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?
Breakthrough of the Year
Stem Cells Show Their Potential
Mesenchymal stem/stromal cells
MESENCHYMYAL STEM CELLS ARE RENOTROPIC, HELPING TO REPAIR THE KIDNEY AND IMPROVE FUNCTION IN ACUTE RENAL FAILURE

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 (5x10^6 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
- 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

*MSCs

Imberti, Morigi et al., J Am Soc Nephrol, 2007
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
### Hematologic Malignancies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of patients</th>
<th>MSC source</th>
<th>Follow up</th>
<th>Tumor development</th>
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<tbody>
<tr>
<td>Graft-versus-Host-Disease</td>
<td>3</td>
<td>BM</td>
<td>Allogeneic</td>
<td>Not indicated</td>
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<tr>
<td>Graft-versus-Host-Disease</td>
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<td>BM</td>
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<tr>
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<tr>
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<td>18</td>
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<td>1 yr</td>
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<tr>
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<td>BM*</td>
<td>Allogeneic</td>
<td>730 d</td>
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<tr>
<td>Graft-versus-Host-Disease</td>
<td>31</td>
<td>BM*</td>
<td>Allogeneic</td>
<td>28 d</td>
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<tr>
<td>Graft-versus-Host-Disease</td>
<td>4</td>
<td>BM</td>
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<td>Not indicated</td>
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<tr>
<td>Hematopoietic stem cell Tx</td>
<td>6</td>
<td>BM</td>
<td>Allogeneic</td>
<td>4.8 yr</td>
</tr>
<tr>
<td>Umbilical Cord Blood Tx</td>
<td>15</td>
<td>BM</td>
<td>Allogeneic</td>
<td>6.8 yr</td>
</tr>
<tr>
<td>Hematopoietic stem cell Tx</td>
<td>7</td>
<td>BM</td>
<td>Allogeneic</td>
<td>29 mo</td>
</tr>
<tr>
<td>Hematopoietic stem cell Tx</td>
<td>14</td>
<td>BM</td>
<td>Allogeneic</td>
<td>28 mo</td>
</tr>
<tr>
<td>Aplastic anemia/HSC Tx</td>
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<td>BM</td>
<td>Allogeneic</td>
<td>2 yr</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>28</td>
<td>BM</td>
<td>Autologous</td>
<td>2 yr</td>
</tr>
<tr>
<td>Hematologic malignancies</td>
<td>10</td>
<td>BM</td>
<td>Allogeneic</td>
<td>3 yr</td>
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<tr>
<td>Hematologic malignancies</td>
<td>46</td>
<td>BM</td>
<td>Allogeneic</td>
<td>688 d</td>
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<tr>
<td>Hematologic malignancies</td>
<td>15</td>
<td>BM</td>
<td>Autologous</td>
<td>Not indicated</td>
</tr>
</tbody>
</table>
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

*\( p < 0.01 \text{ vs saline} \)

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-MS Cs

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
PARACRINE ACTION OF MSC IN KIDNEY REPAIR

- Oxidative stress
- Hypoxia
- Inflammation
- Apoptosis/necrosis
- Fibrosis/scarring

- Growth factors
- pAKT
- Proliferation
- Vascular protection
- Mitochondrial homeostasis
- Tissue regeneration

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

BUN (mg/dl)

Control
Cisplatin + saline
Cisplatin + RPCs

Imberti et al., Scientific Report, 2015
## iPS CELLS PROGRESSING TO THE CLINIC

<table>
<thead>
<tr>
<th>Condition</th>
<th>Stem Cells</th>
<th>Differentiated Cells</th>
<th>Clinical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-related macular degeneration</td>
<td>iPS Cs/ESC</td>
<td>Retinal pigment epithelium</td>
<td>Clinical Phase I-II</td>
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<tr>
<td>Parkinson disease</td>
<td>iPS Cs/ESC</td>
<td>A9 dopaminergic neuron</td>
<td>Clinical Phase I</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>iPS Cs/ESC</td>
<td>Oligodendrocyte Progenitor</td>
<td>Clinical Phase I</td>
</tr>
<tr>
<td>Diabetes</td>
<td>iPS Cs/ESC</td>
<td>Pancreatic islet β-cell progenitor</td>
<td>Clinical Phase I-II</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>iPS Cs/ESC</td>
<td>Cardiomyocytes</td>
<td>Clinical Phase I</td>
</tr>
</tbody>
</table>

*Trounson et al., Nature Review 2016*
Significant demographic changes in the transition between the 20th and 21st centuries
CHRONIC KIDNEY DISEASE: AN IMPORTANT CONTRIBUTOR TO THE NCD BURDEN

CKD attributable deaths - 2015

1,234,900

- 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

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.
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

Control
ADR + saline
ADR + ucMSCs
ADR + CM
ADR + bmMSCs

ADR + ucMSCs
ADR + kMSCs*
ADR + CM° - ucMSCs

* 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
\[ \Delta \text{GFR} = -0.44 \pm 0.54 \]

\[ \Delta \text{GFR} = -0.10 \pm 0.50 \]

\[ \Delta \text{GFR} = -0.81 \pm 1.12 \]

\[ \Delta \text{GFR} = -0.14 \pm 0.87 \]

Ruggenenti et al., Lancet, 1998
REGRESSION

10 patients with increasing GFR

\[ \Delta GFR \text{ (ml/min/month)} = -0.21 \pm 0.09 \text{ to } +0.49 \pm 0.19 \]

\[ P = 0.01 \]

Change in proteinuria (post vs pre break point)

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

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

**Extra renal cells**

- Endothelial progenitor and/or bone marrow-derived stem
Bowman’s capsule at SEM
NCAM* co-expresses progenitor cell marker CD24

*Neural Cell Adhesion Molecule: a protein expressed in metanephric mesenchyme
NCAM+ 76 %

NCAM+ WT1+ 12 %

WT1+ 12 %

Claudin1+ 100 %

Progenitor cells  Transitional cells  Podocytes

Benigni et al., Am J Pathol, 2011
Control  MWF Untreated  MWF ACEi-treated

NCAM

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.
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.

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
Gjertson et al., 1992

% 1-Year Graft Survival

Year of Transplant

Acute rejection (%)

- 60 CsA 1982
- 40-45 CsA + ST 1983
- 45 FK + ST 1991
- 40 RAPA + ST + AZA 1995
- 15 CsA + ST + MMF 1995
- 10 FK + ST + MMF 1996
- 10 CsA + ST + RAPA 1996

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)

**Mycophenolate**  
(specific suppressor of T and B lymphocytes)

**Daclizumab**  
(humanized monoclonal antibody against IL-2 R)

**Sirolimus**  
(m-TOR T cell proliferation inhibitor)

**Everolimus**  
(m-TOR T cell proliferation inhibitor)

- **Kidney Tx**  
  - Nashan et al., *Lancet*  
  - Vincenti et al., *N Engl J Med*  
  - Calne et al., *Lancet*

- **Heart Tx**  
  - Eisen et al., *N Engl J Med*  

- **Kidney Tx**  
  - Kahan et al., *Lancet*  

- **Kidney Tx**  
  - Vincenti et al., *N Engl J Med*  

- **Kidney Tx**  
  - *Lancet*
Activated CD8+ memory

IFN-γ

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

<table>
<thead>
<tr>
<th>Memory T cell response</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MMF</td>
</tr>
<tr>
<td></td>
<td>Basiliximab</td>
</tr>
<tr>
<td></td>
<td>Sirolimus, everolimus</td>
</tr>
<tr>
<td></td>
<td>Alemtuzumab*</td>
</tr>
</tbody>
</table>

*Campath-1

*Inhibition (partial)  
*Stimulation
Belatacept

1,420 - 11,300 euro per month depending on treatment phase
• **CD8^+CD28^- T cells harbor effector-memory phenotype**

![Diagram of CD8^+CD28^- T cells](image)

Belatacept resistant cell activation

- TNFα
- IFNγ

CD8^+CD28^- T cells cell proliferation

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

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

MSC

- NK cells
  - Cytotoxicity
  - Proliferation

- B cells
  - Cell activation
  - Ig release

- T cells
  - Proliferation
  - Cell activation

- DC
  - Differentiation
  - Maturation

- Memory T cells
  - Inhibition

- T reg
  - Expansion

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

Recipient MSC

Donor MSC

no cell infusion

% surviving allografts

days after transplant

% CD4+CD25+Foxp3+ cells

Casiraghi et al, J Immunol, 2008
Timing is important
LIVING TRANSPLANT RECIPIENTS

Engraftment syndrome: YES
Acute graft rejection: NO
IL-2 dependent Treg expansion

Engraftment syndrome: NO
Acute graft rejection: YES (1)
IL-2 dependent Treg expansion

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.

MSC infusion

Serum creatinine (mg/dl)

-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

day

months

10.3 4.0 1.8 1.3 1.2 1.2 1.2 1.2 1.2 1.0 1.0 1.0 1.0

Protocol biopsy

Perico, Casiraghi et al, **Transplant Int**, 2013
CD4+FoxP3+

% on CD4+

pre-tx | 7 | 14 | 30 | 180 | 360

Pt #1 DD | Pt #2 GU | with Basiliximab | Pt #3 CM | Pt #4 DA | without Basiliximab

Control Bas/RATG (n=6) | Control RATG alone (n=6)

Perico, Casiraghi et al, Transplant Int, 2013
Patient #1
Patient #2
Patient #3
Patient #4

Casiraghi, Personal communication, 2015
LONG-LASTING COMPLETE AND DONOR-SPECIFIC SUPPRESSION OF CD8+ T CELL CYTOTOXICITY

Cell mediated lympholysis (% of specific lysis)

<table>
<thead>
<tr>
<th>Patients</th>
<th>pre-tx</th>
<th>1st yr</th>
<th>2nd yr</th>
<th>3rd yr</th>
<th>5th yr</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>don third-party</td>
<td>don third-party</td>
<td>don third-party</td>
<td>don third-party</td>
<td>don third-party</td>
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<tr>
<td>1</td>
<td>3.9 26.0</td>
<td>0 6.0</td>
<td>0.3 41.2</td>
<td>0 21.0</td>
<td>0.4 41.4</td>
</tr>
<tr>
<td>2</td>
<td>4.0 29.0</td>
<td>0 7.6</td>
<td>0.5 0</td>
<td>0 22.5</td>
<td>3.0 26.5</td>
</tr>
<tr>
<td>3</td>
<td>4.6 13.6</td>
<td>0 6.3</td>
<td>0 18.7</td>
<td>0 28.5</td>
<td>1.2 61.5</td>
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<tr>
<td>4</td>
<td>2.4 4.9</td>
<td>0 4.9</td>
<td>0 3.7</td>
<td>0 25.9</td>
<td></td>
</tr>
</tbody>
</table>

CTR Mean (n=6) SE
8.0 15.5 6.3 10.8 5.6 4.3 1.9 13.8 3.0 23.9
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

<table>
<thead>
<tr>
<th>Patients</th>
<th>pre-tx</th>
<th>1(^{st}) year</th>
<th>2(^{nd}) year</th>
<th>3(^{rd}) year</th>
<th>5(^{th}) year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
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<tr>
<td>2</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
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<tr>
<td>3</td>
<td>NEG</td>
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<tr>
<td>4</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>CTR (n=6)</td>
<td>NEG</td>
<td>NEG</td>
<td>5 NEG</td>
<td>1 POS</td>
<td>3 NEG</td>
</tr>
</tbody>
</table>

By Luminex

Threshold for DSA positivity: Mean Fluorescence Intensity > 2000
RENAL GRAFT FUNCTION IN MSC-TREATED PATIENTS

**MSC:** mGFR slope (n=4)
- $0.278 \text{ ml/min/1.73 m}^2/\text{year}$

**Controls:** mGFR slope (n=6)
- $1.326 \text{ ml/min/1.73 m}^2/\text{year}$

Values are median of individual GFR slopes

Measured GFR by iohexol plasma clearance
# RENAL GRAFT FUNCTION IN MSC-TREATED PATIENTS

**GFR slope (ml/min/1.73 m²/year)**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Last follow-up (range 5-8 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>- 0.157</td>
</tr>
<tr>
<td>2</td>
<td>- 0.398</td>
</tr>
<tr>
<td><strong>3</strong></td>
<td><strong>+ 2.301</strong></td>
</tr>
<tr>
<td>4</td>
<td>- 1.442°</td>
</tr>
</tbody>
</table>

**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**

**Kidney Tx**

*Low MMF 750 mg x 2/day*

*Low CsA*
- total dose (mg/day) 225 150 125 100 60 20 20
- trough levels (ng/ml) 135 75 49 53 20 <10

**Recipient MSC**

↓ MP  ↓ L-RATG *(0.5 mg/kg)*

*Dose: 2 x 10^6/kg i.v.*
REGISTERED CLINICAL TRIALS OF MSCs IN SOLID ORGAN TRANSPLANTATION

MSC and subclinical kidney graft rejection (*Leiden, Netherlands*)

MSC after renal or liver Tx (*Liege, Belgium*)

MSC in liver Tx (*Regensburg, Germany*)

MSC in kidney Tx (*Chandigarh, India*)

MSC for kidney acute rejection with donors after cardiac death (*Fujian, China*)

Induction therapy with MSC for kidney allograft

- MSC + Standard CNI (*n* = 53)
- MSC + Low CNI (*n* = 52)

Endpoint: acute graft rejection

**MSC groups** *n* = 105
**Control groups** *n* = 51

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

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IRCCS- Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

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