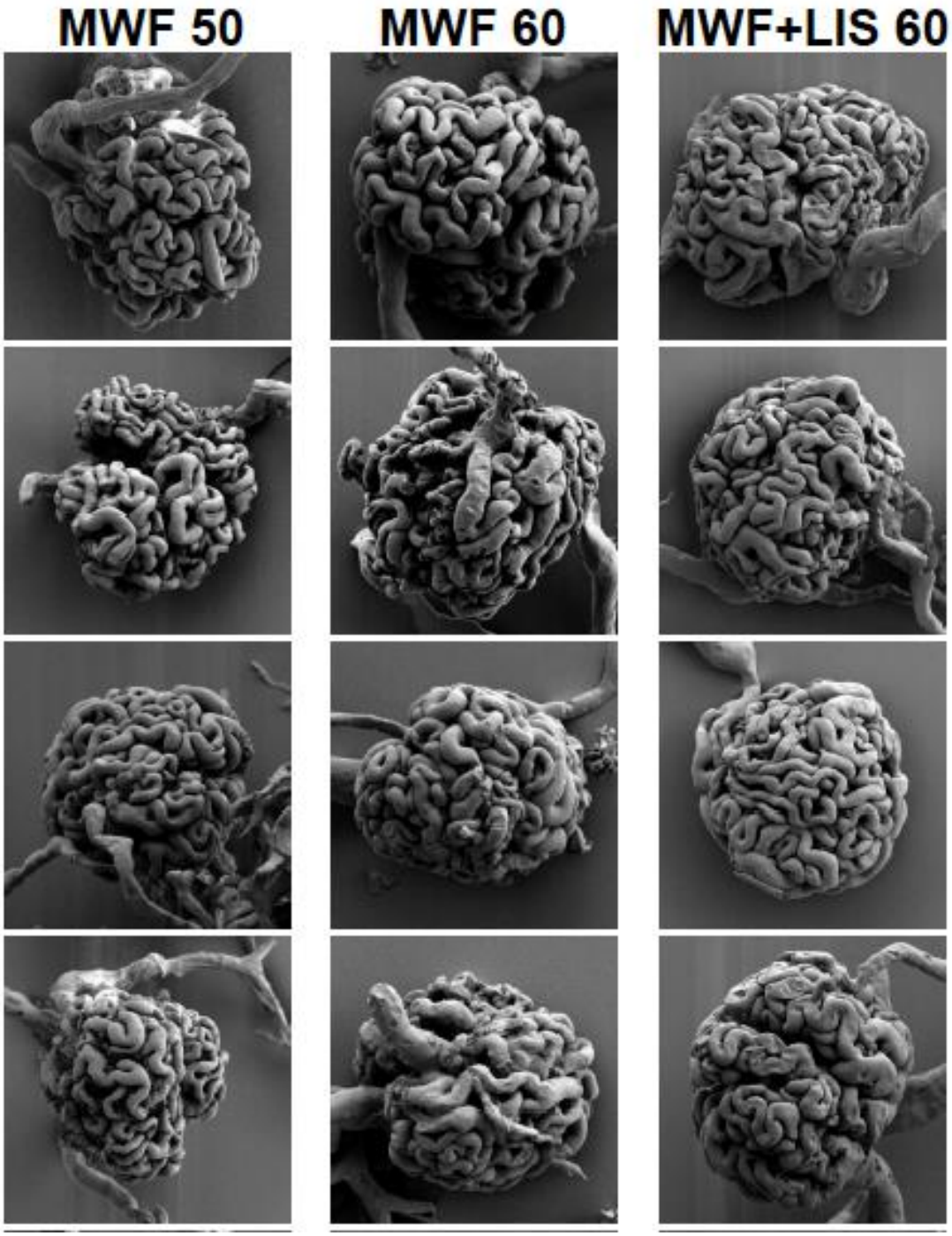
HUMAN PROGENITOR CELLS RARELY EXPRESS AT1R IN NORMAL KIDNEY

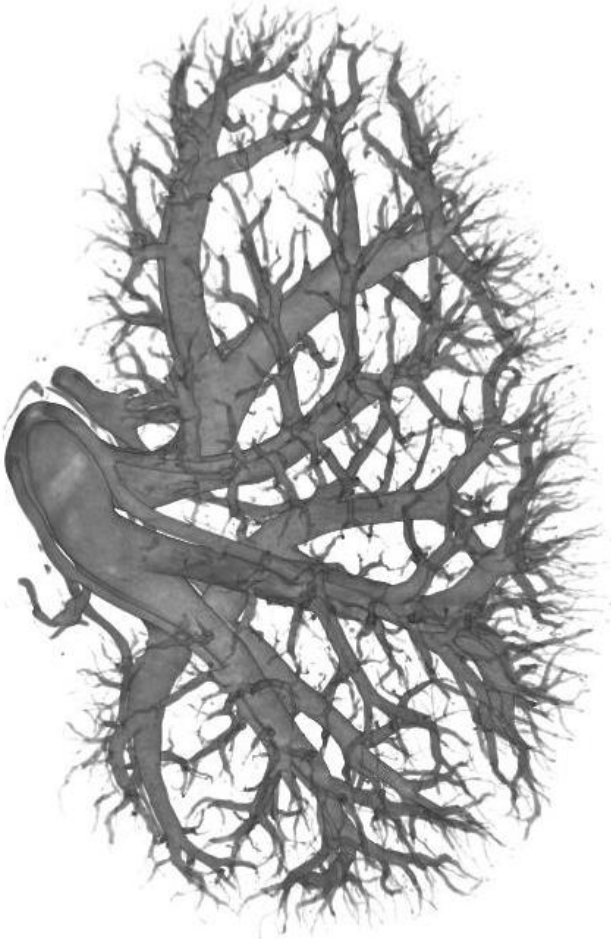


Rizzo et al., *Am J Pathol,* ***2013***

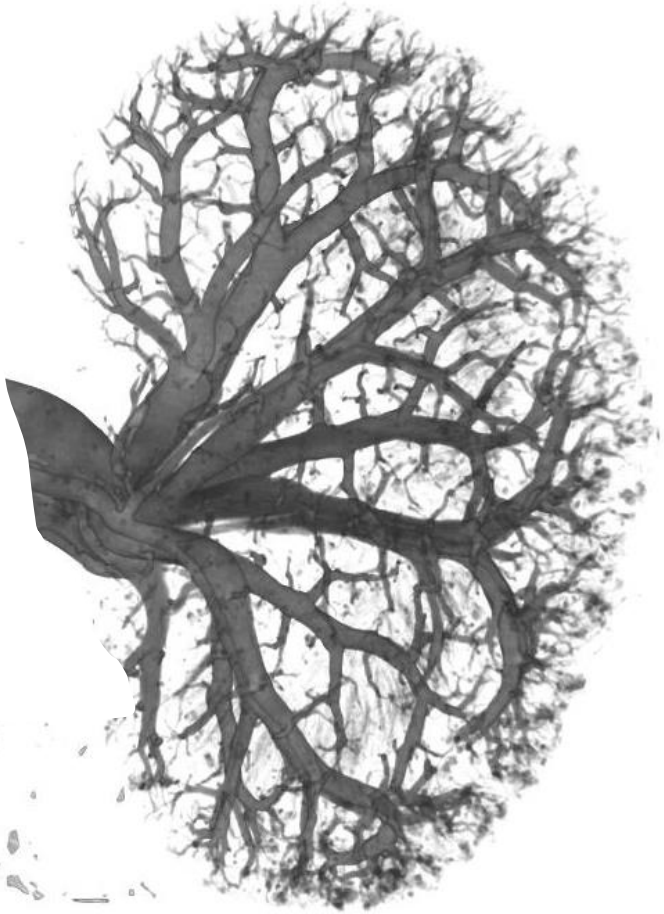
Migration of parietal cells from the Bowman's capsule to capillary tuft




MWF 50
MWF 60
MWF+LIS 60

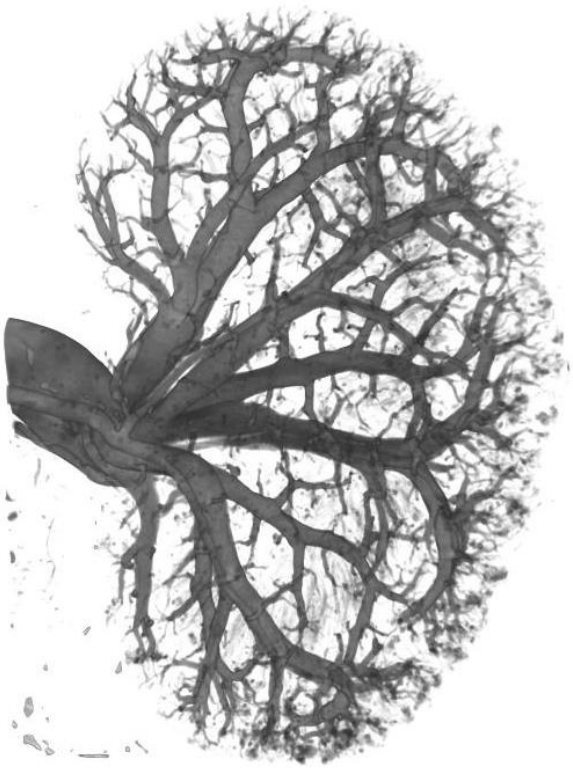


Wistar 25wk

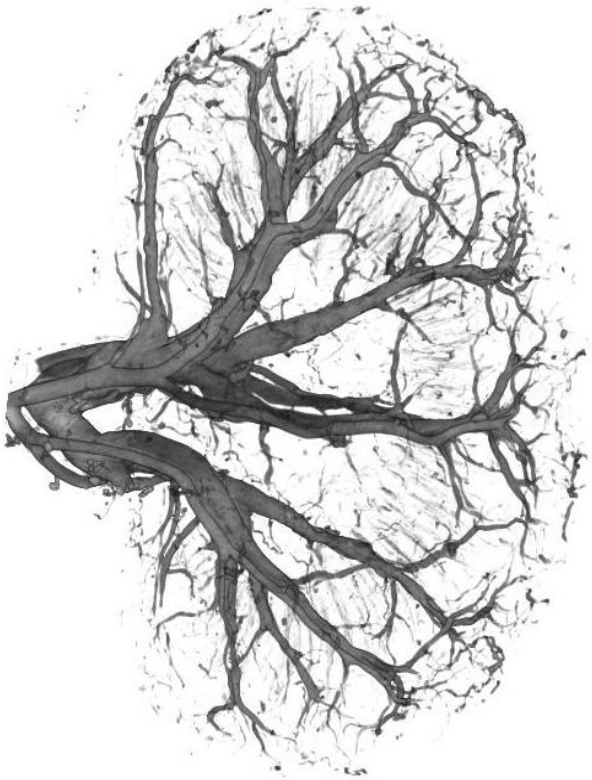


MWF 25wk

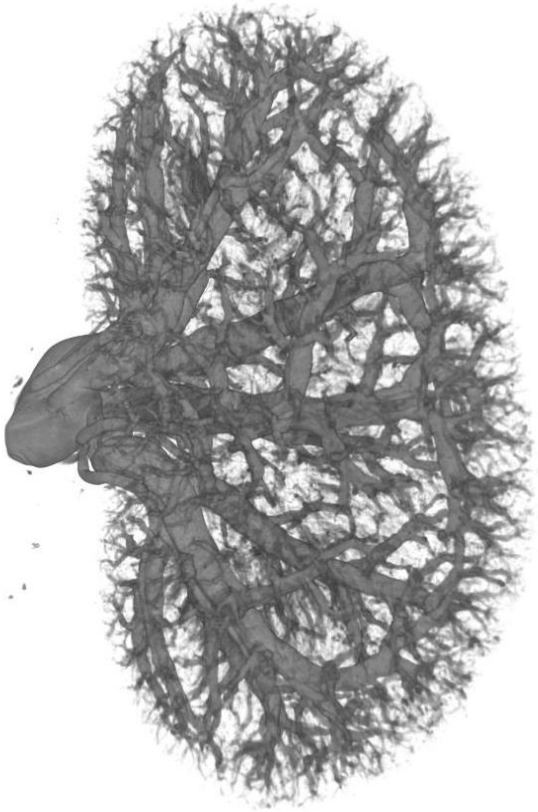
Remuzzi A. et al., *J Am Soc Nephrol,* ***2015***



MWF 25wk



MWF 50wk

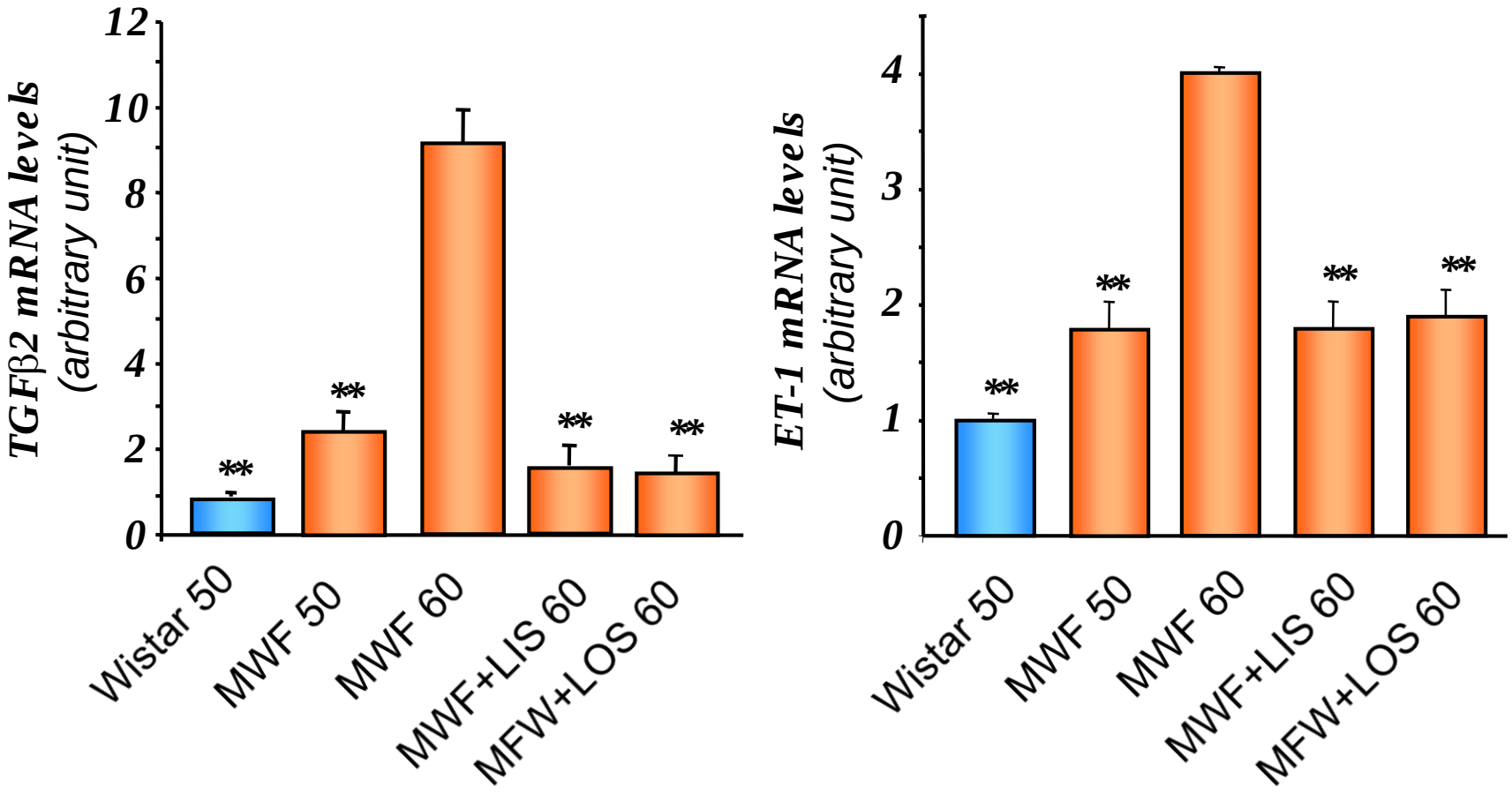


MWF + Lisinopril 50-60wk

Remuzzi A. et al., *J Am Soc Nephrol,* ***2015***

Unexpectedly, gene expression of vascular growth promoting factors, such as VEGF and related receptors or angiopoietin-1 and angiopoietin-2, did not change between MWF 60 and Wistar rats, while genes related to fibrosis, inflammation and extracellular matrix remodeling, including TGFβ2 and ET-1, were differentially expressed between the two strain

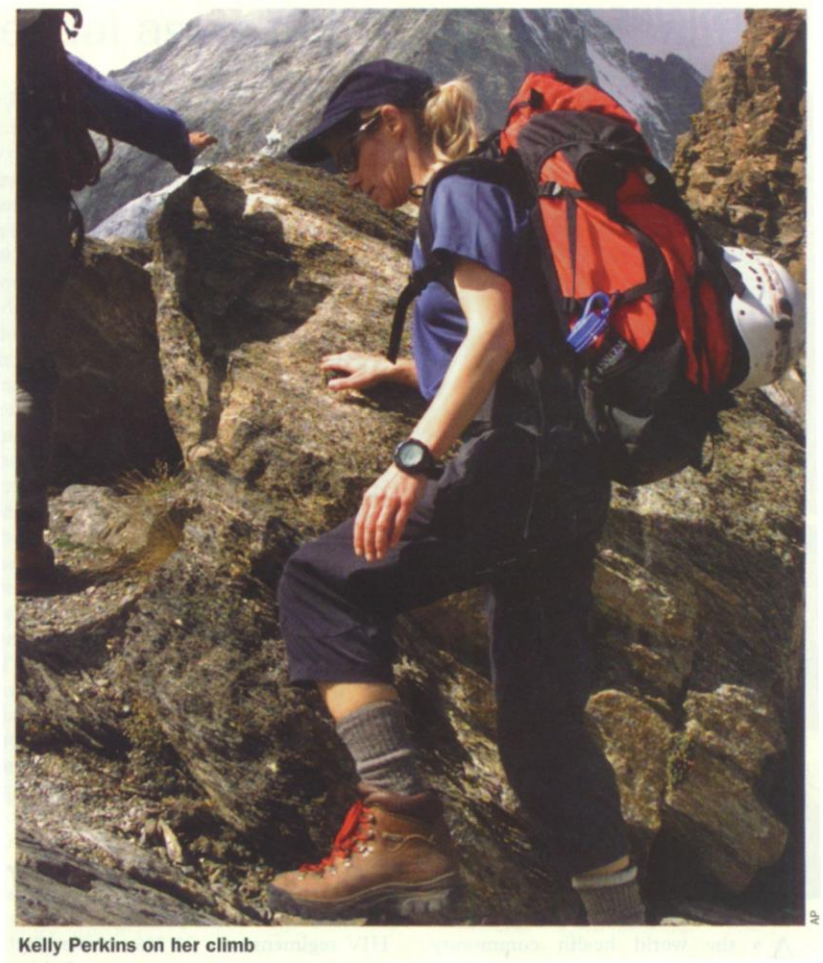
Remuzzi A. et al., J Am Soc Nephrol, 2015



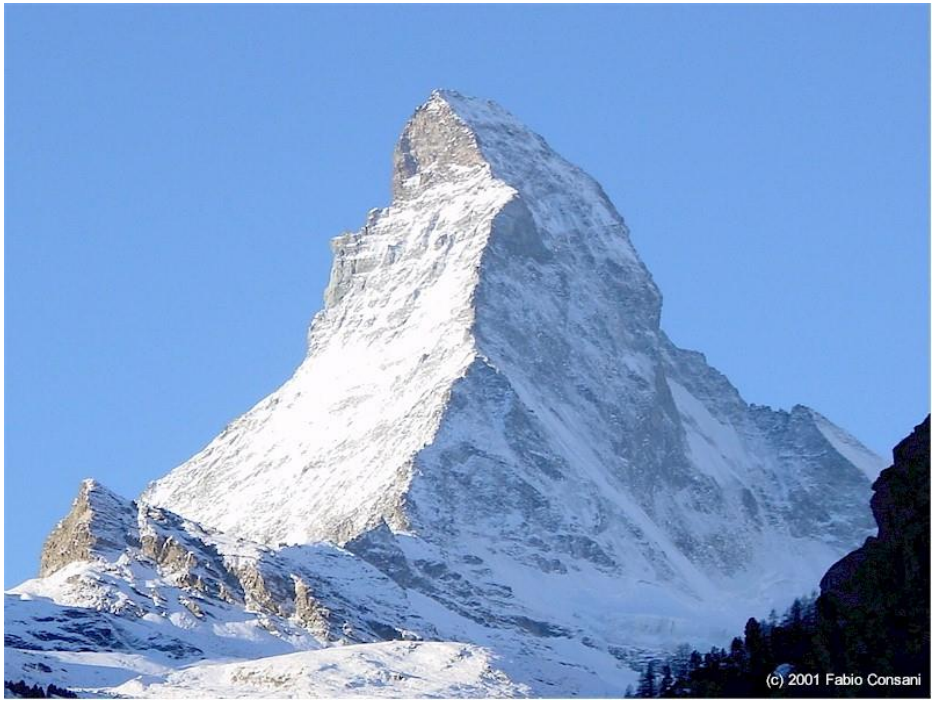
Remuzzi A. et al., *J Am Soc Nephrol,* ***2015***

HEART TRANSPLANT RECIPIENT CLIMBS THE MATTERHORN (Swiss Alps)

42-year-old Kelly Perkins becomes the first person with a heart transplant to ascend the 4478-m peak

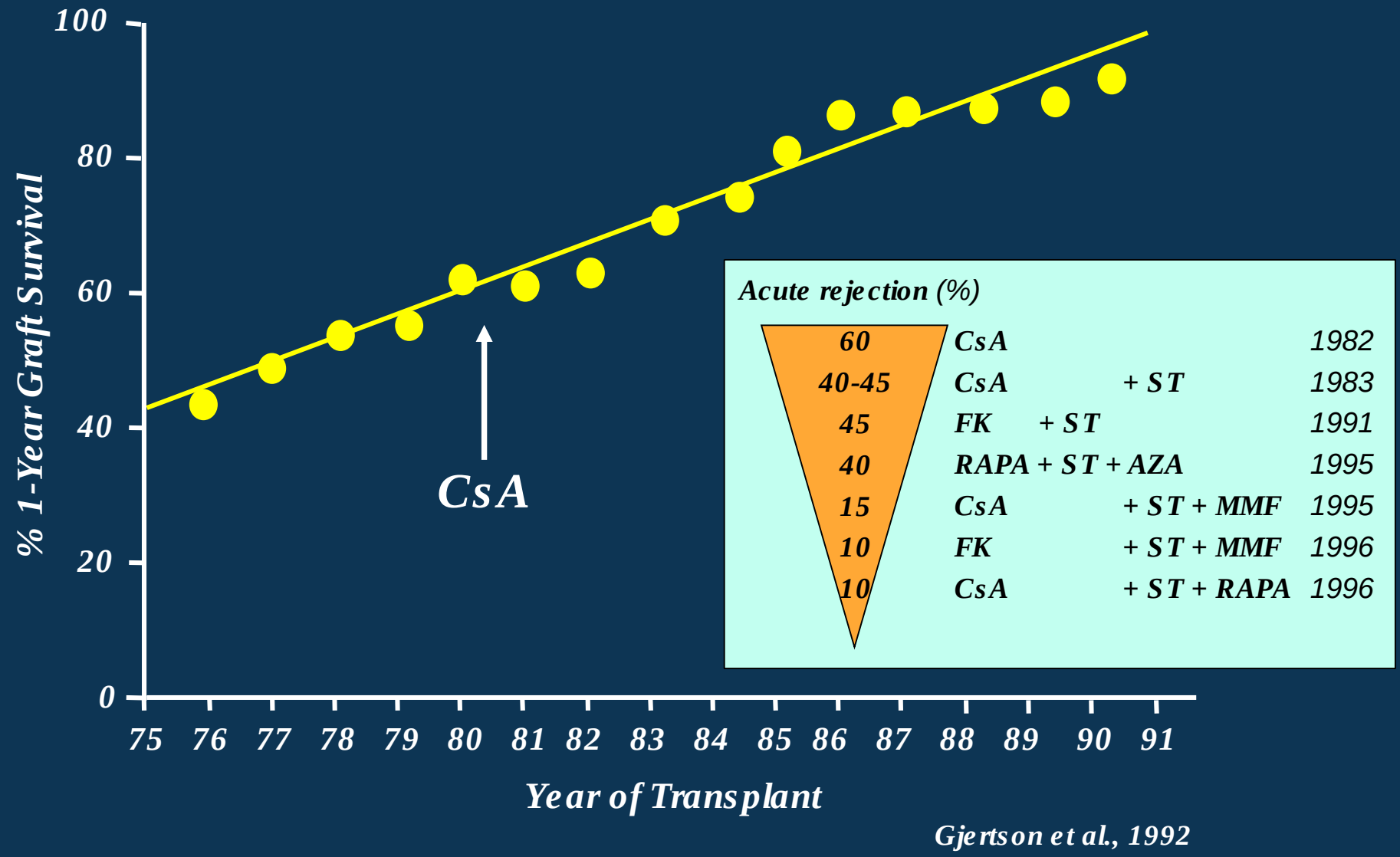


Kelly Perkins on her climb



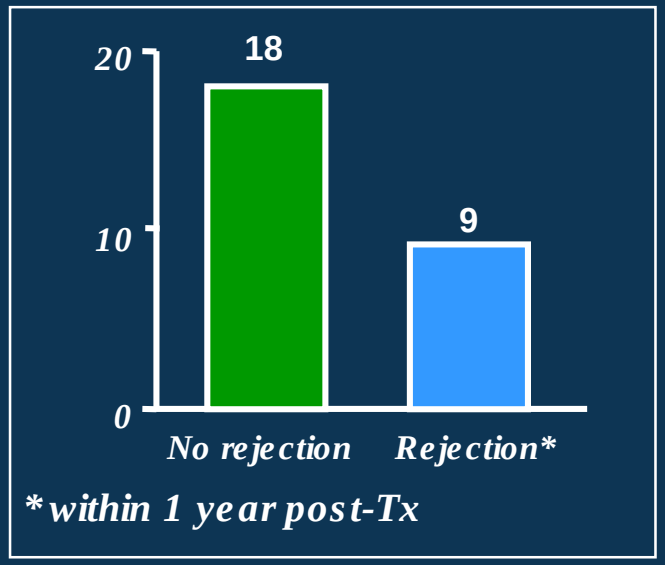
The Matterhorn in Zermatt, Switzerland

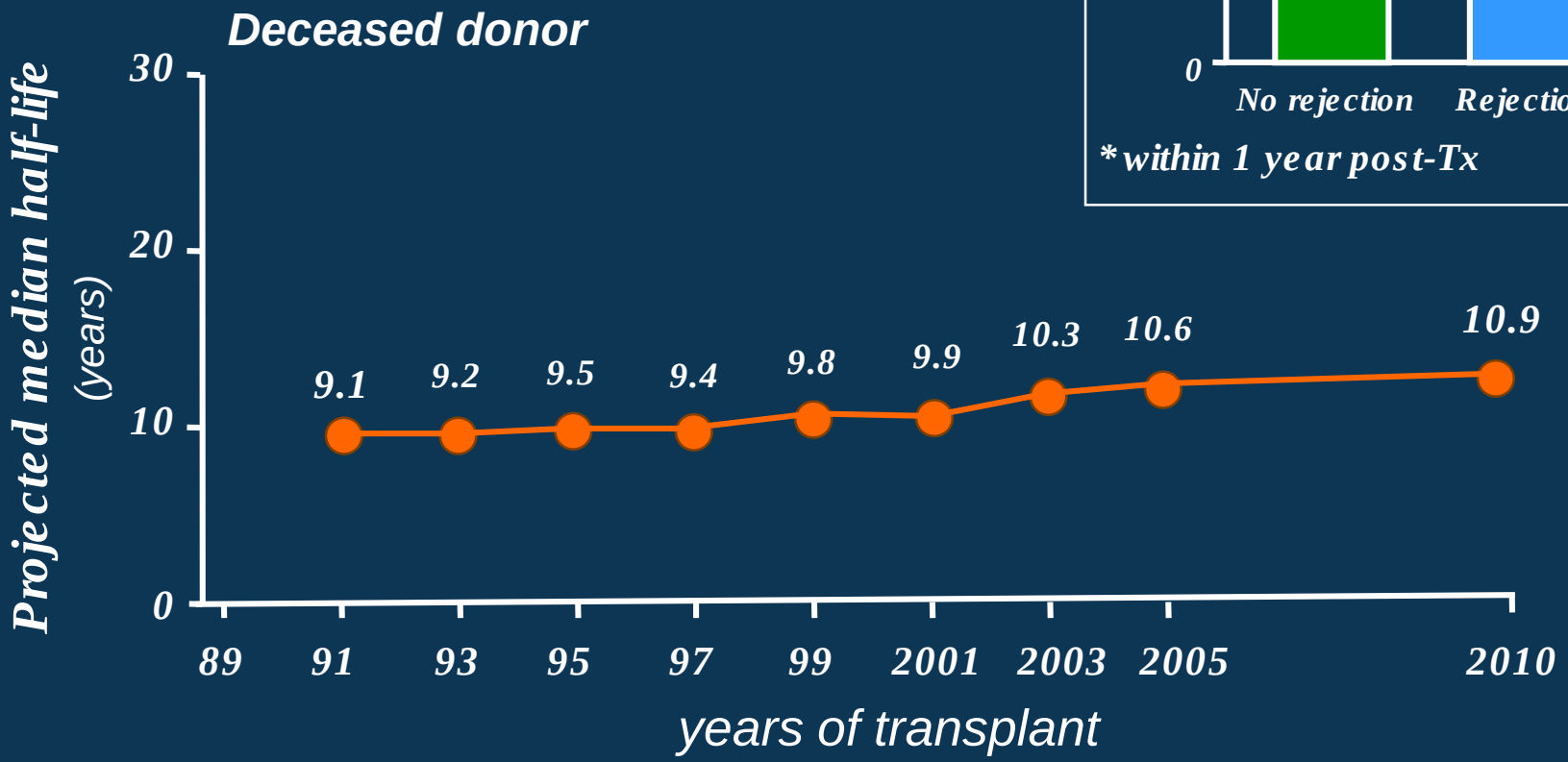
*Lancet, **2003***



Gjertson et al., 1992

LONG TERM GRAFT SURVIVAL AFTER RENAL TRANSPLANTATION HAS NOT SIGNIFICANTLY IMPROVED IN THE PERIOD 1991-2010





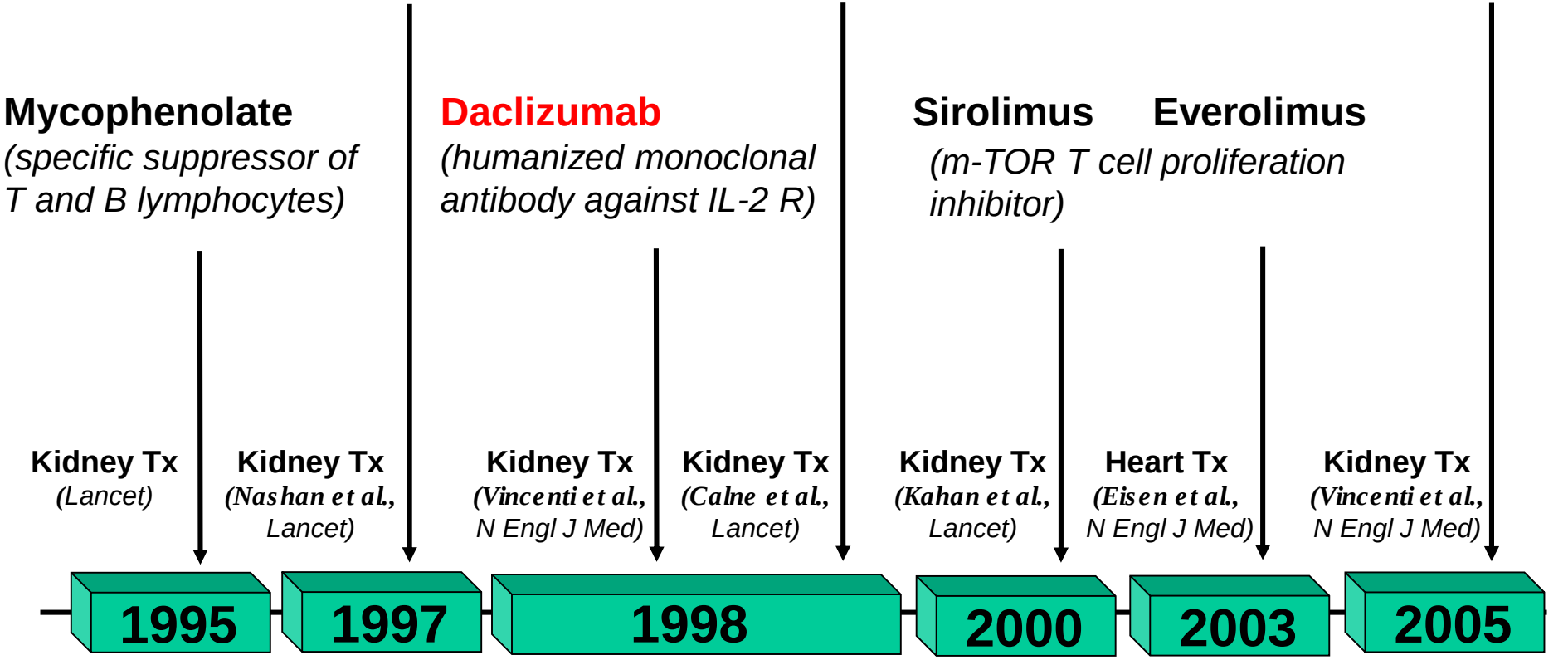
OPNT/SRTR 2012 Annual Data Report

THE PROMISE OF NOVEL IMMUNOSUPPRESSIVE AGENTS

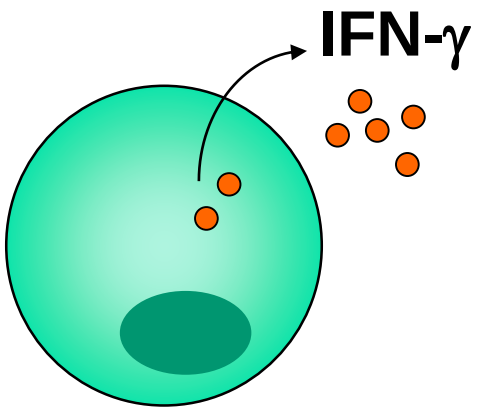
Basiliximab
(chimeric monoclonal antibody against IL-2 R)

CAMPATH-1H
(humanized anti-CD52 antibody - T and B cells depletion)

Belatacept
(IgG/CTLA4 fusion protein selective blocker of T cell activation)



THE SPECIAL PROBLEM OF MEMORY



Activated CD8⁺ memory

	IFN-γ blockade (in vitro)
- Calcineurin inhibitors	*partial*
- Hydrocortisone	*partial*
- Azathioprine	*negligible*

Jones et al., *Transplantation,* ***2006***

Memory T cells contribute to allograft rejection through:

- Activation endothelial cells

- Help naïve CD8, CD4 T cells and B cells

THE SPECIAL PROBLEM OF MEMORY

	Memory T cell response
- MMF	*Inhibition (partial)*
- Basiliximab	*Inhibition (partial)*
- Sirolimus, everolimus	*Stimulation*
- Alemtuzumab*	*Stimulation*

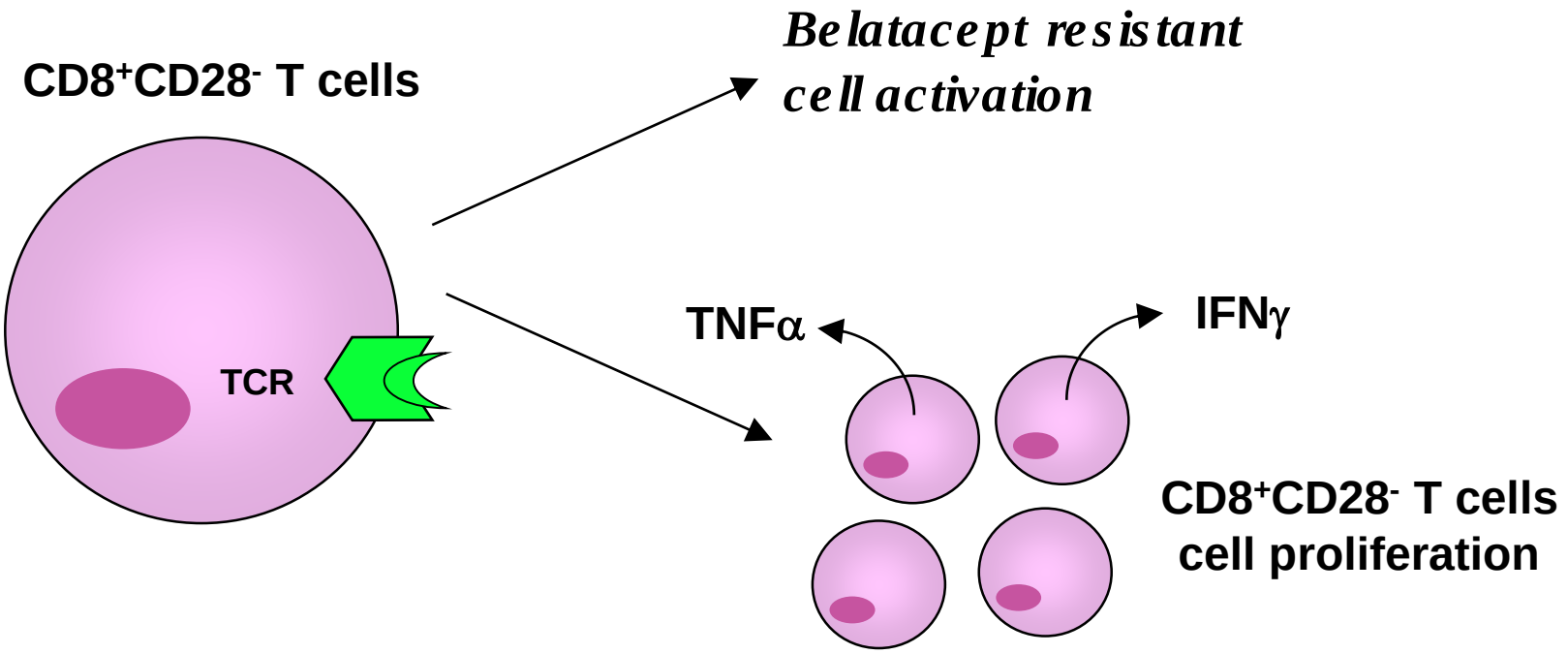
**** Campath-1***

Page AJ et al., *Curr OpOrgan Transplant, 2009*

Belatacept

1,420 -11,300 euro per month depending on treatment phase

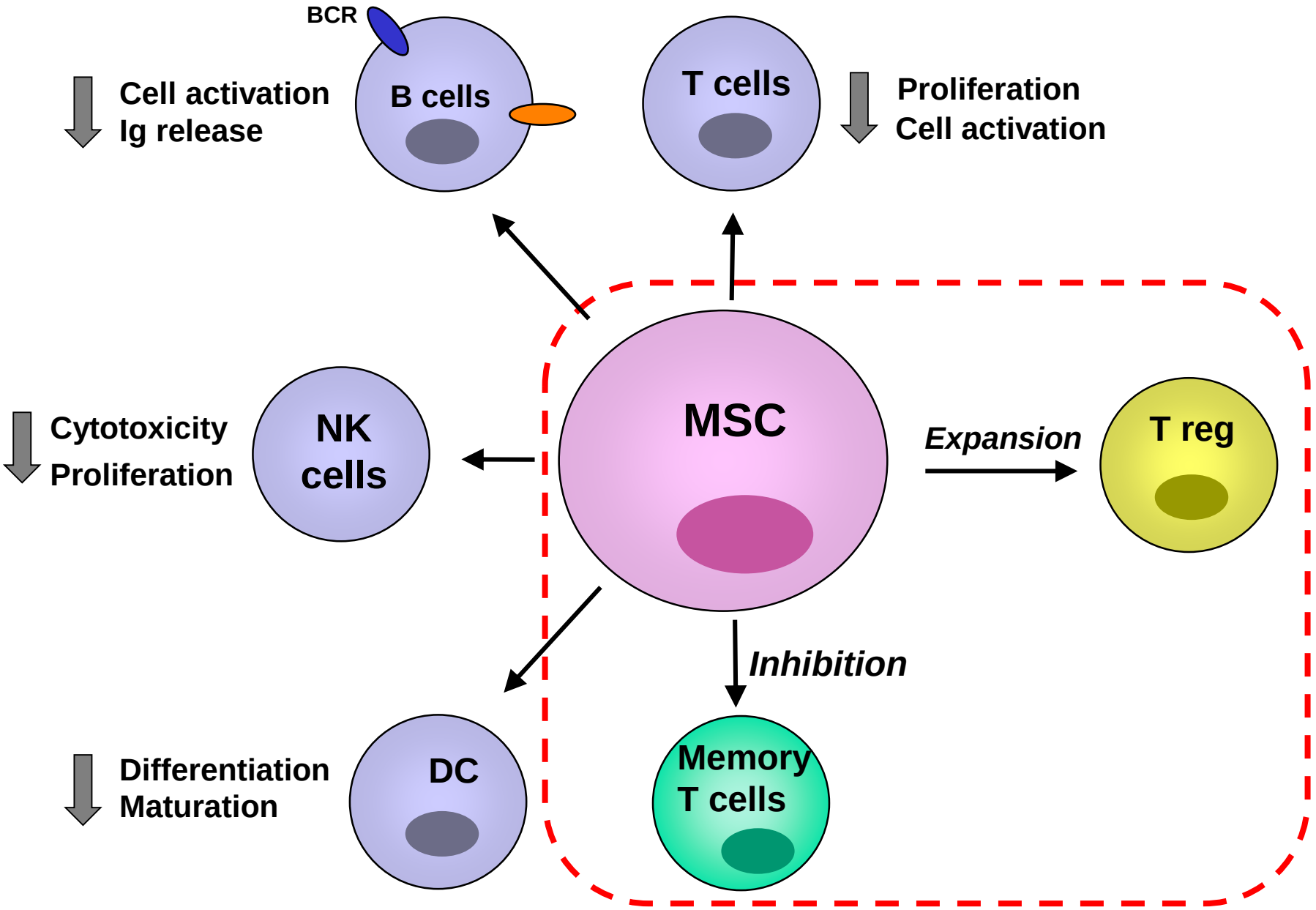
- ***$CD8^+CD28^-$ T cells harbor effector-memory phenotype***



Traitanou et al., *Am J Transpl,* ***2014***

- ***$CD4^+CD28^-CD57^+$ T cells underlie belatacept-resistant allograft rejection***

Espinosa et al., *Am J Transpl,* ***2016***

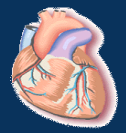


Nauta et al, Blood, 2007

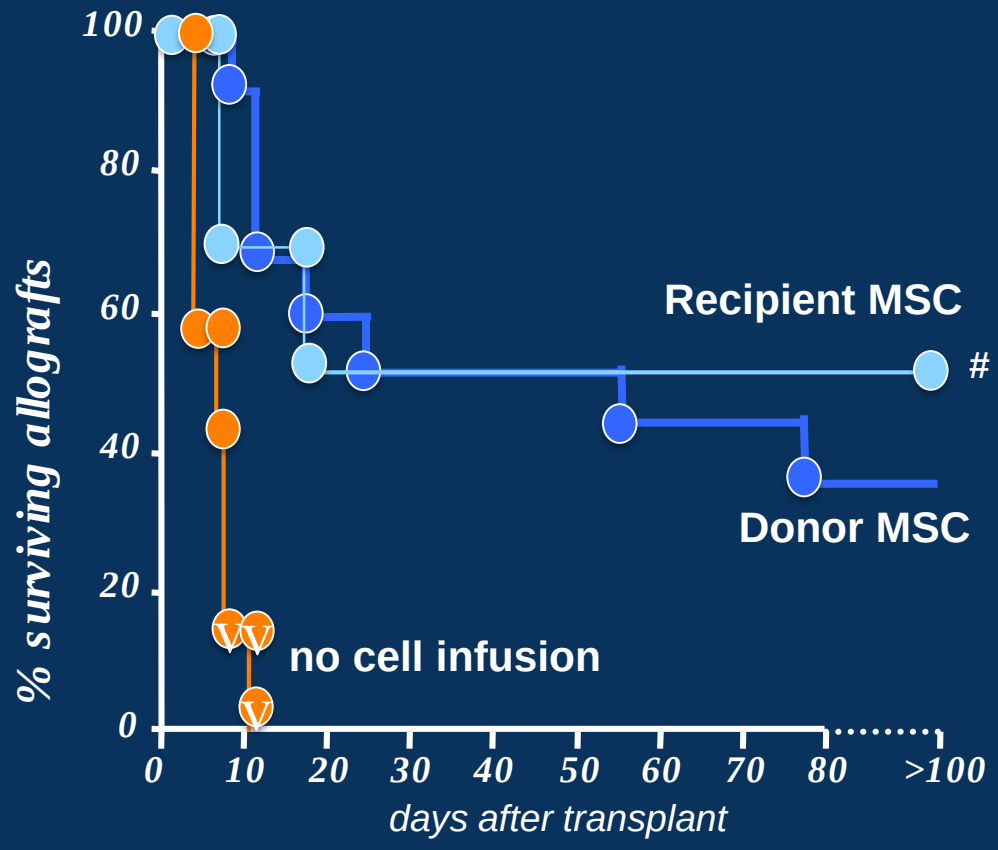
AUTOLOGOUS MSC PROLONG HEART TRANSPLANT SURVIVAL MEDIATED BY CD4+CD25+Foxp3+ REGULATORY T CELLS

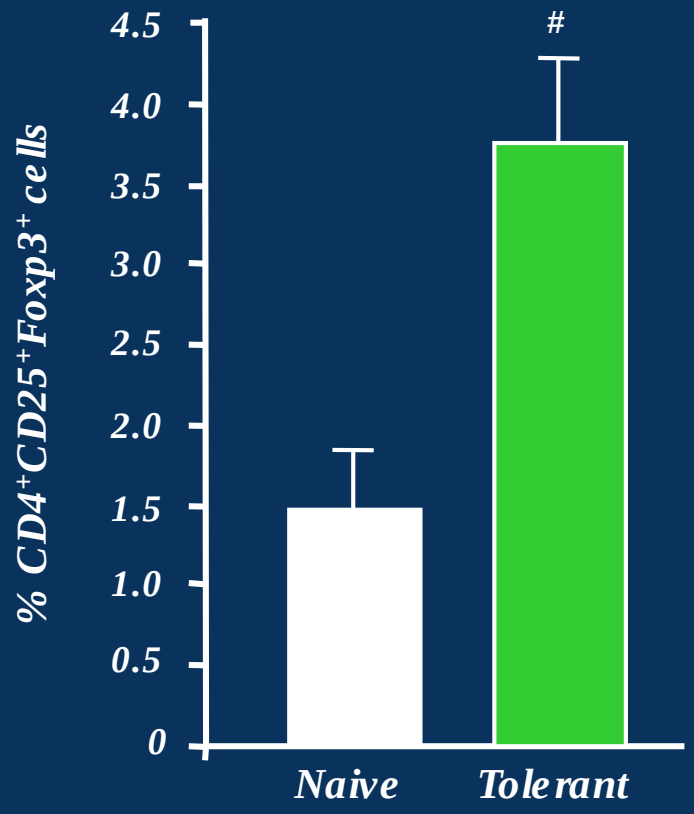
B6 MSC infusion
(0.5 x 10^6 cells)

B6 recipient mice



B6C3



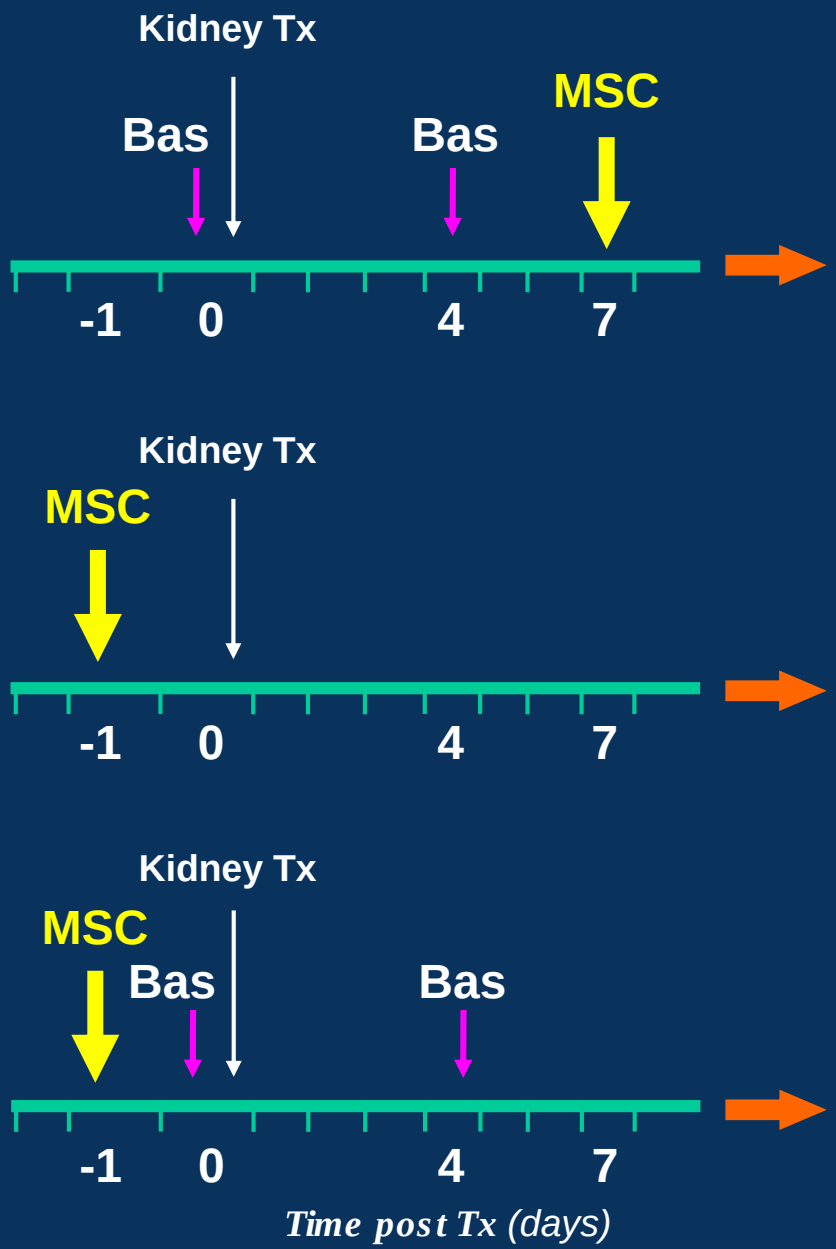


# P<0.05

Casiraghi et al, J Immunol, 2008

Timing is important

LIVING TRANSPLANT RECIPIENTS



2 *

Engraftment syndrome: YES

Acute graft rejection: NO

IL-2 dependent Treg expansion ↑

2 *

Engraftment syndrome: NO

Acute graft rejection: YES (1)

IL-2 dependent Treg expansion ↑

2 * *Ongoing*

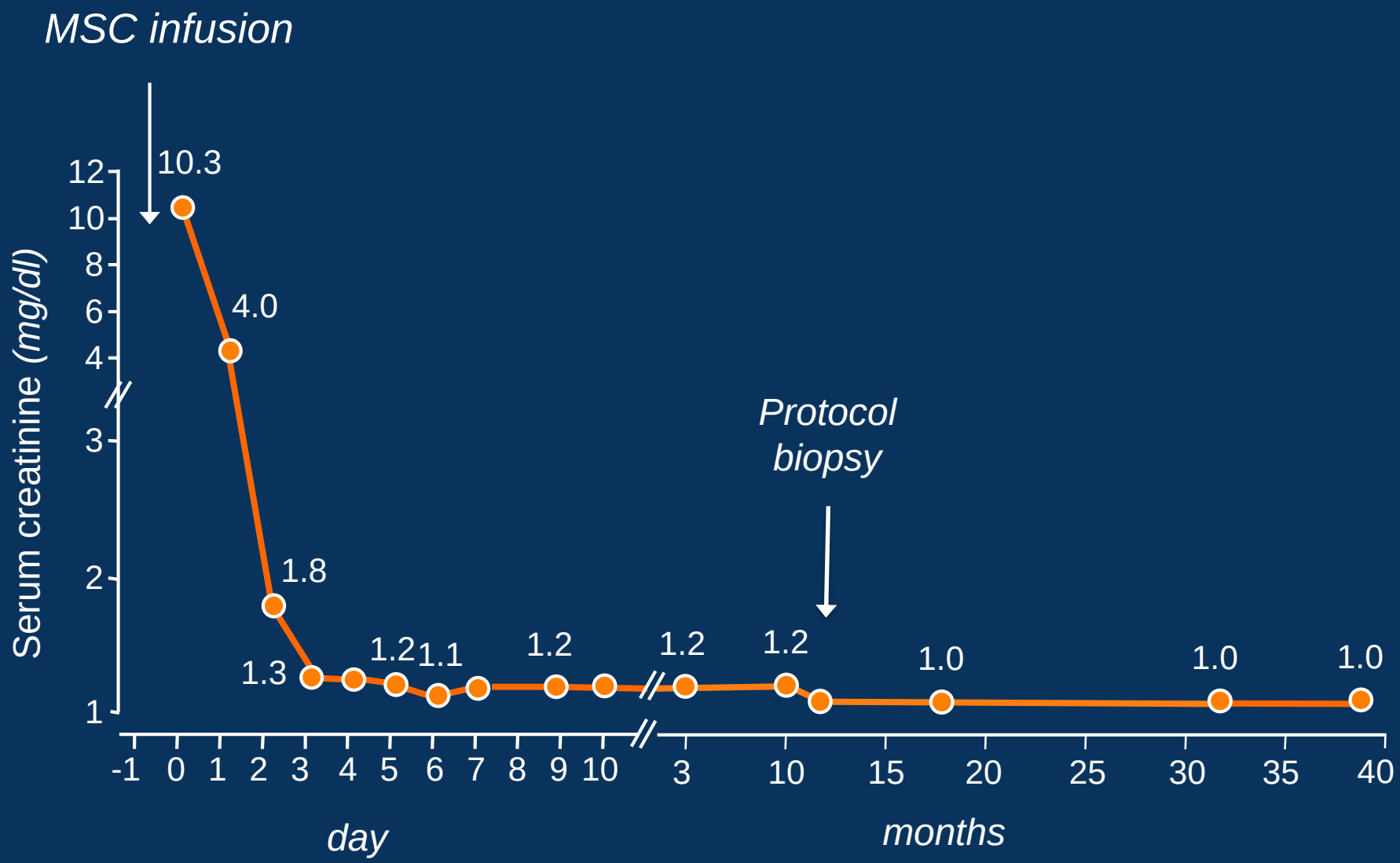
Engraftment syndrome: NO

Acute graft rejection: NO

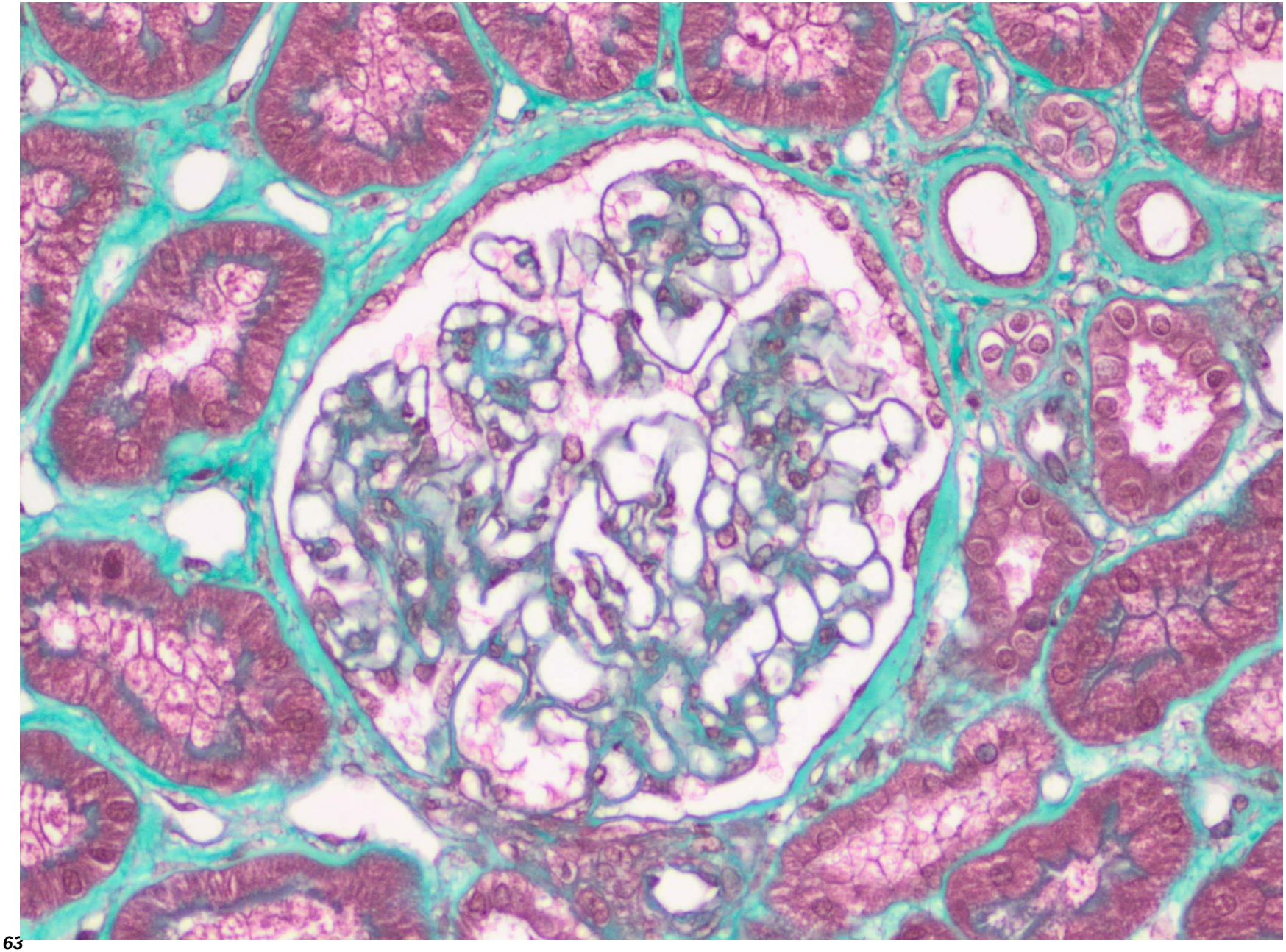
IL-2 dependent Treg expansion ?

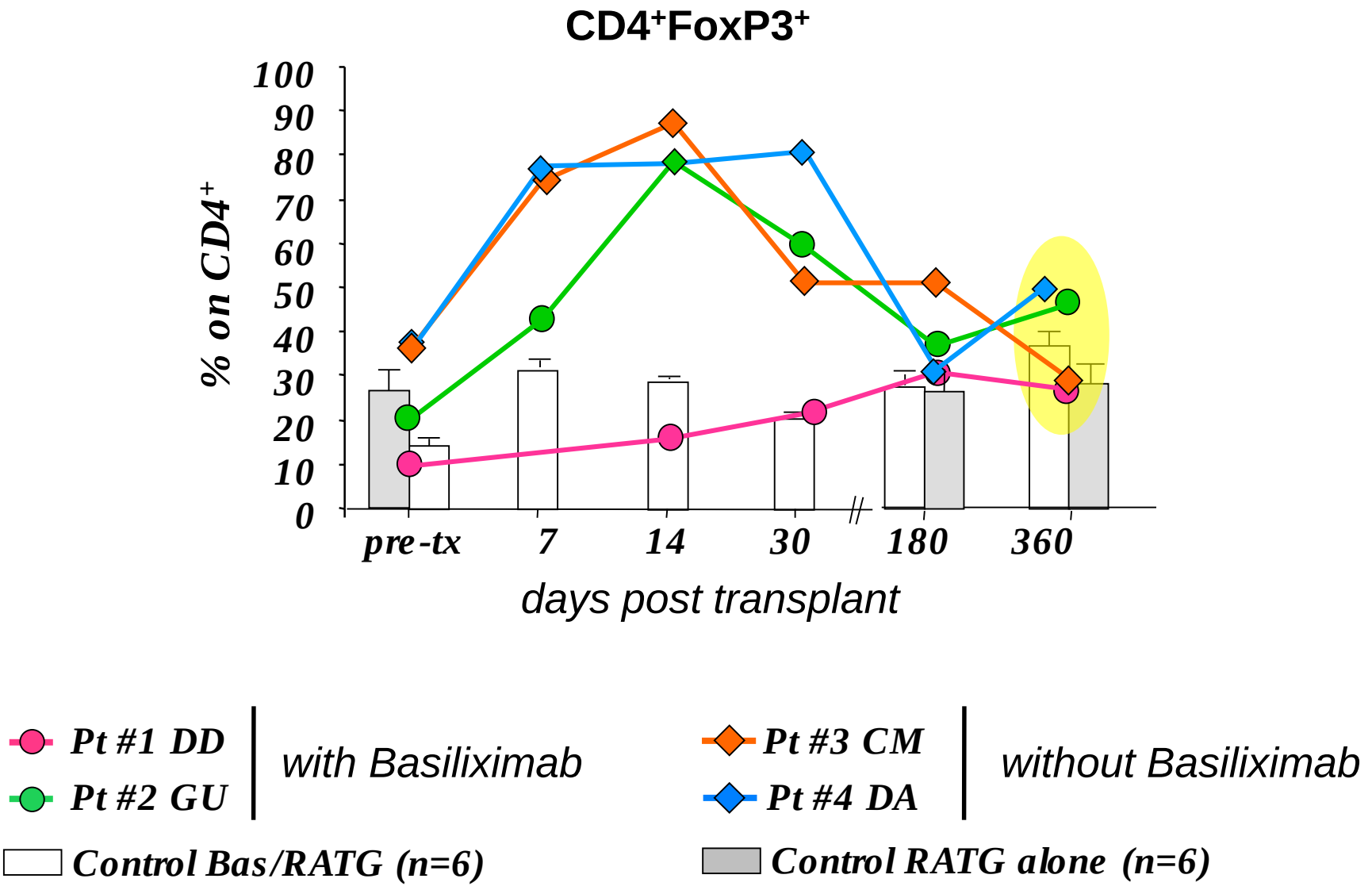
* *On top of RATG (0.5 mg/kg for 7 days)*

Patient #3 C.M.



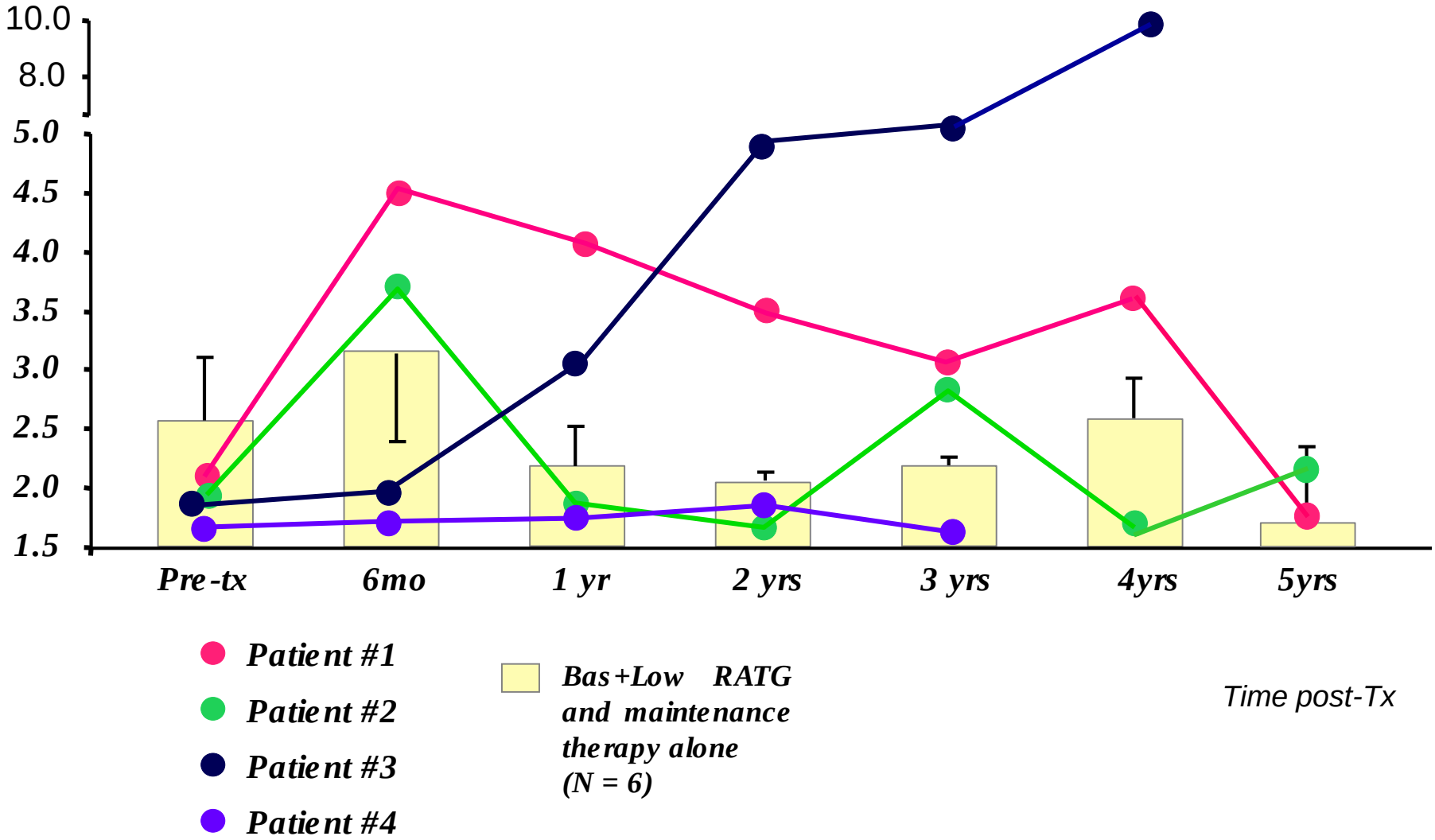
Perico, Casiraghi et al, *Transplant Int,* 2013





Perico, Casiraghi et al, Transplant Int, 2013

Treg/memory CD8^{+} T cells *(ratio)*



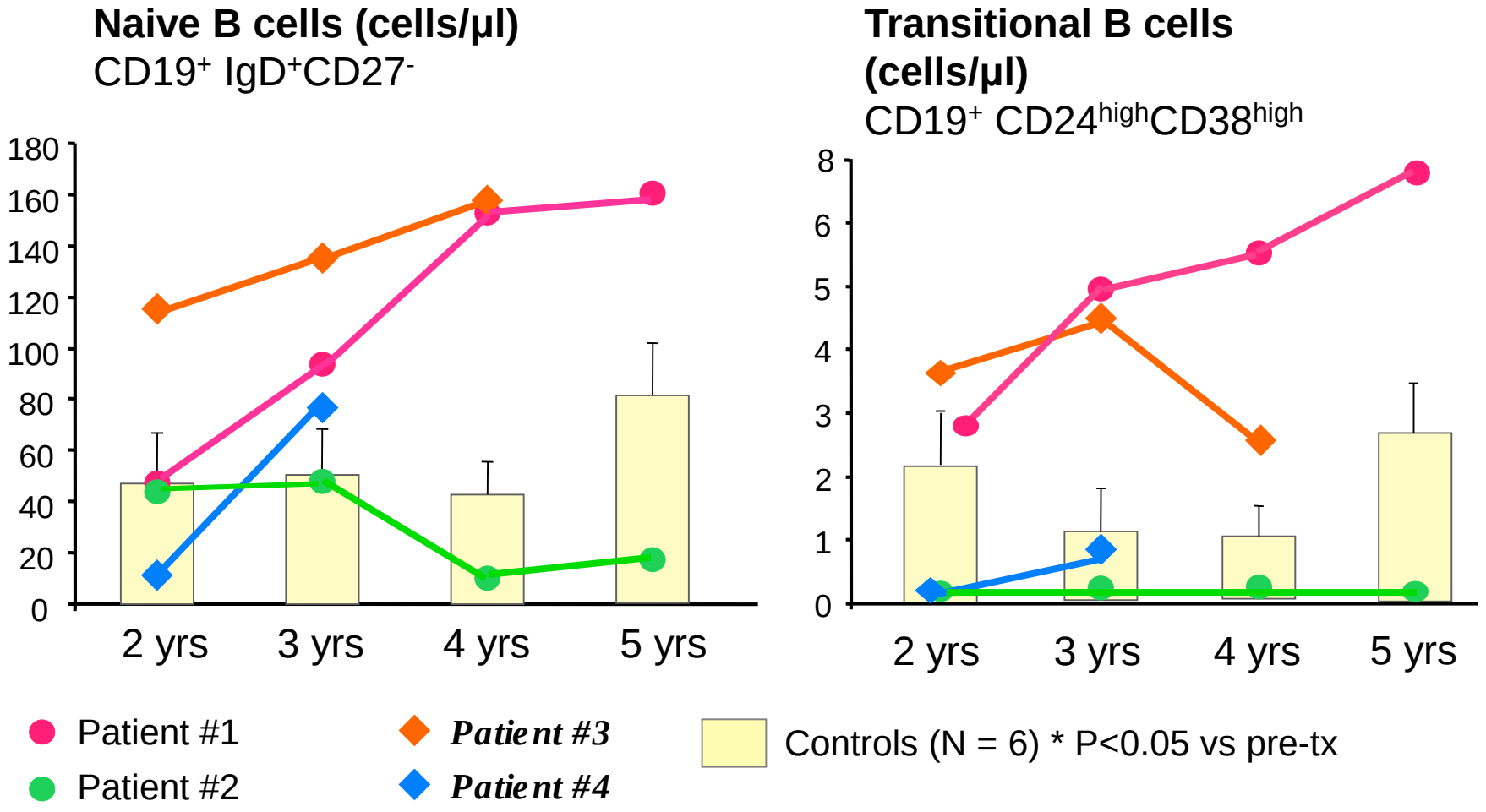
Casiraghi, Personal communication, 2015

LONG-LASTING COMPLETE AND DONOR-SPECIFIC SUPPRESSION OF CD8$^+$ T CELL CYTOTOXICITY

Cell mediated lympholysis (% of specific lysis)

Patients	pre-tx		1st yr		2nd yr		3th yr		5th yr	
	don	*third-party*	*don*	*third-party*	*don*	*third-party*	*don*	*third-party*	*don*	*third-party*
1	**3.9**	26.0	**0**	6.0	**0.3**	41.2	**0**	21.0	**0.4**	41.4
2	**4.0**	29.0	**0**	7.6	**0.5**	0	**0**	22.5	**3.0**	26.5
3	**4.6**	13.6	**0**	6.3	**0**	18.7	**0**	28.5	**1.2**	61.5
4	**2.4**	4.9	**0**	4.9	**0**	3.7	**0**	25.9		
CTR *(n=6)* Mean	**8.0**	15.5	**6.3**	10.8	**5.6**	4.3	**1.9**	13.8	**3.0**	23.9
SE	**5.5**	7.6	**3.5**	5.3	**2.0**	1.8	**1.3**	7.9	**4.6**	7.1

Casiraghi, *Personal communication,* ***2016***



Spontaneous operational tolerance to kidney allograft is associated with elevated number of naïve and transitional B cells with regulatory properties suggesting a critical role for these B cells subsets in the regulation of alloimmune response

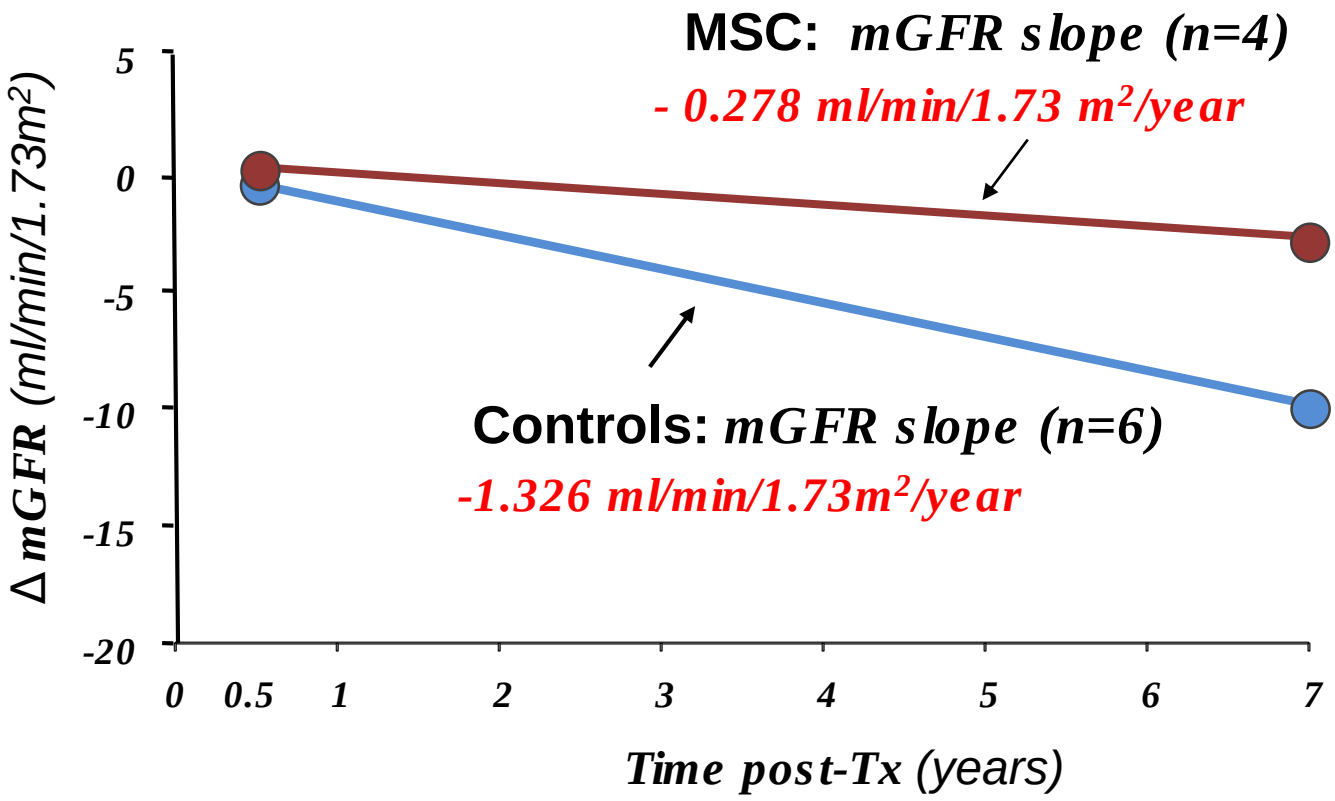
DONOR HLA-SPECIFIC ANTIBODIES

Patients	pre-tx	1st year	2nd year	3rd year	5th year
1	NEG	NEG	NEG	NEG	NEG
2	NEG	NEG	NEG	NEG	NEG
3	NEG	NEG	NEG	NEG	NEG
4	NEG	NEG	NEG	NEG	NEG
CTR *(n=6)*	NEG	NEG	5 NEG 1 POS	NEG	3 NEG 3 POS

By Luminex
Threshold for DSA positivity: Mean Fluorescence Intensity > 2000

Casiraghi,** Personal communication, **2016

RENAL GRAFT FUNCTION IN MSC-TREATED PATIENTS



Values are median of individual GFR slopes

Measured GFR by iohexol plasma clearance

Casiraghi, *Personal communication,* ***2016***

RENAL GRAFT FUNCTION IN MSC-TREATED PATIENTS

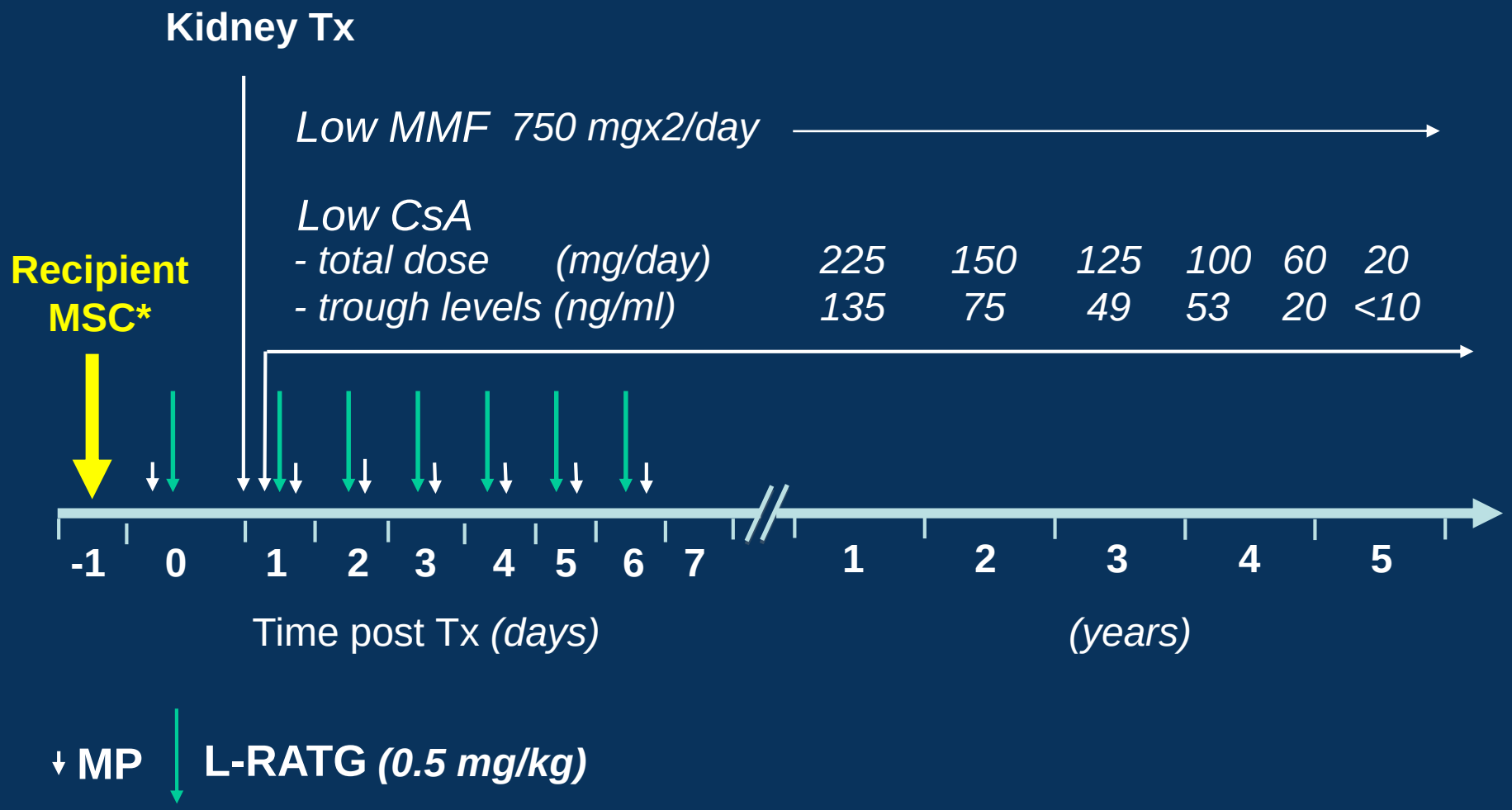
GFR slope *(ml/min/1.73 m² /year)*

Patients	Last follow-up (range 5-8 years)	
1	- 0.157	Engraftment syndrome
2	- 0.398	
3	+ 2.301	
4	- 1.442°	Acute rejection
CTR *(n=6, median)*	- 1.326	

Measured GFR by iohexol plasma clearance

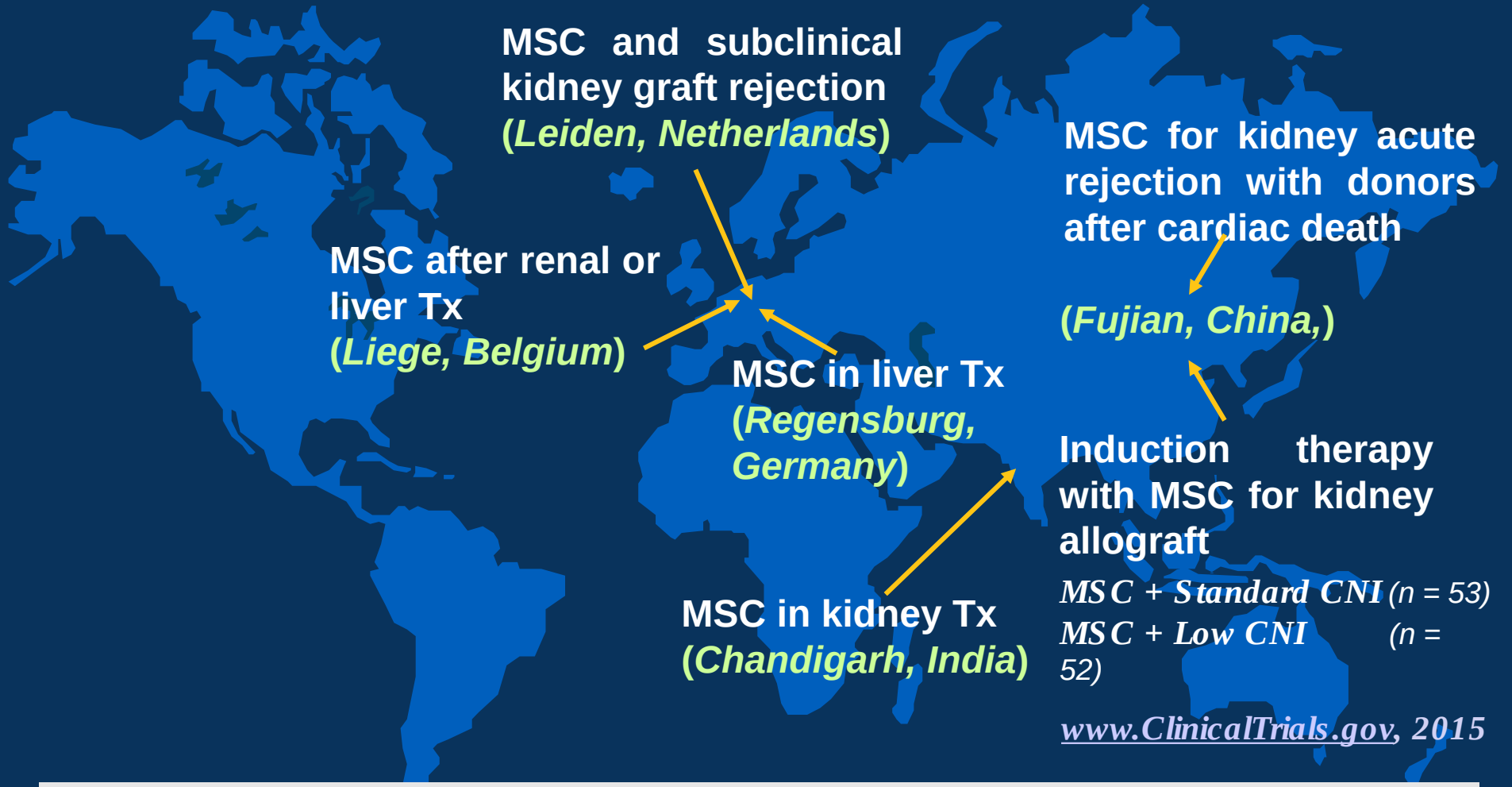
Casiraghi, *Personal communication,* ***2015***

IMMUNOSUPPRESSIVE DRUG TAPERING IN PATIENT #3 OVER 5 YEAR FOLLOW-UP



* Dose: 2 x 10^6/kg i.v.

REGISTERED CLINICAL TRIALS OF MSCs IN SOLID ORGAN TRANSPLANTATION



MSC groups n = 105
Control groups n = 51

Endpoint: acute graft rejection

Riella et al., *JAMA,* ***2012***
Casiraghi et al., *Nat Rev Nephrol,* ***2016***


AUGUST 7, 2006
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STEM CELLS
THE HOPE, THE HYPE AND WHAT IT MEANS FOR YOU
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