ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI IRCCS

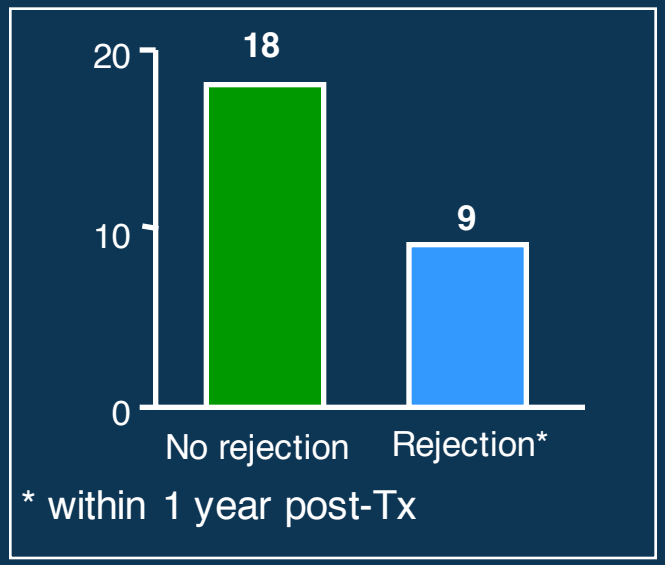
E' possibile creare un ambiente tollerogenico dopo il trapianto d'organo utilizzando cellule staminali come se fossero farmaci?

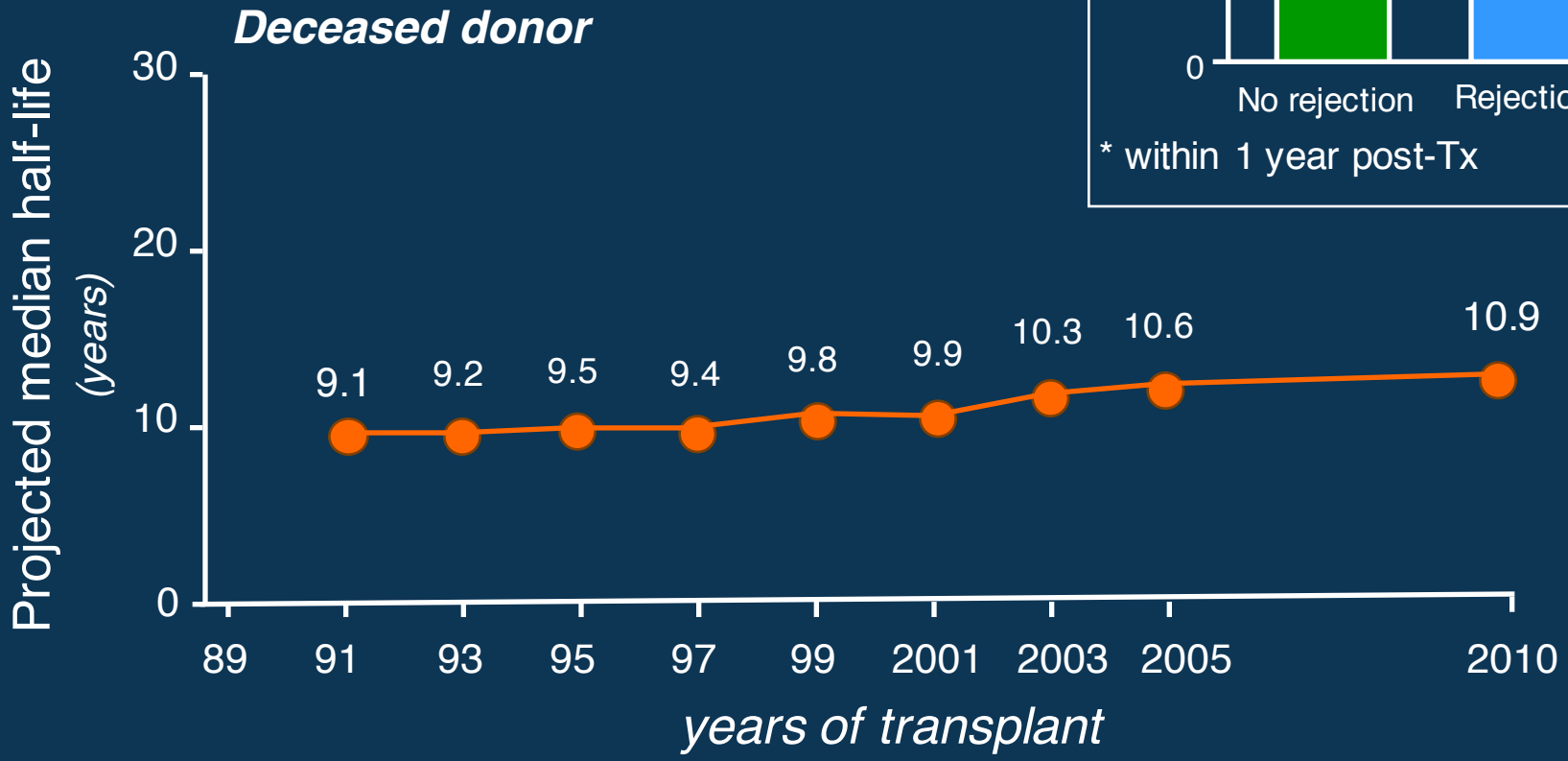
Giuseppe Remuzzi

Infections & Transplantation

Varese, 18 maggio 2017

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)

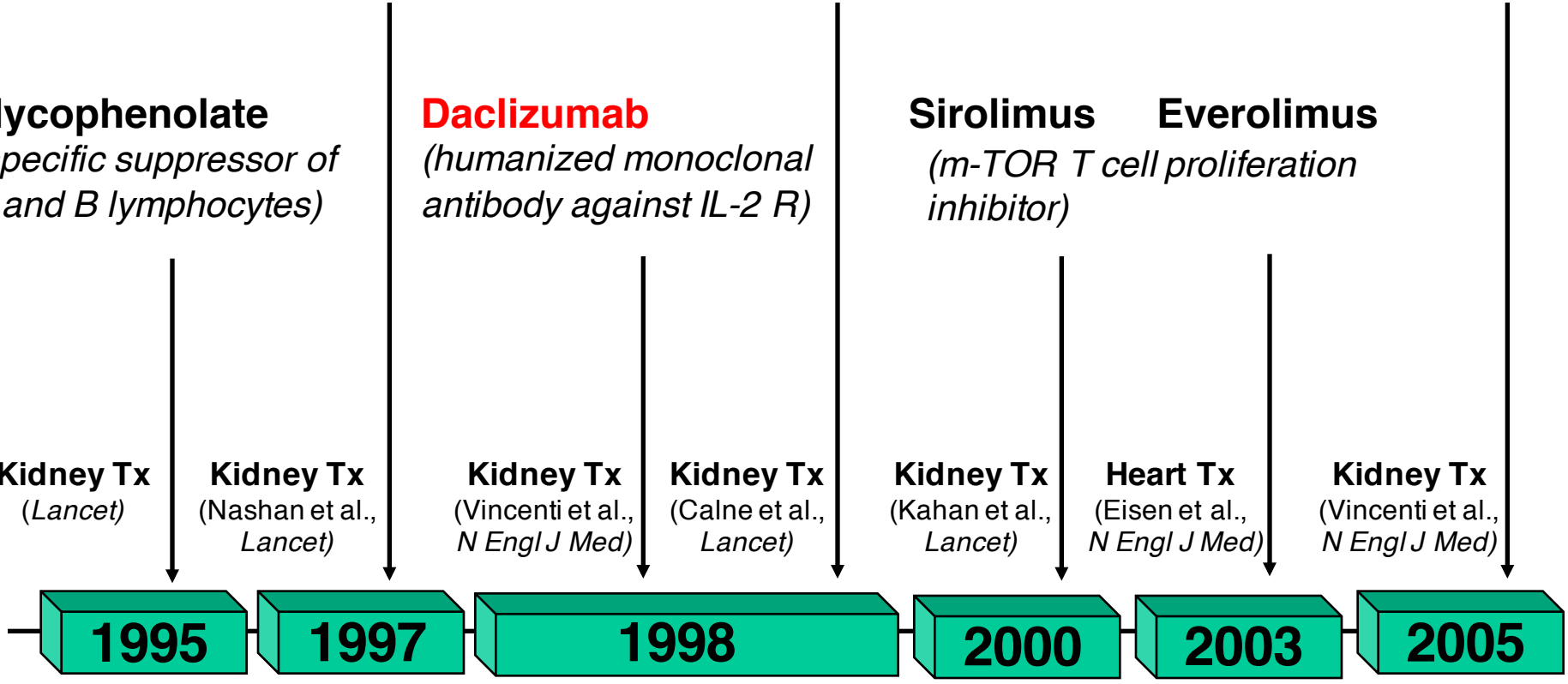
CAMPATH-1H
(humanized anti-CD52 antibody - T and B cells depletion)

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

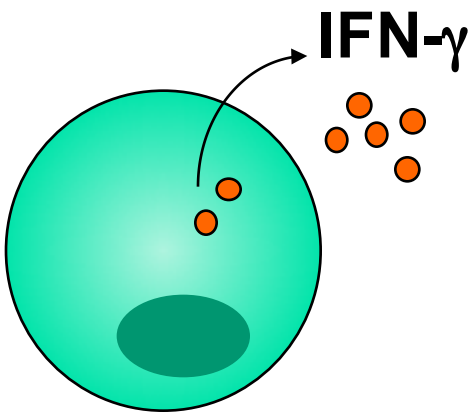
Mycophenolate
(specific suppressor of T and B lymphocytes)

Daclizumab
(humanized monoclonal antibody against IL-2 R)

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



THE SPECIAL PROBLEM OF MEMORY



Activated CD8⁺ memory

	IFN-γ blockade (in vitro)
- Calcineurin inhibitors	*partial*
- Hydrocortisone	*partial*
- Azathioprine	*no*
- MMF	*partial*
- Basiliximab	*partial*
- Sirolimus, everolimus	*stimulation*
- Alemtuzumab*	*stimulation*

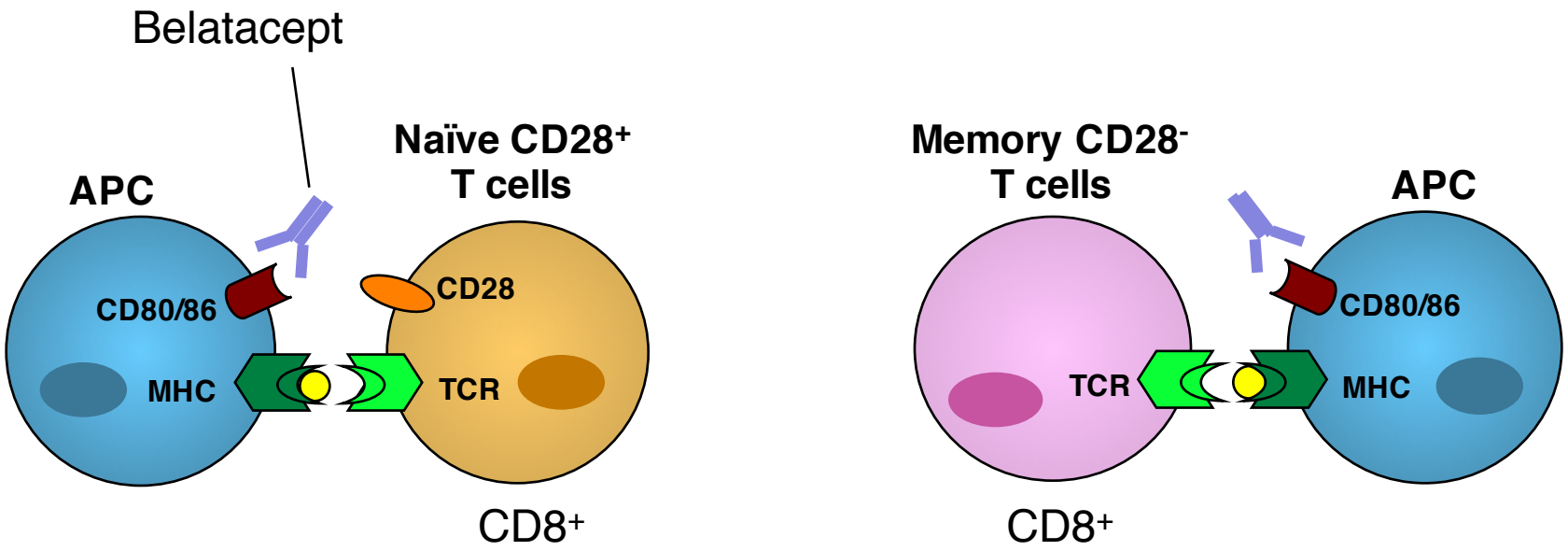
* Campath-1

Jones et al., *Transplantation,* 2006
Page et al., *Curr OpOrgan Transplant,* 2009

Memory T cells contribute to allograft rejection through:

- *Activation endothelial cells*
- *Help naïve CD8, CD4 T cells and B cells*

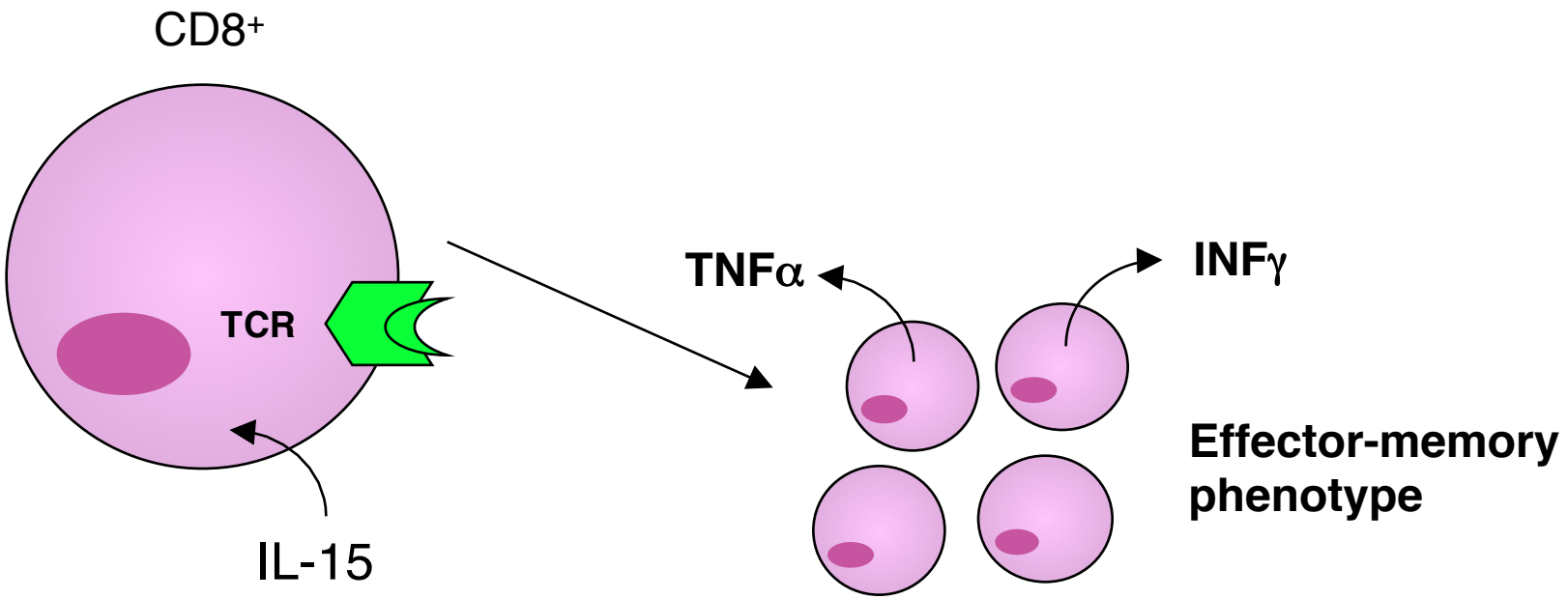
COSTIMULATON BLOCKADE-RESISTANT REJECTION



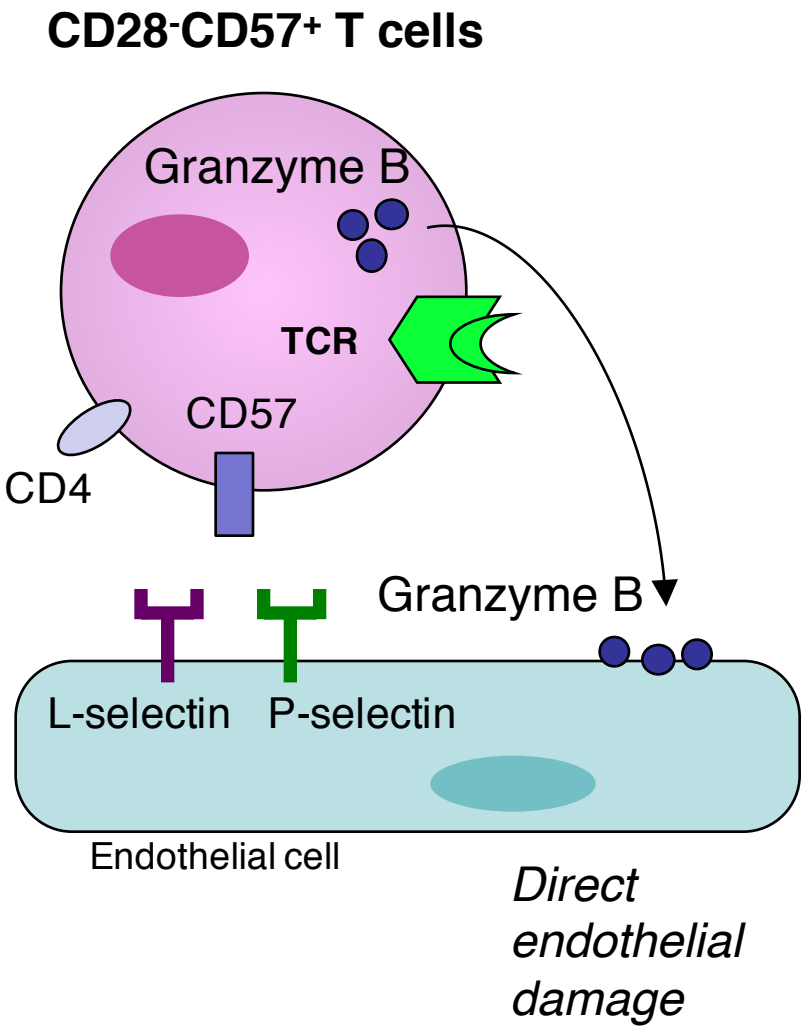
Antigen-experienced T cells, in particular CD8+Tcells, lose CD28 expression and became memory T cells with increased capability of mounting a rapid response upon antigen rechallenge independent of CD80/86-CD28 costimulation

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

BELATACEPT-RESISTANT REJECTION - CD28^{-} CD8^{+}T CELLS

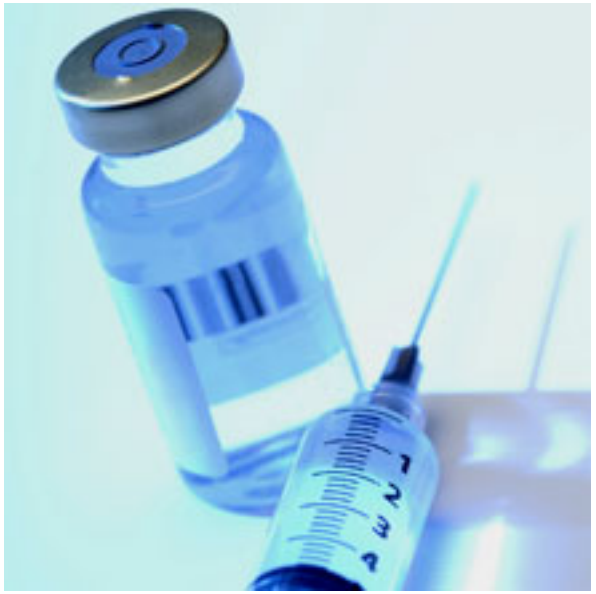


BELATACEPT-RESISTANT REJECTION - CD57+CD4+ T CELLS



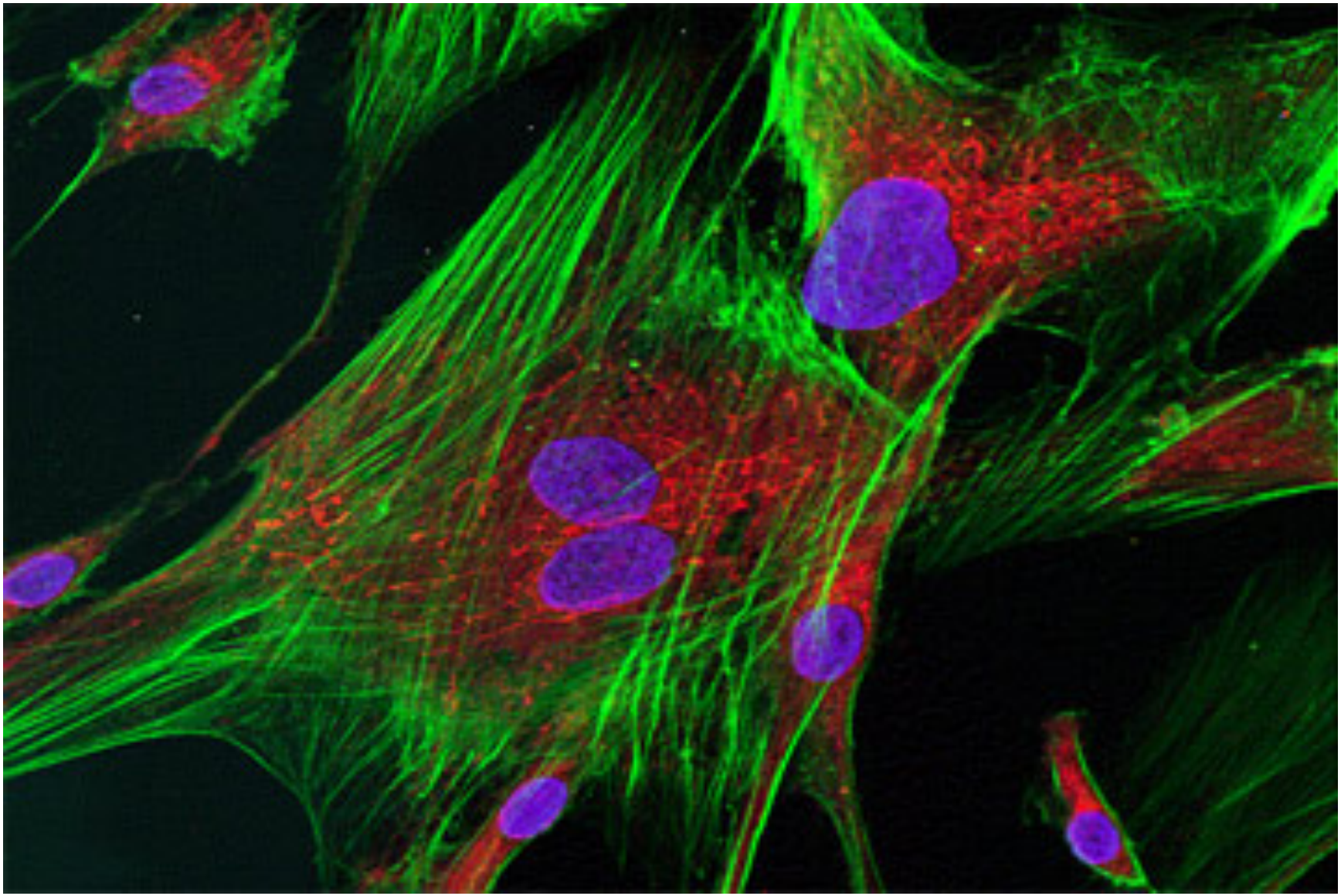
- Infiltrate the allograft during rejection
- May carry allograft-specific memory by antigen cross-reactivity
- This cell subset pre-transplant correlates with costimulation blockade-resistant rejection post-transplant

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

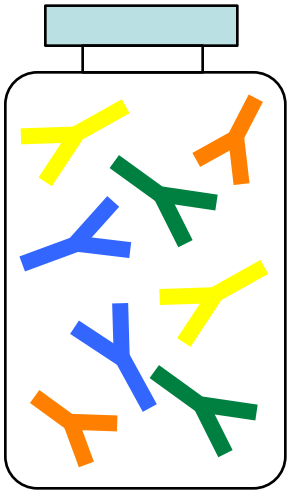


Belatacept

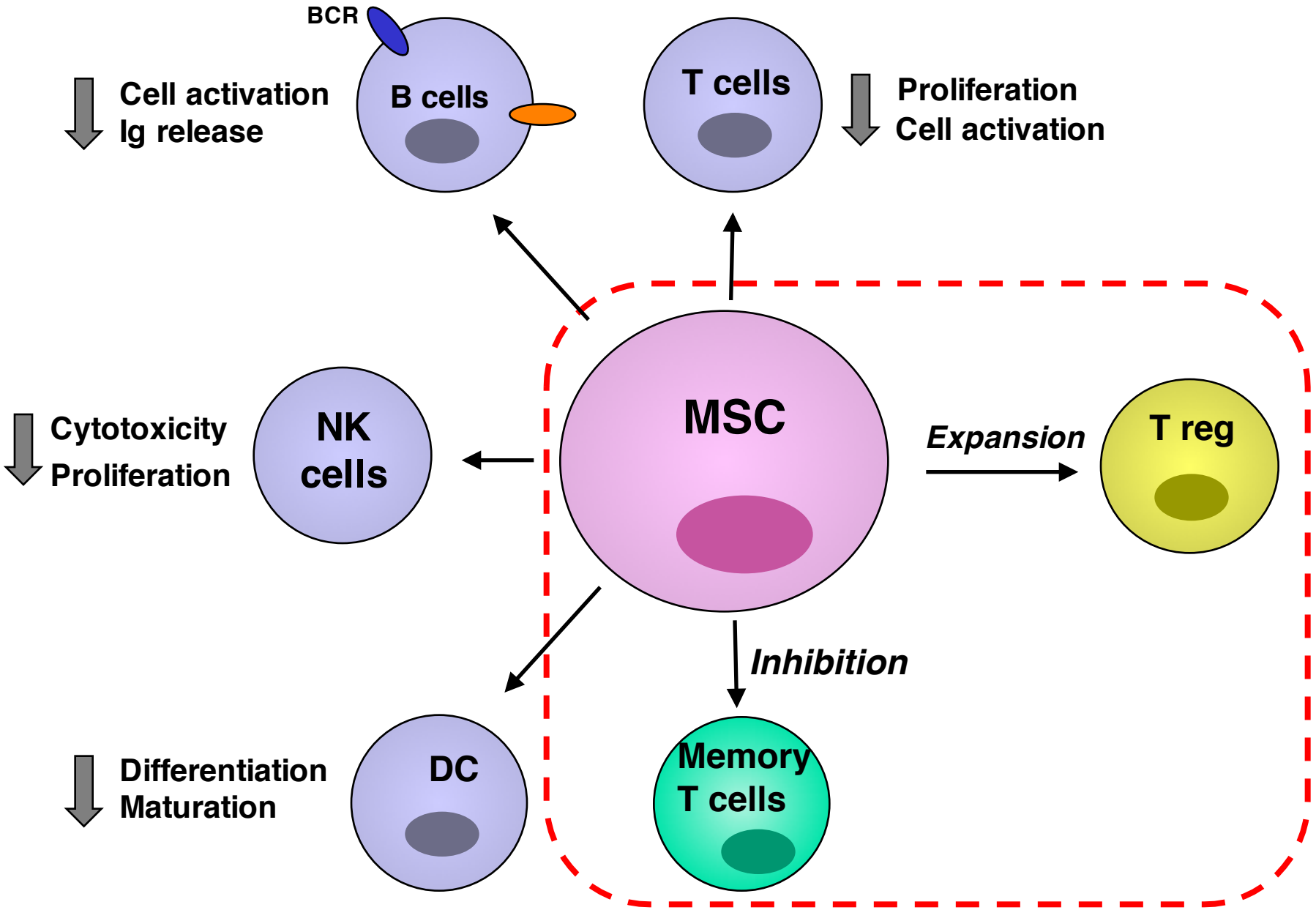
1,420 -11,300 euro per month depending on treatment phase



Casiraghi et al, Curr Opin Organ Transpl, 2010



Primi anni ‘50

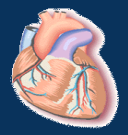


Nauta et al, *Blood*, 2007

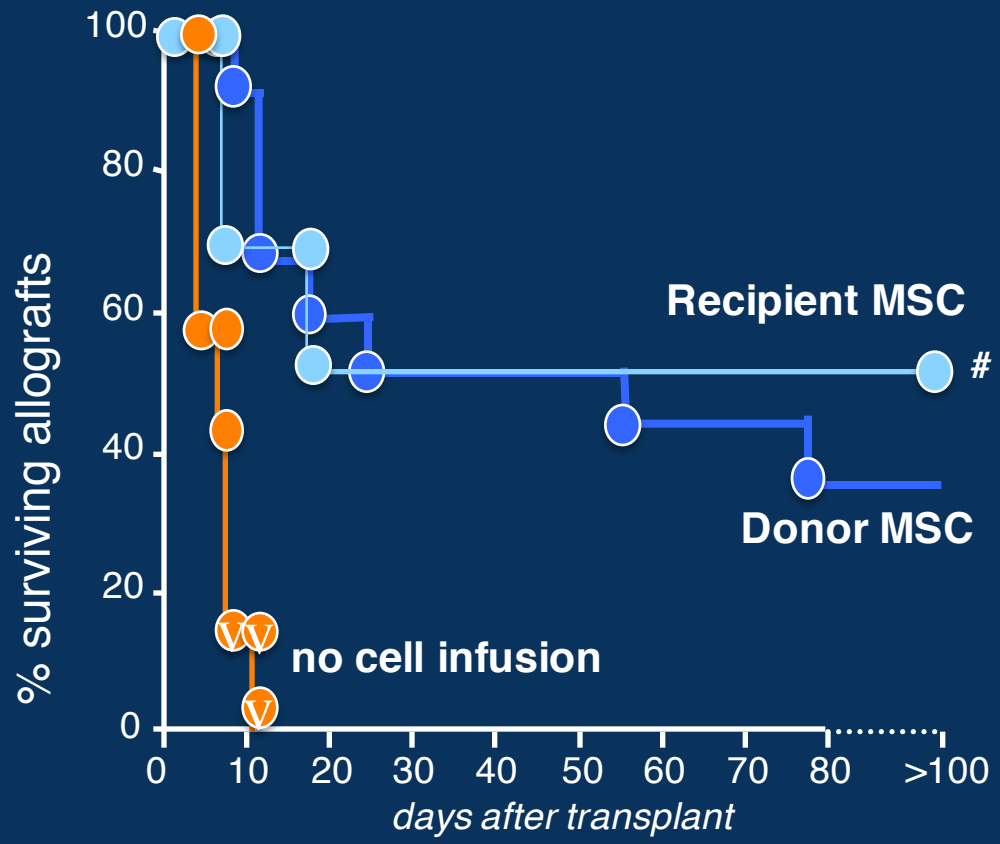
AUTOLOGOUS MSC PROLONG HEART TRANSPLANT SURVIVAL MEDIATED BY CD4+CD25+Foxp3+ REGULATORY T CELLS

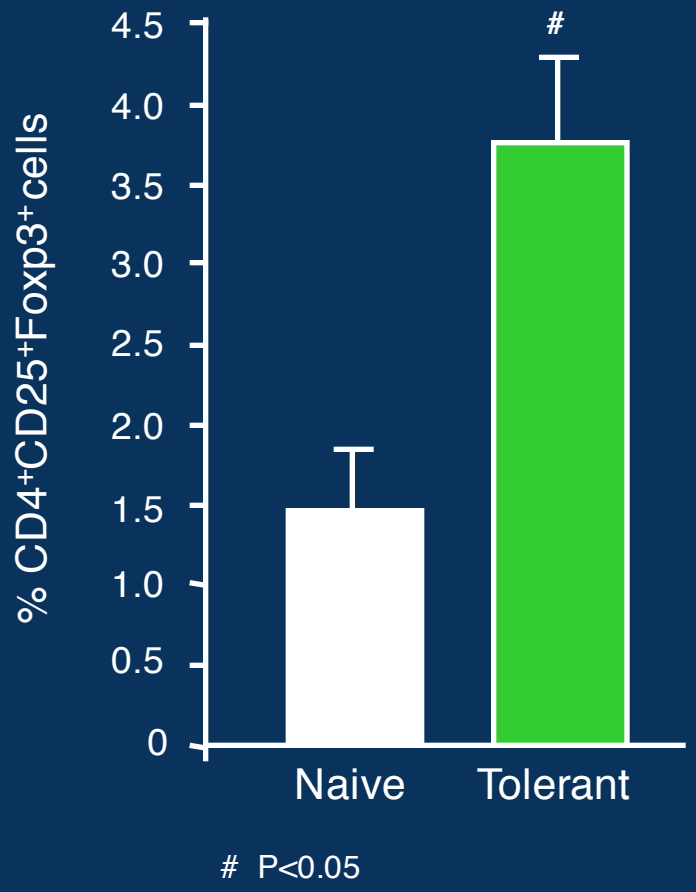
B6 MSC infusion
(0.5 x 10^6 cells)

B6 recipient mice



B6C3





# P<0.05

Casiraghi et al, *J Immunol*, 2008

AUTOLOGOUS BONE MARROW-DERIVED MSC TO INDUCE TOLERANCE IN LIVING-DONOR KIDNEY TRANSPLANT RECIPIENTS

Bergamo

- IRCCS, Mario Negri Institute
  (coordinator/immunomonitoring)

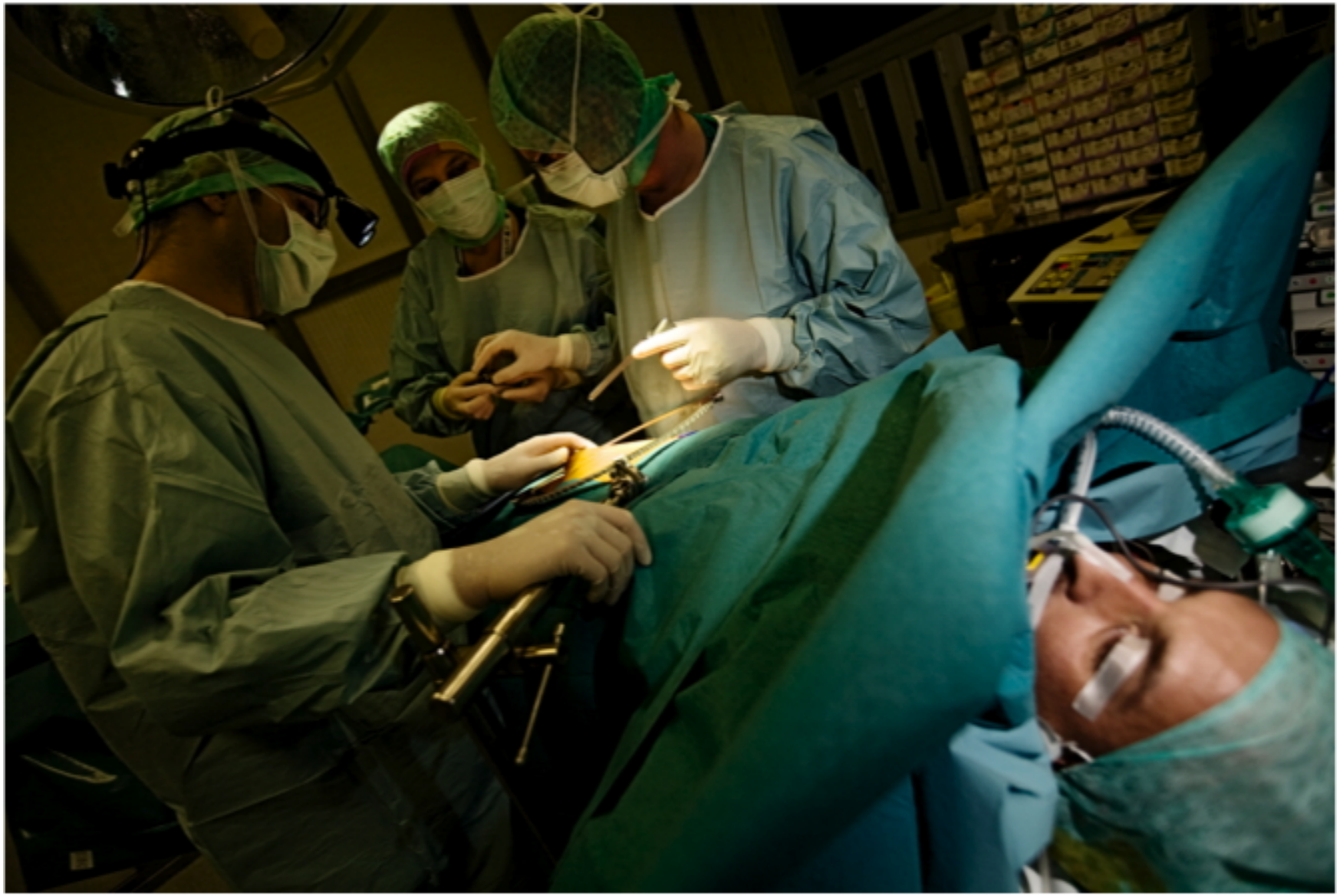
- Azienda Ospedaliera Papa Giovanni XXIII

U.S.C. Chirurgia pediatrica
Kidney Tx

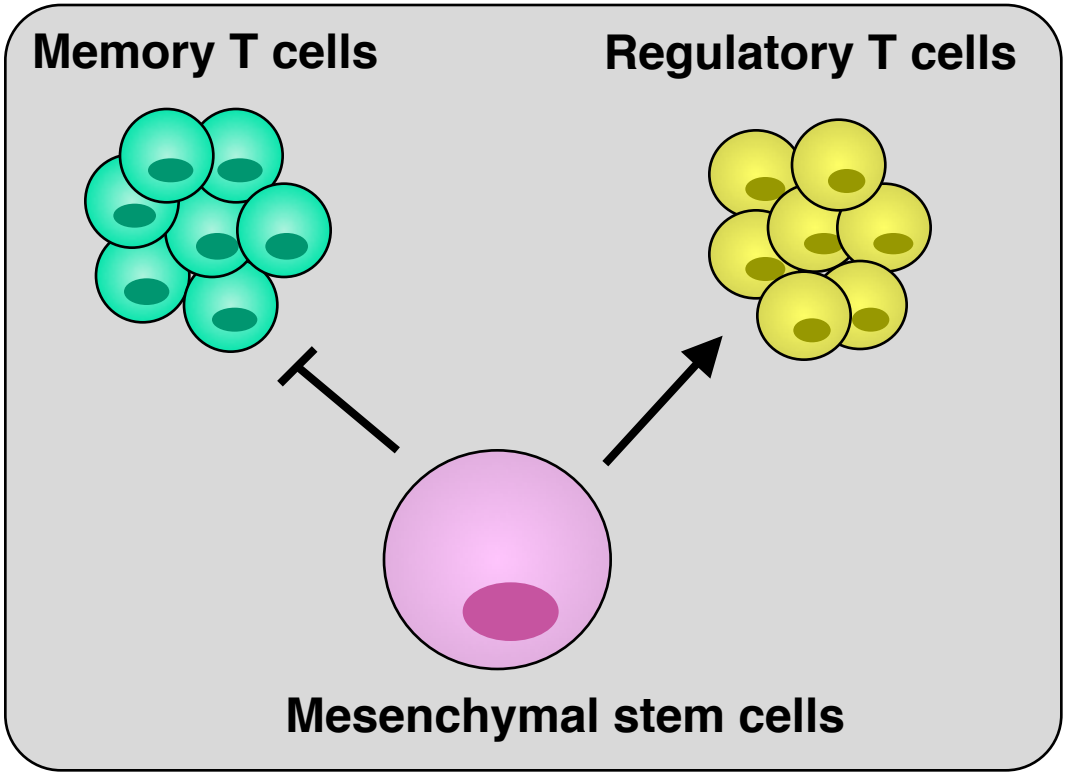
U.S.C. Ematologia
Bone marrow explants

Laboratorio Terapie Cellulari "G. Lanzani"
MSC preparation according to European GMP

U.S.C. Nefrologia
pre- and post-transplant patient monitoring and follow-up



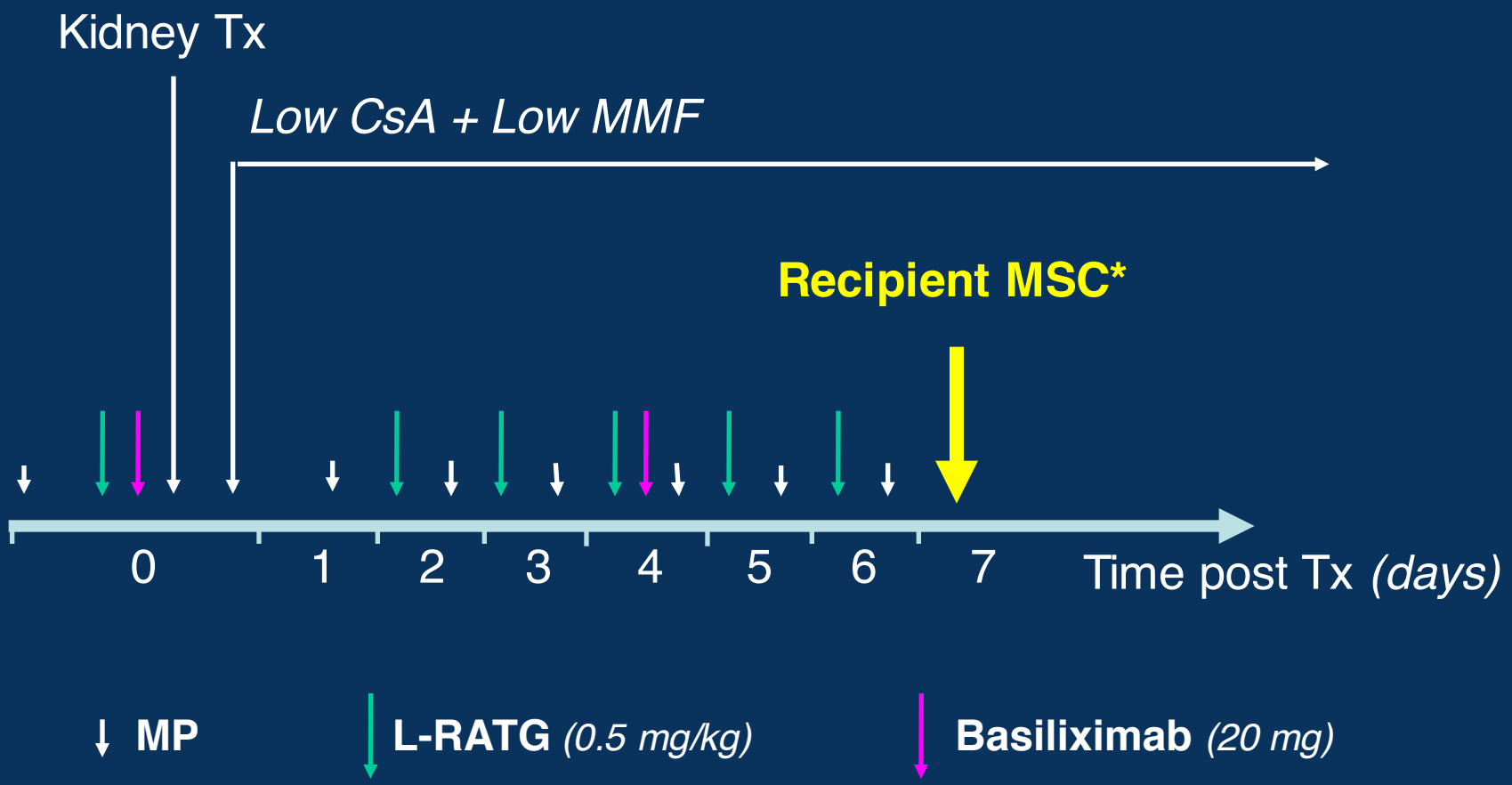
Timing is important



Timing of MSC infusion?

7 days post transplant coincides with the start of homeostatic proliferation induced by lymphopenia

Phase a: 2 patients

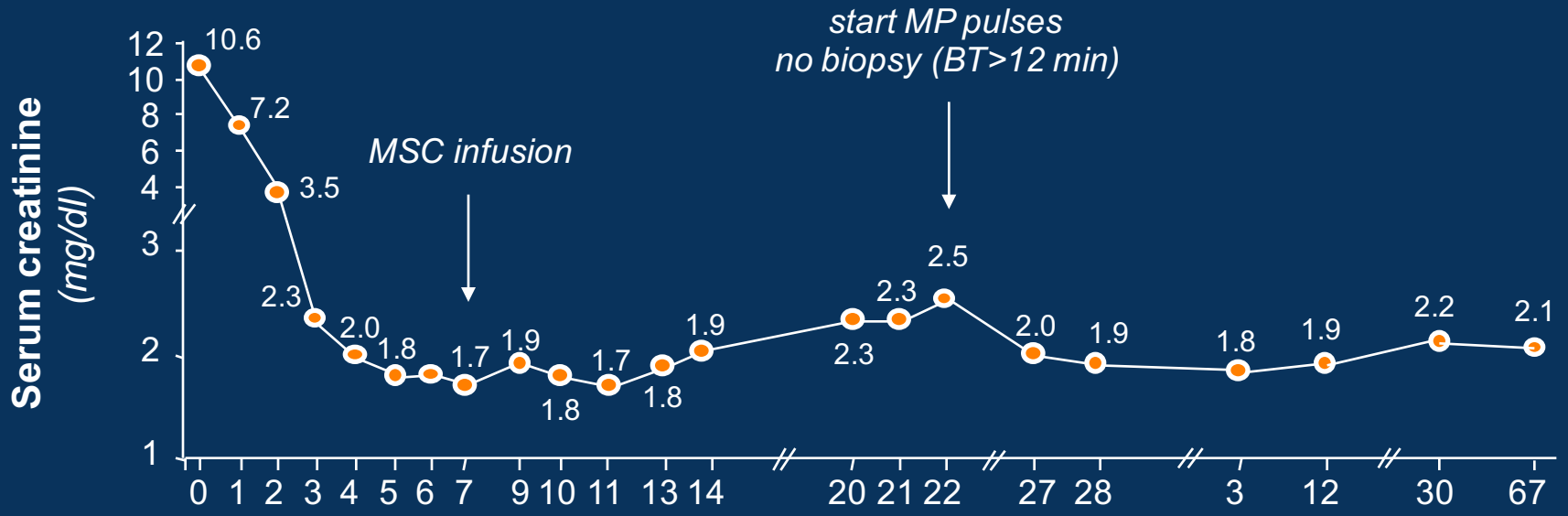


MSC in-vitro expanded for 2 passages

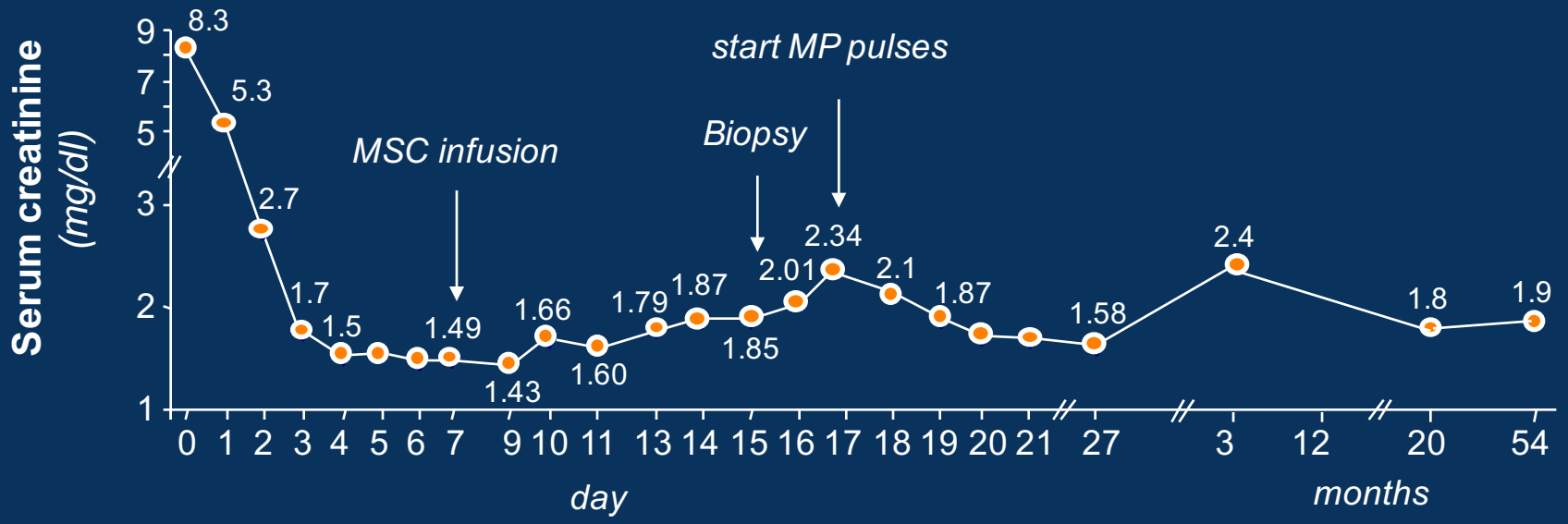
* Dose: 2 x 10^6/kg i.v.

Perico, Casiraghi et al, *Clin J Am Soc Nephrol*, 2011

Patient #1 D.D.



Patient #2 G.U.



Perico, Casiraghi et al, *Clin J Am Soc Nephrol*, 2011

GRAFT INFILTRATING CELLS *(day +15)*

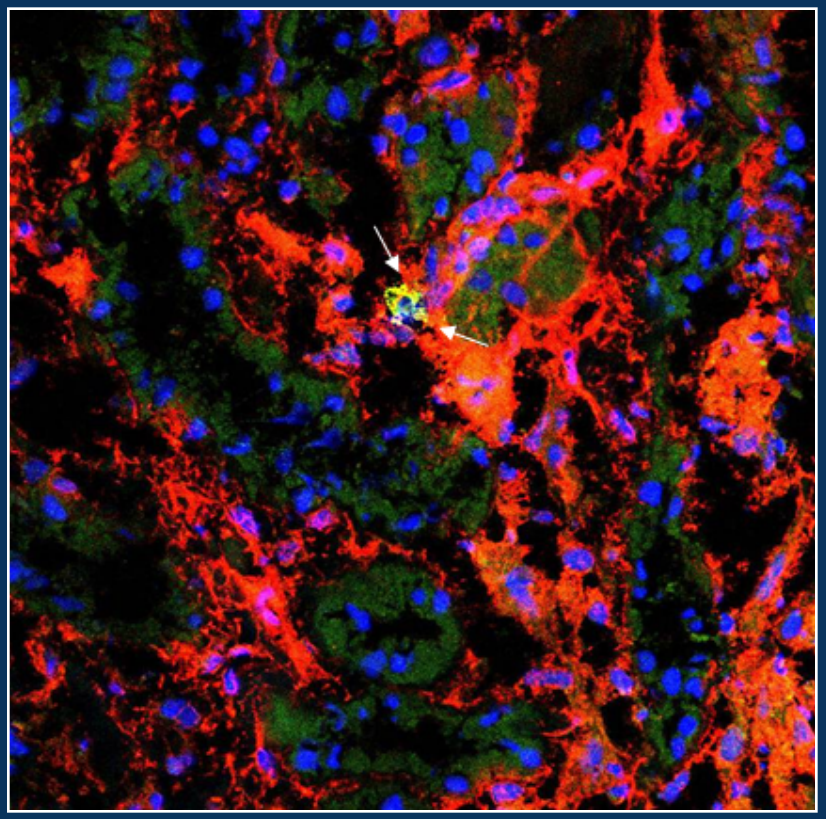
(cells/field)	Patient #2: G.U.	*Controls* *acute rejection (n=3)*
CD4⁺ T cells	1.5	80 ± 35
CD8⁺ T cells	9.8	103 ± 35
CD20⁺ B cells	1.2	20 ± 16
CD68⁺ macrophages	26.1	58 ± 2
granulocytes	30.7	5.5 ± 1.2
Complement C3	0.98	0.2 ± 0.3

Values are mean of 30 fields

Perico et al., *CJASN,* 2011

MSC INFILTRATE THE GRAFT *(day +15)*

	CD105⁺ CD44⁺ cells (cells/3 mm²)
Patient #2: G.U.	17
Controls	
Acute rejection:	
15 day post-tx	0
Native control kidneys (n=4)	1.5 ± 2.6



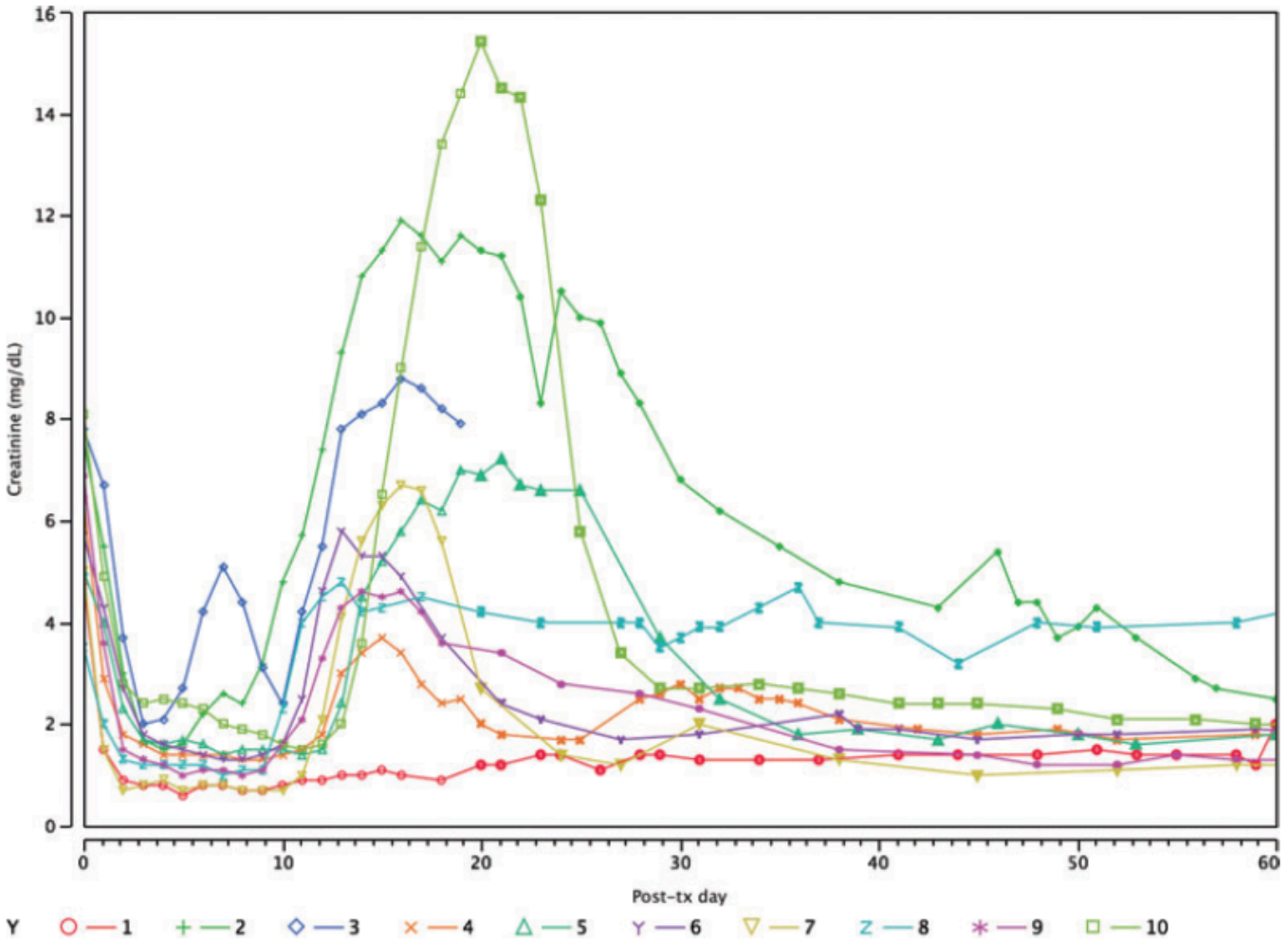
A high number of CD105⁺CD44⁺ MSC was found into the kidney graft biospy of MSC infused patient

Perico, Casiraghi et al, *Clin J Am Soc Nephrol*, 2011

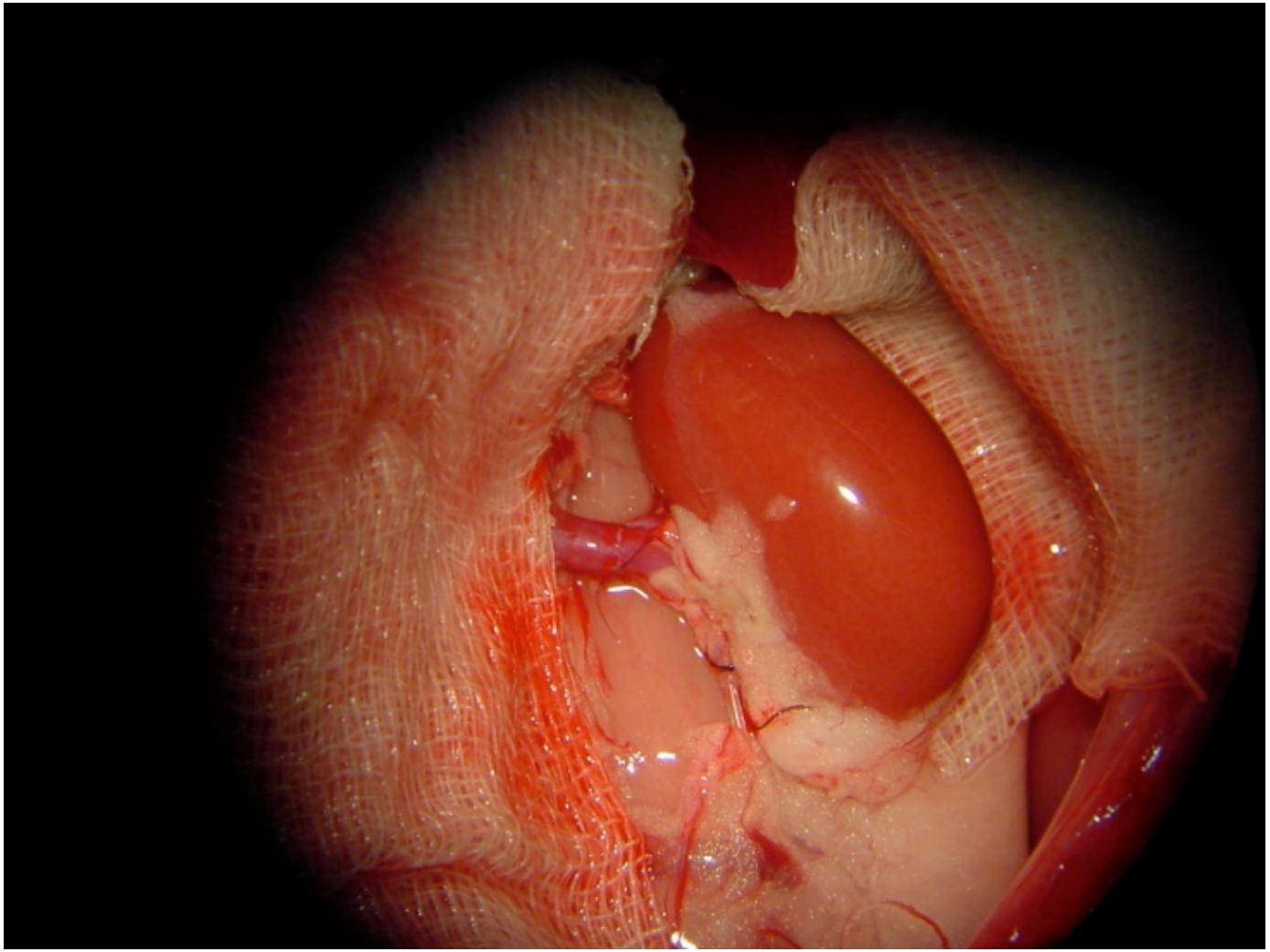
American Journal of Transplantation, 2011

Acute Renal Endothelial Injury During Marrow Recovery in a Cohort of Combined Kidney and Bone Marrow Allografts

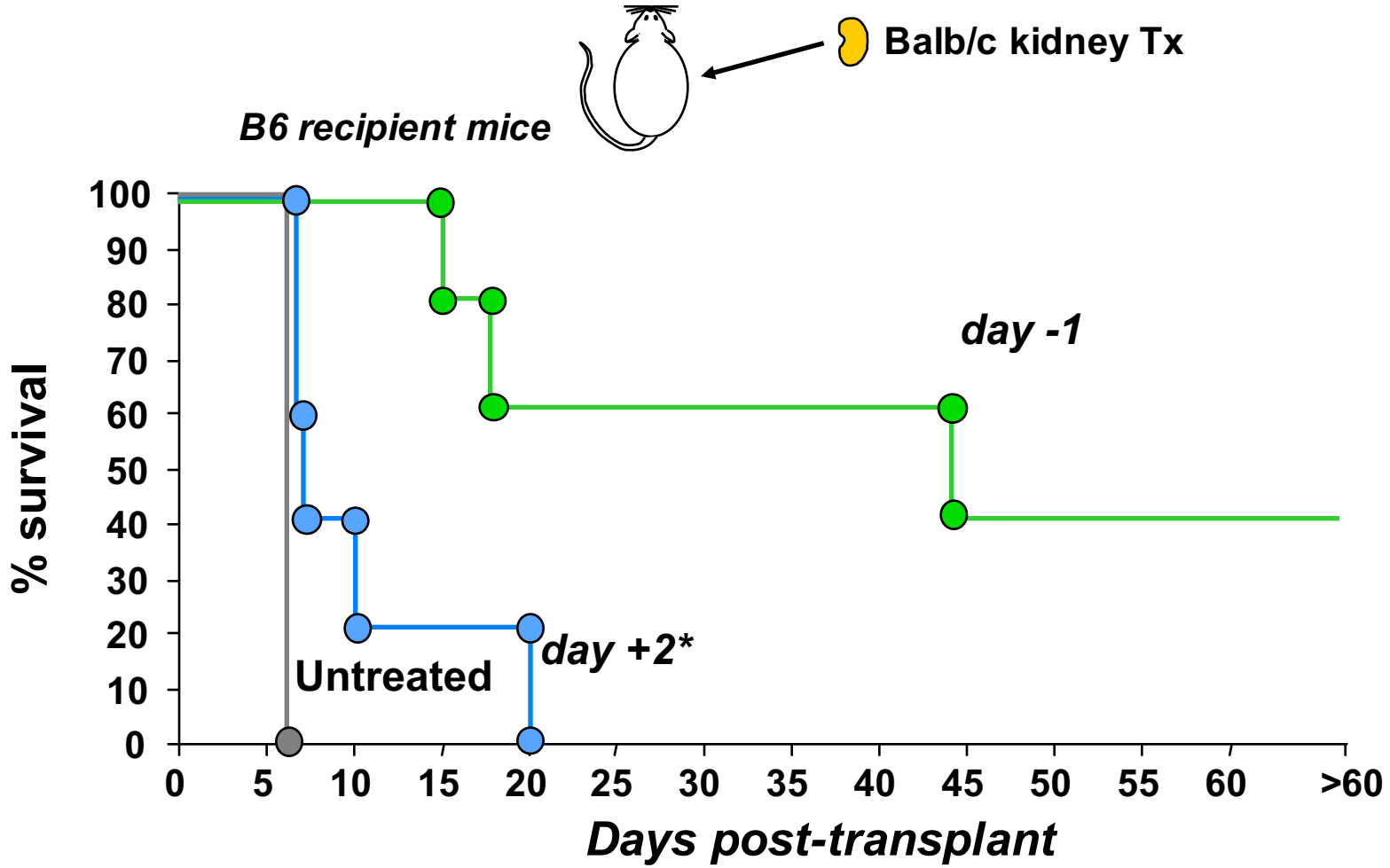
A.B. Farris et al.




ZEISS
OPMI 6-CFR
Carl Zeiss



PRE-TRANSPLANT INFUSION OF MSC IN SENSITIZED MICE



5 animals for each group

Balb/c donor splenocyte (1,000,000/mouse i.v.)

autologous MSC (500,000/mouse i.v.)

* day+7 in mice is unfeasible because of graft loss for acute rejection in all recipient mice

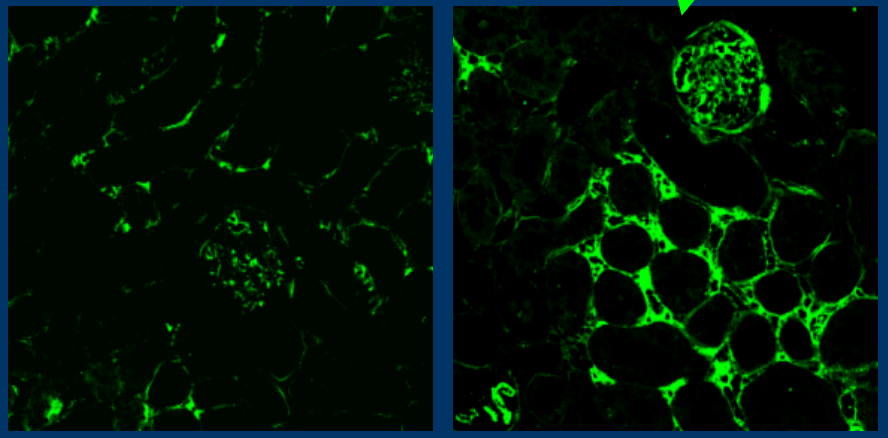
Casiraghi et al, *Am J Transplant,* 2012

LOCALIZATION OF MSC DICTATES THEIR IMMUNE OR PROINFLAMMATORY EFFECTS IN KIDNEY TRANSPLANTATION IN MICE

	PKH 26⁺ MSC (cells/mm²)	
	kidney	spleen
MSC day +2	26.6 ± 4.0*	58.9 ± 20.9*
MSC day -1	4.7 ± 0.2	290 ± 107

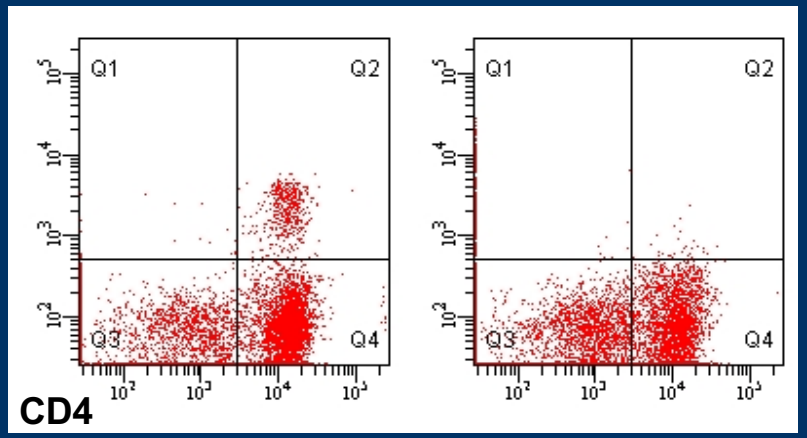
* P<0.05 vs MSC day-1

C3 deposition



MSC day -1 MSC day +2

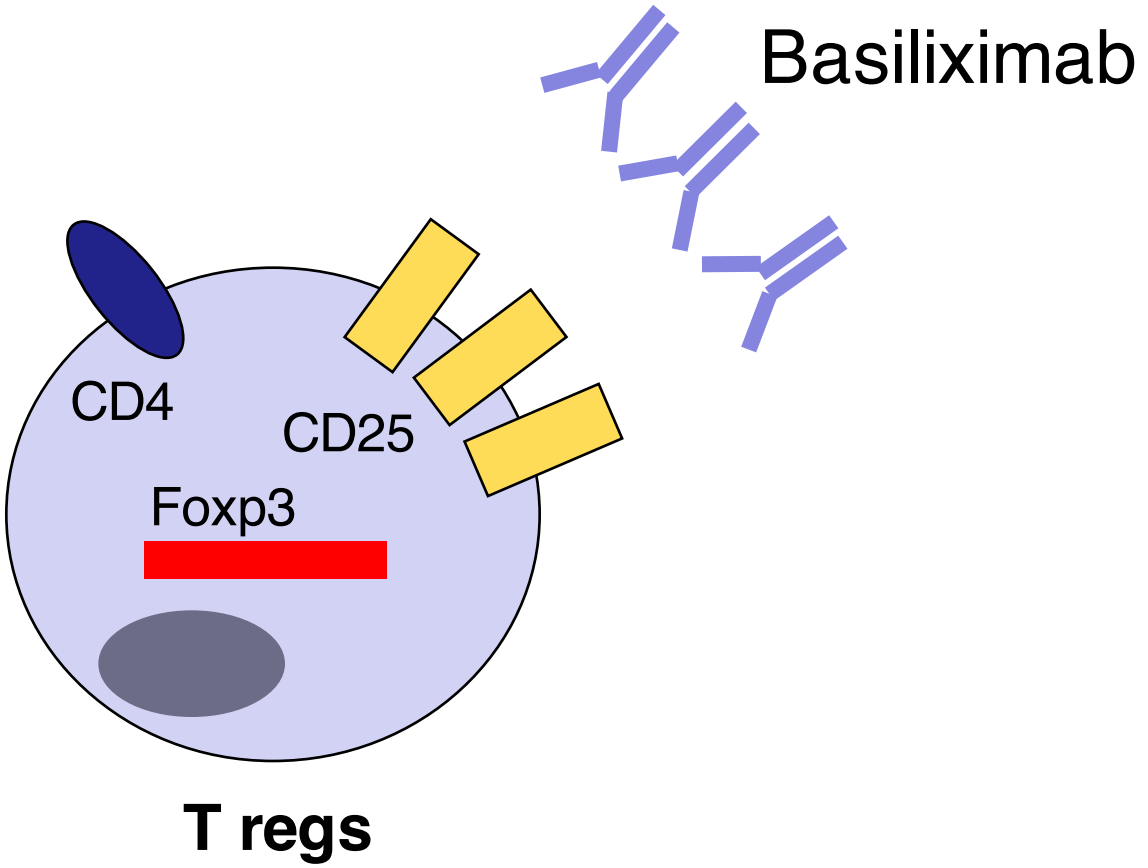
Treg induction



MSC day -1 MSC day +2

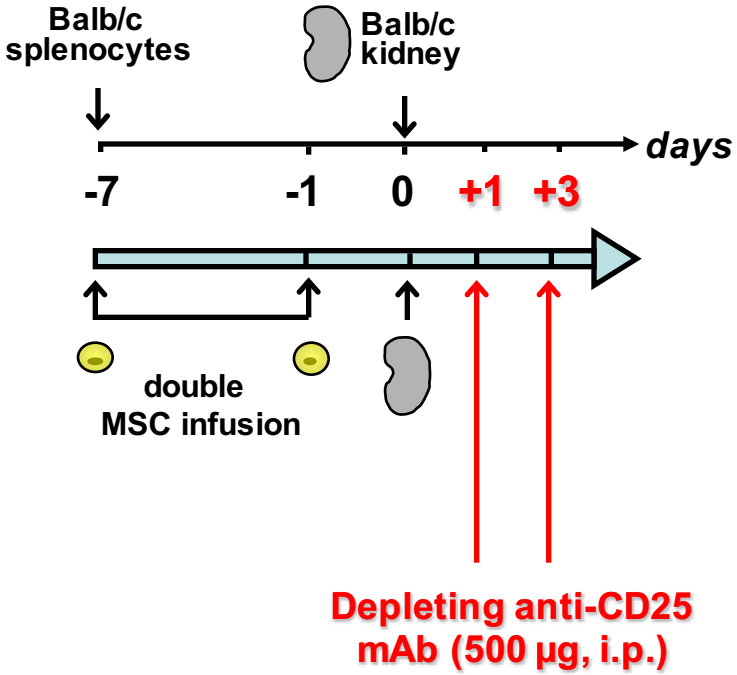
Casiraghi et al., *Am J Transpl.* 2012

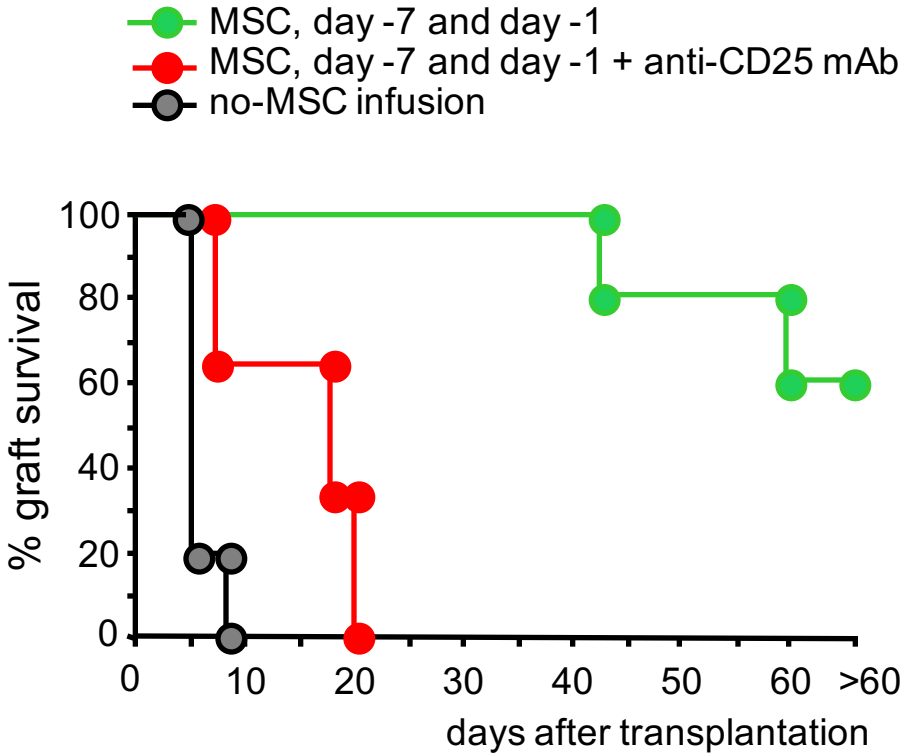
IS BASILIXIMAB PERMISSIVE FOR T REG MEDIATED TOLERANCE?



ANTI-CD25 ANTIBODY ABROGATES TOLERANCE (BY DEPLETING TREGs)

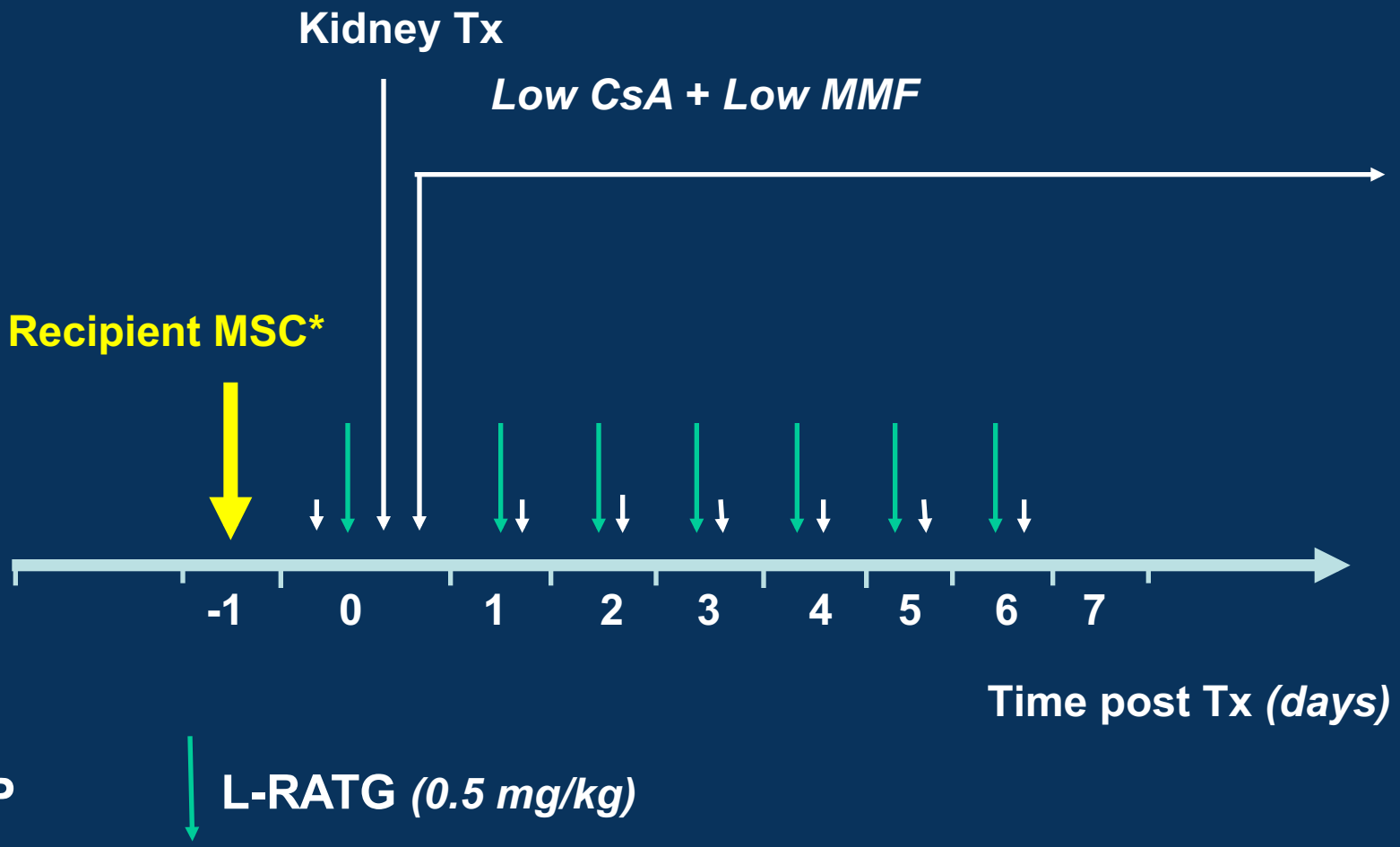
C57 recipient mice





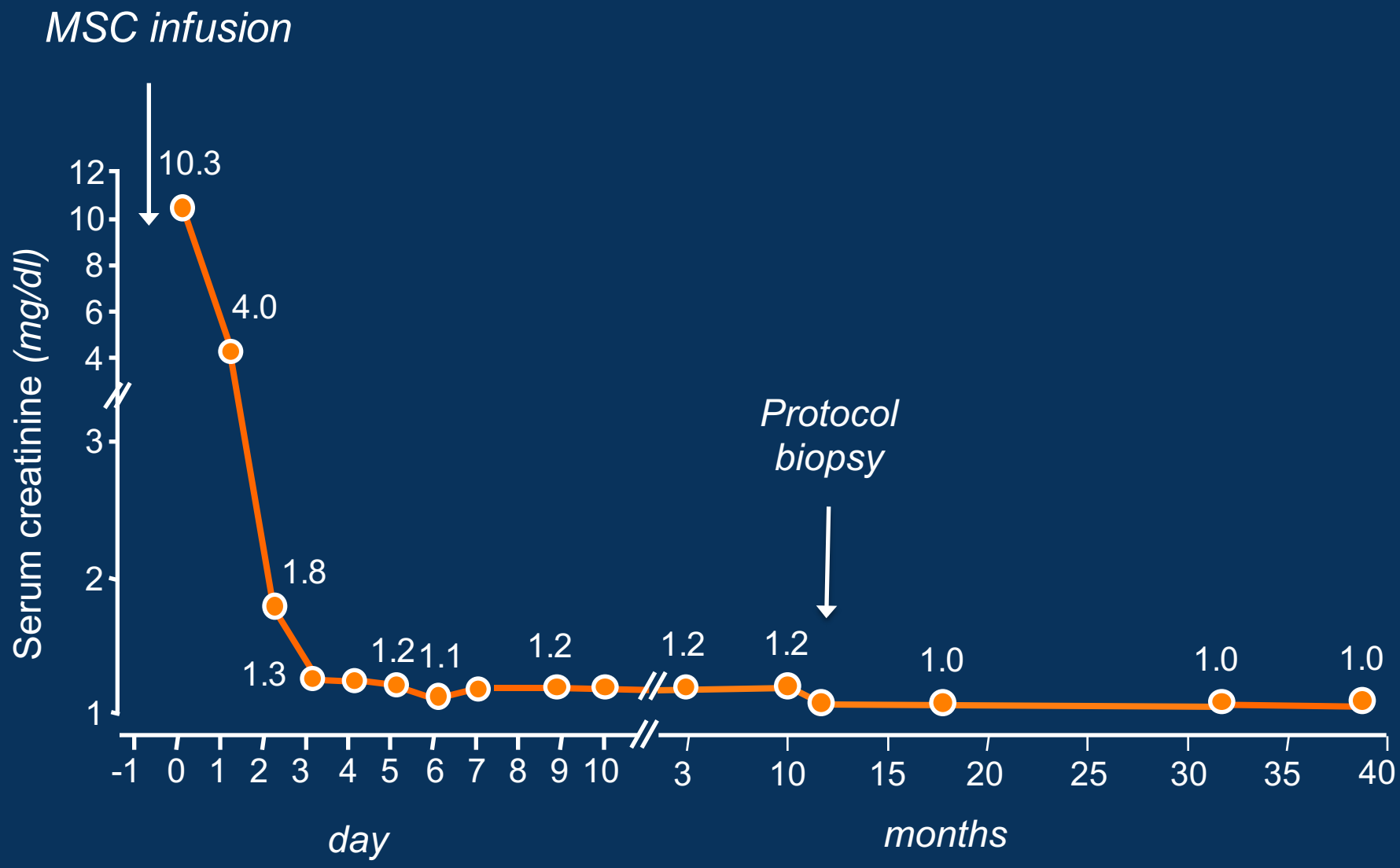
Casiraghi et al, *Am J Transpl.* 2012

Phase b: 2 patients



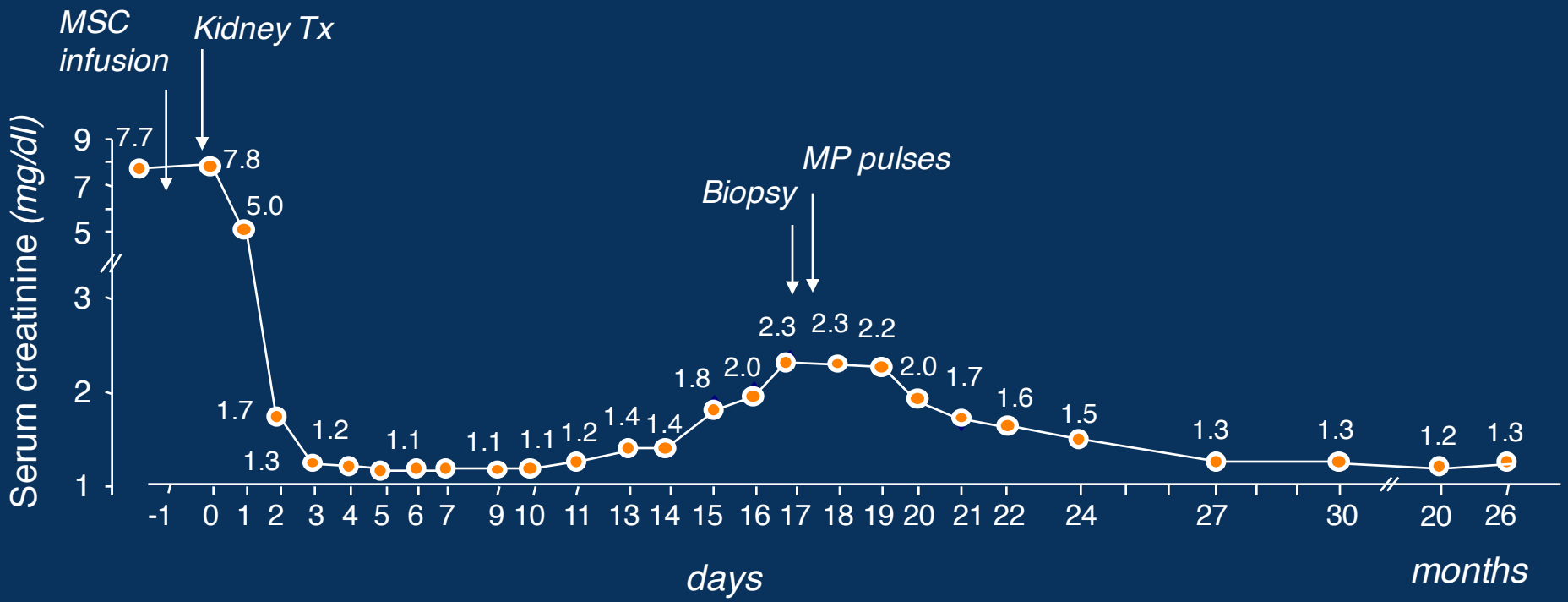
* Dose: 2 x 10^6/kg i.v.

Patient #3 C.M.

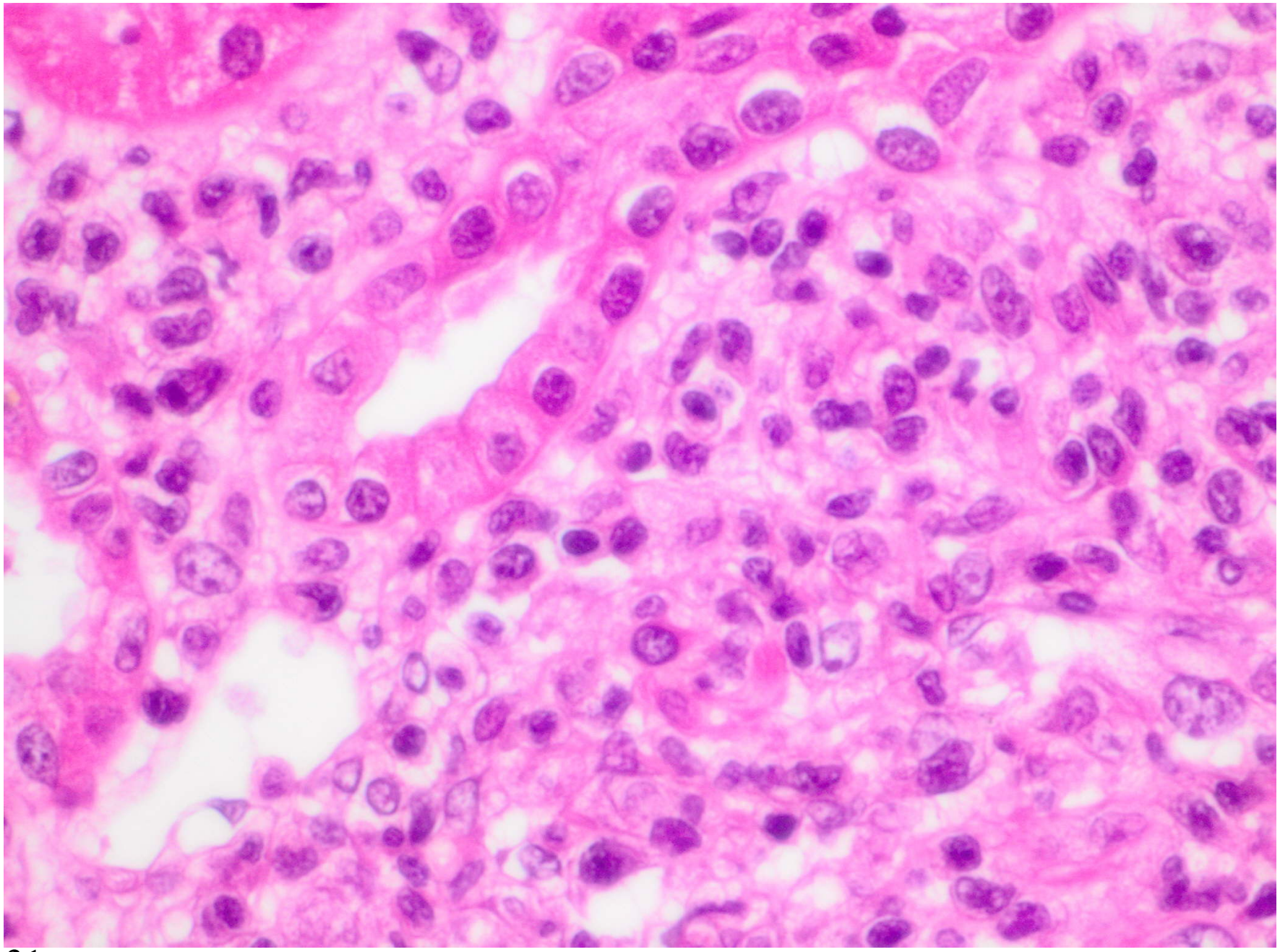


Perico, Casiraghi et al, *Transplant Int,* 2013

Patient #4 D.A.



Perico, Casiraghi et al, *Transplant Int* 2013



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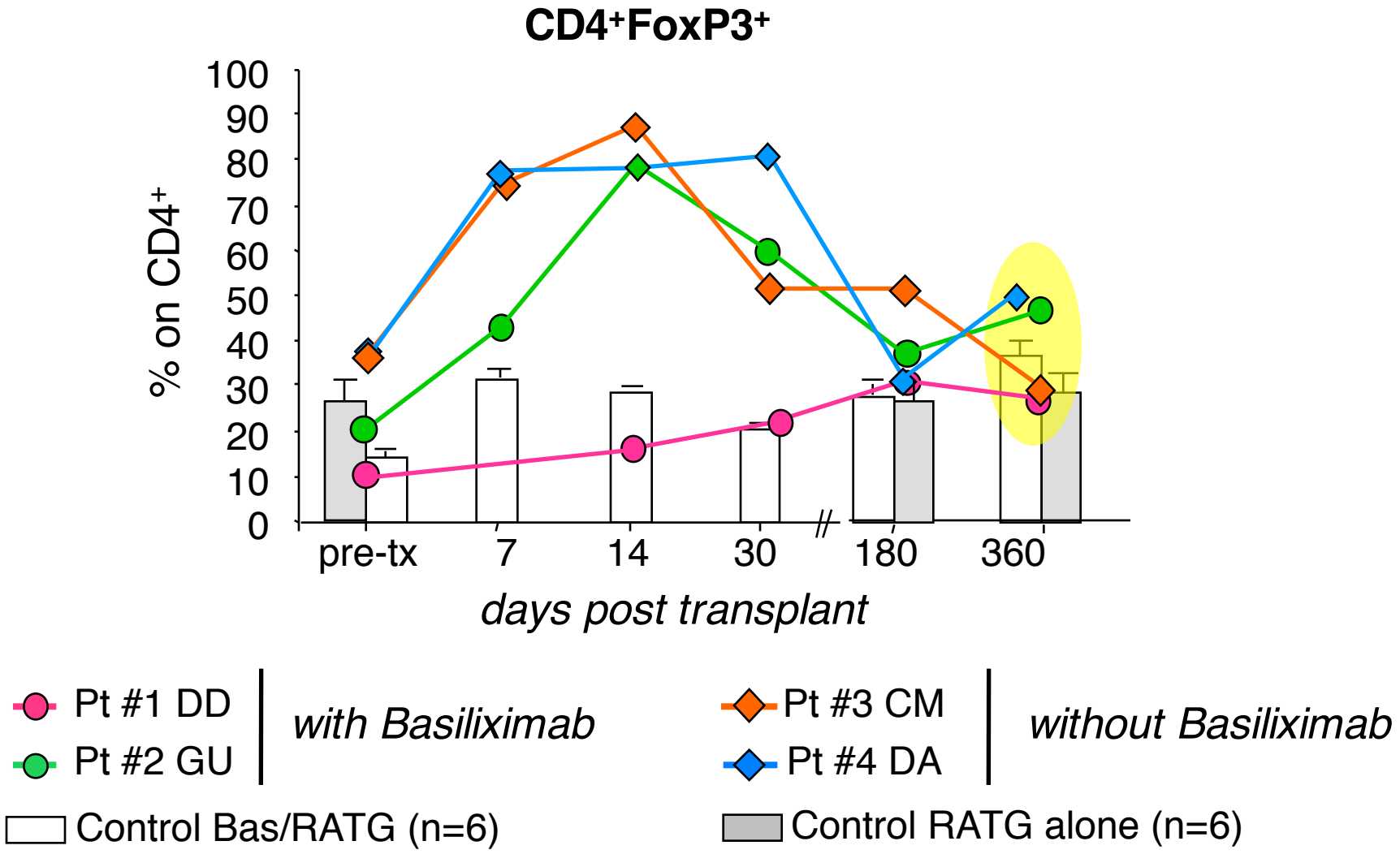
GRAFT INFILTRATING CELLS *(day +17)*

(cells/field)	Patient #4: D.A.	*Controls* *acute rejection (n=3)*
CD4⁺ T cells	19.3	80 ± 35
CD8⁺ T cells	20.2	103 ± 35
CD20⁺ B cells	8.6	20 ± 16
CD68⁺ macrophages	37.3	58 ± 2
granulocytes	9.7	5.5 ± 1.2

Values are mean of 30 fields

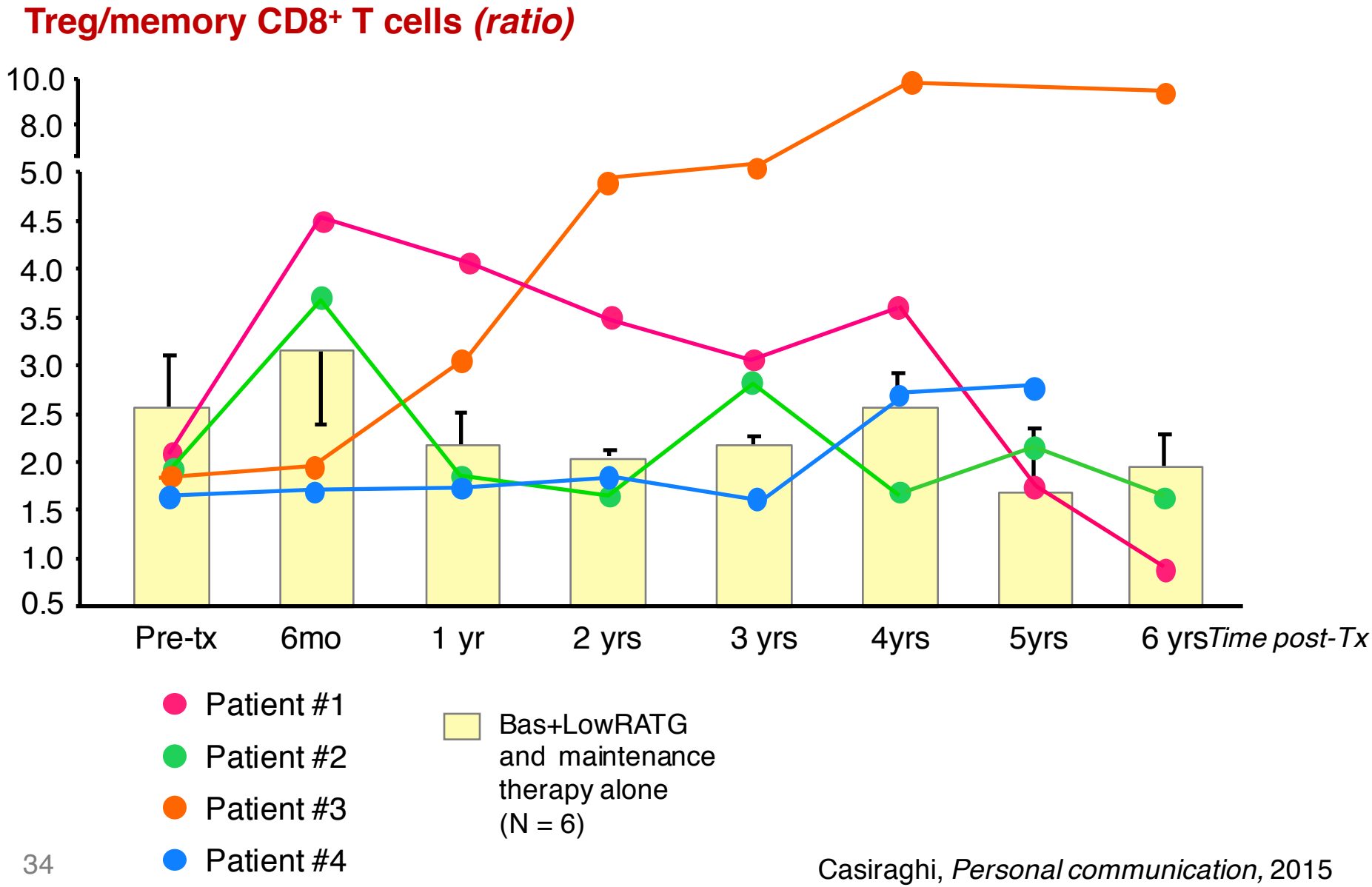
Perico et al., *CJASN,* 2011

EFFECT OF INDUCTION REGIMEN WITH OR WITHOUT BASILIXIMAB ON FoxP3+ T CELL PROFILE



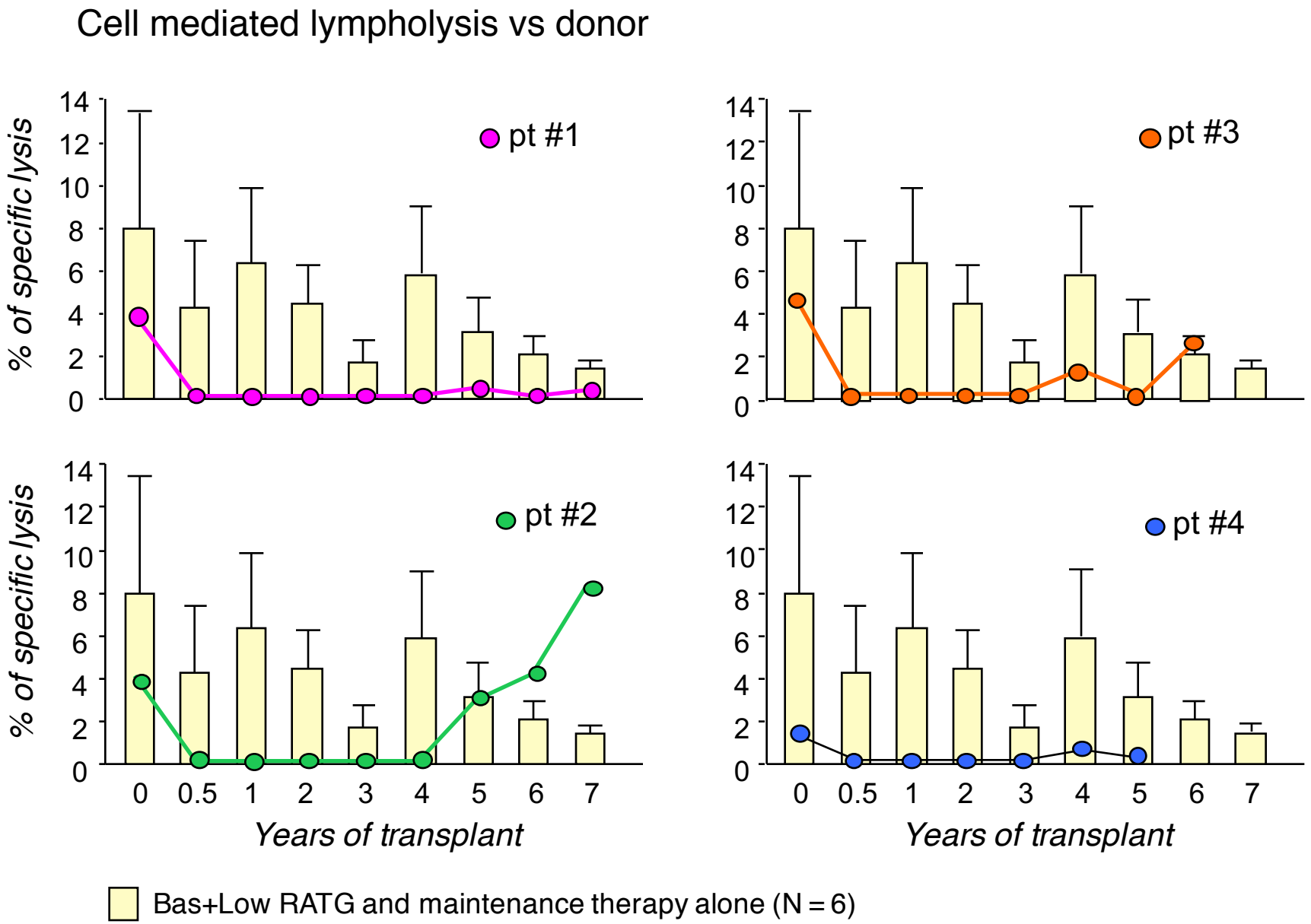
Perico, Casiraghi et al, *Transplant Int,* 2013

THE HIGH RATIO OF Treg/Tmemory COULD FAVOUR A STATE OF IMMUNE REGULATION

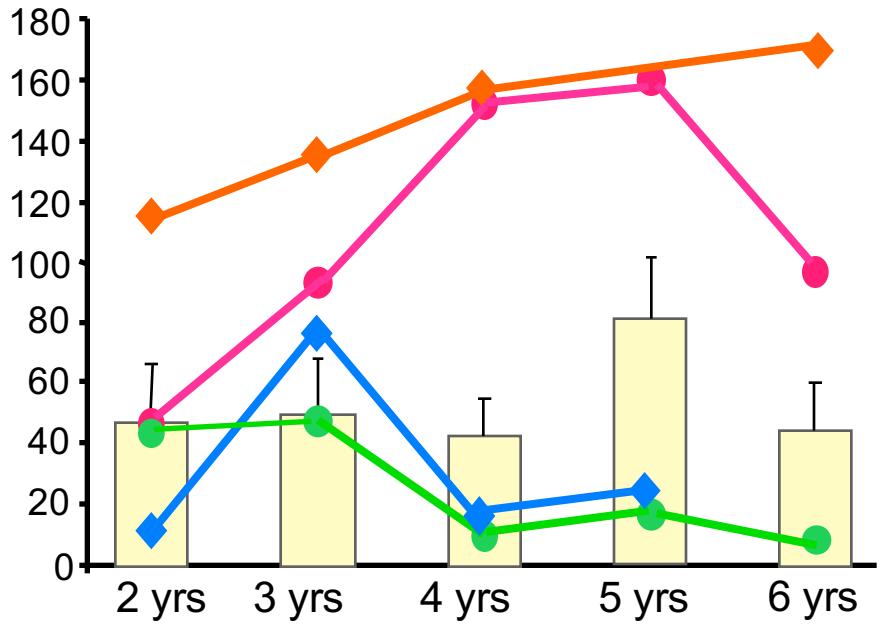


Casiraghi, *Personal communication,* 2015

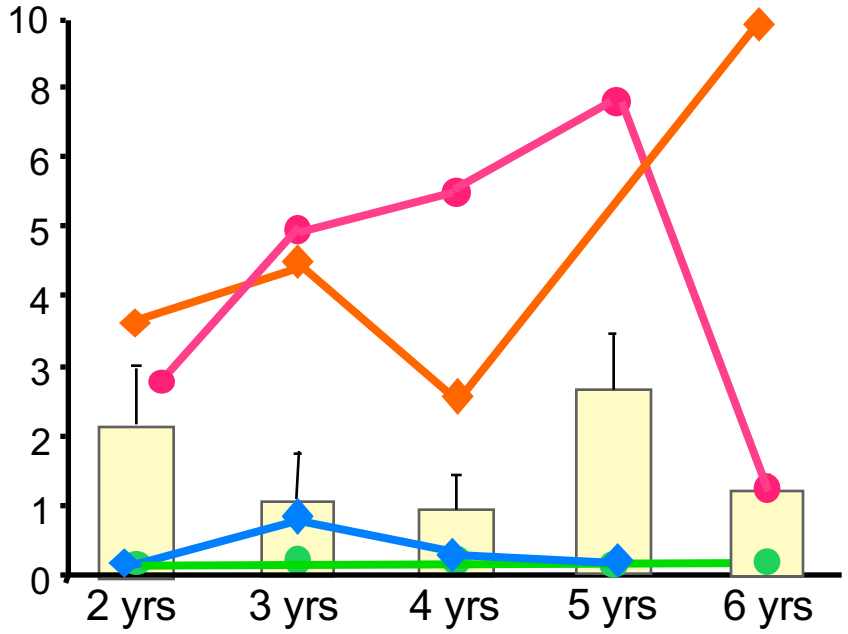
LONG-LASTING COMPLETE AND DONOR-SPECIFIC SUPPRESSION OF CD8+ T CELL CYTOTOXICITY



Naive B cells (cells/µl)
$CD19^+$ IgD^+CD27^-



Transitional B cells (cells/µl)
$CD19^+$ $CD24^{high}CD38^{high}$

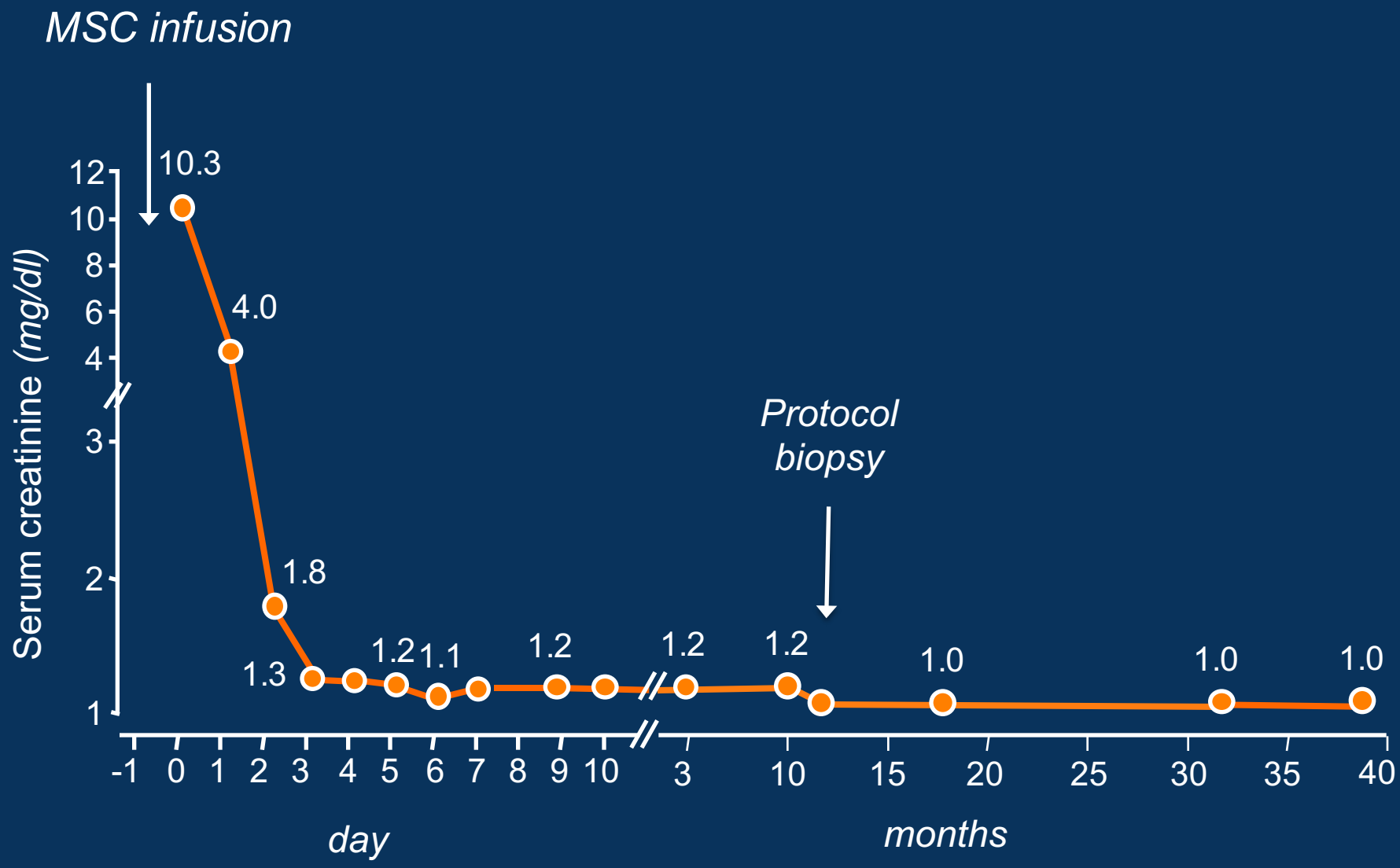


Patient #1
Patient #2
Patient #3
Patient #4
Controls (N = 6) * P<0.05 vs pre-tx

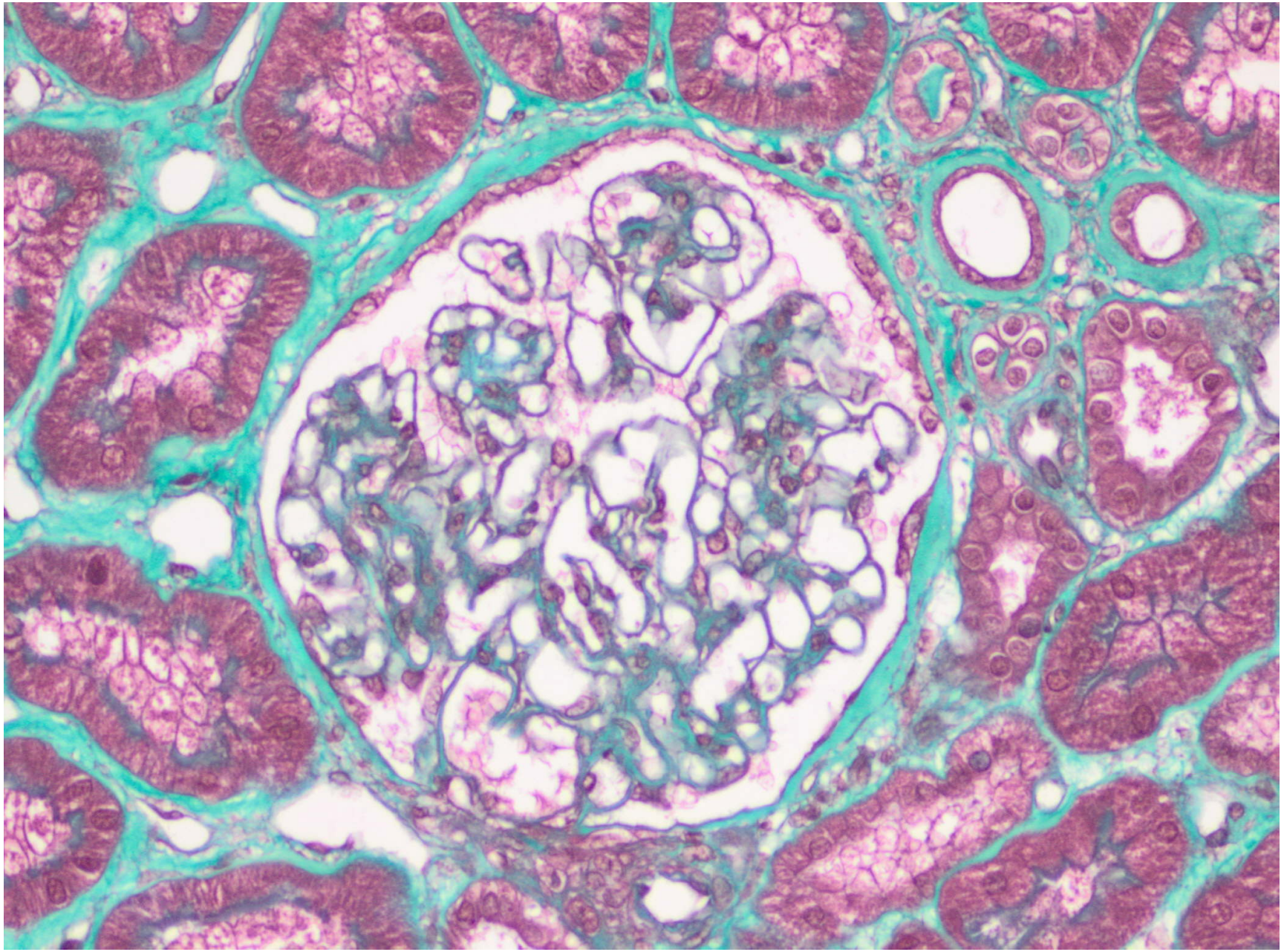
Spontaneous operational tolerance to kidney allograft is associated with elevated number of naïve and transitional B cells with regulatory properties suggesting a critical role for these B cells subsets in the regulation of alloimmune response

Newell et al., J Clin invest, 2010

Patient #3 C.M.



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



RENAL GRAFT FUNCTION IN MSC-TREATED PATIENTS

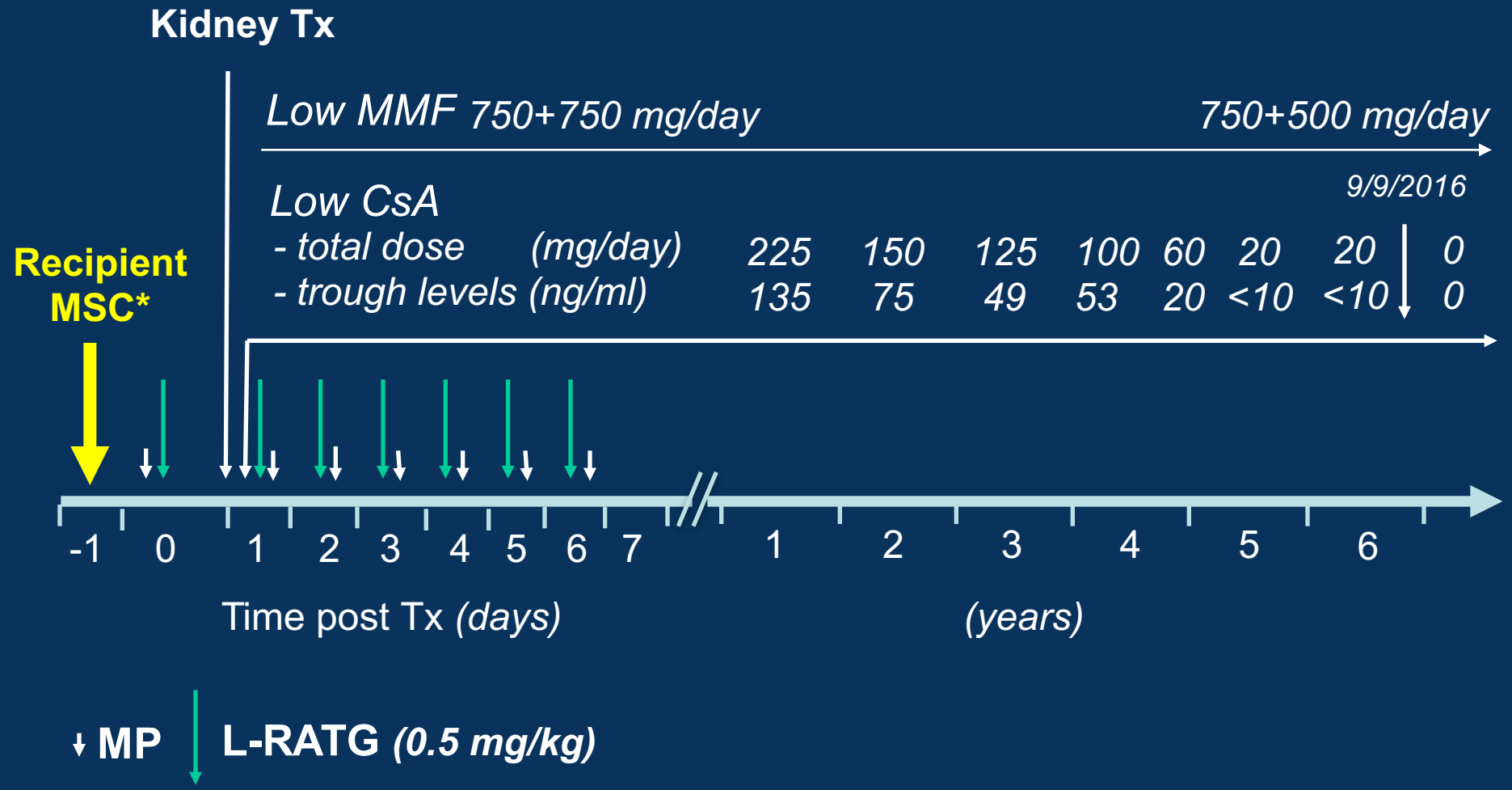
GFR slope *(ml/min/1.73 m^2 /year)*

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

Measured GFR by iohexol plasma clearance

Casiraghi, *Personal communication,* 2017

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



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

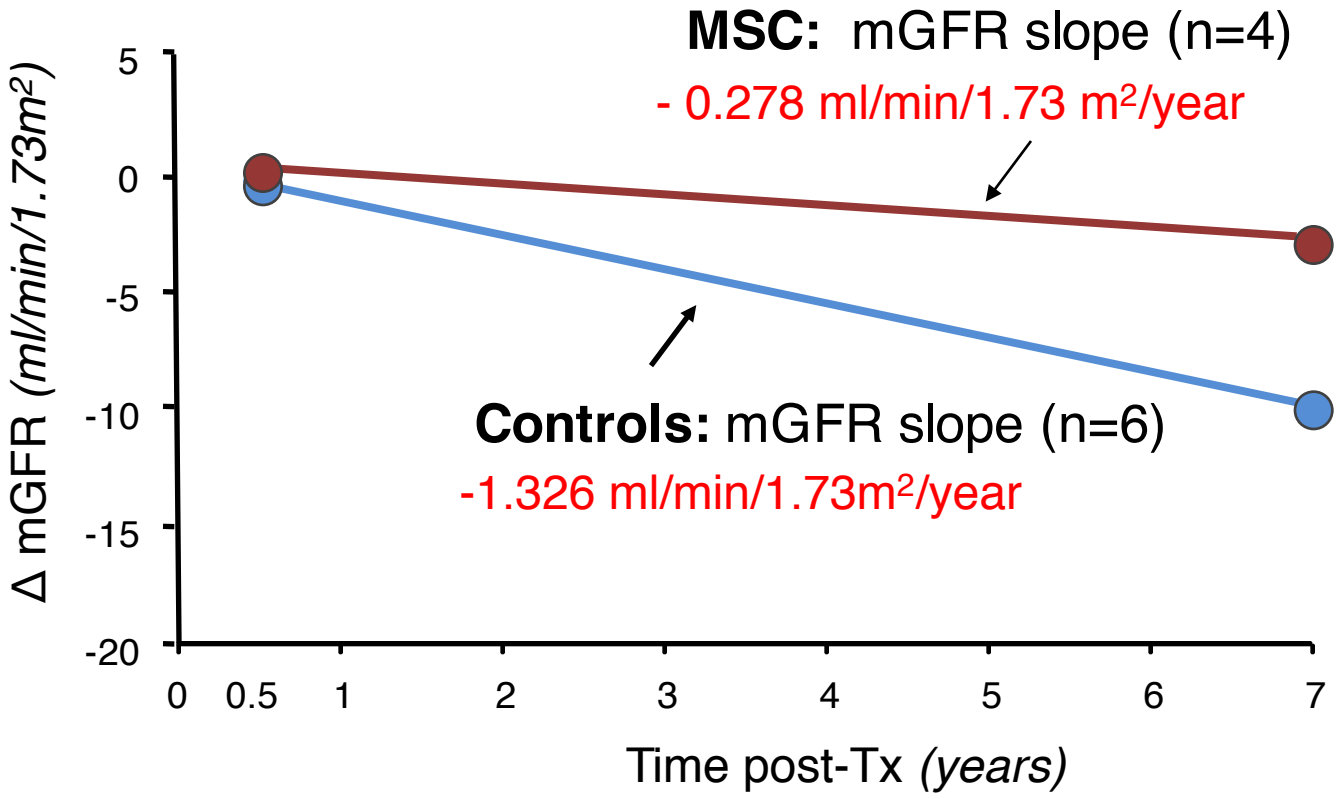
DONOR HLA-SPECIFIC ANTIBODIES

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,* 2017

RENAL GRAFT FUNCTION IN MSC-TREATED PATIENTS

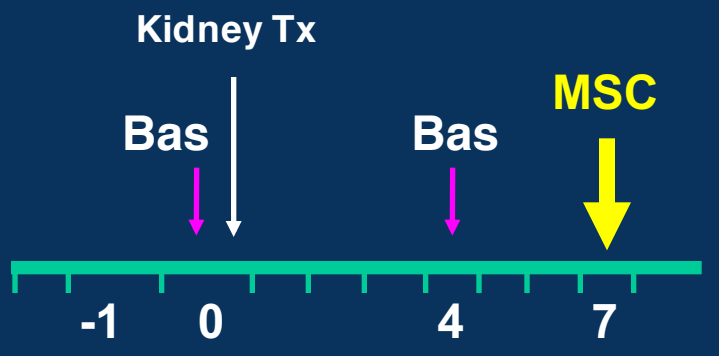


Values are median of individual GFR slopes

Measured GFR by iohexol plasma clearance

Casiraghi, *Personal communication,* 2017

LIVING TRANSPLANT RECIPIENTS

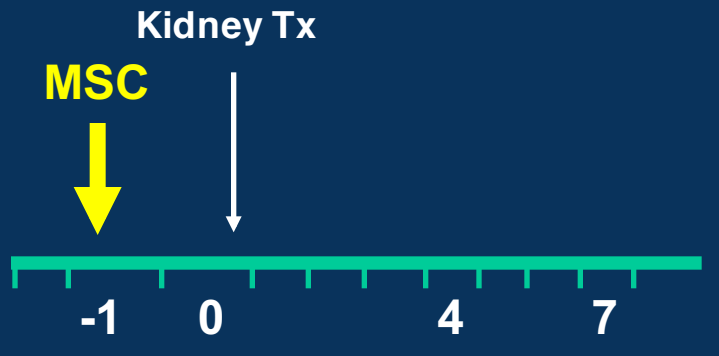


2 *

Engraftment syndrome: YES

Acute graft rejection: NO

IL-2 dependent Treg expansion ↑

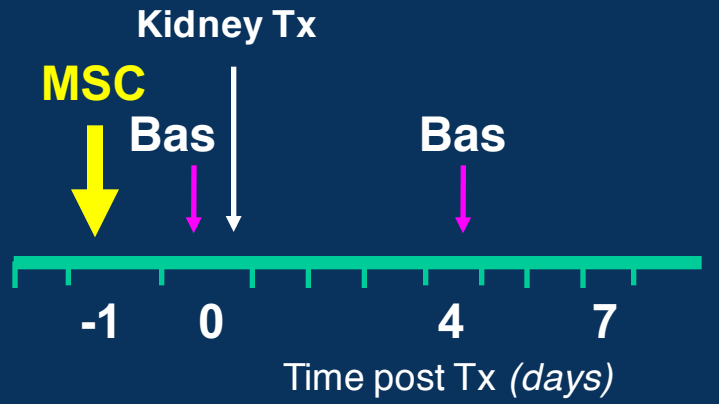


2 *

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)*

THIRD-PARTY BONE MARROW-DERIVED MSC TO INDUCE TOLERANCE IN LIVER TRANSPLANT RECIPIENTS

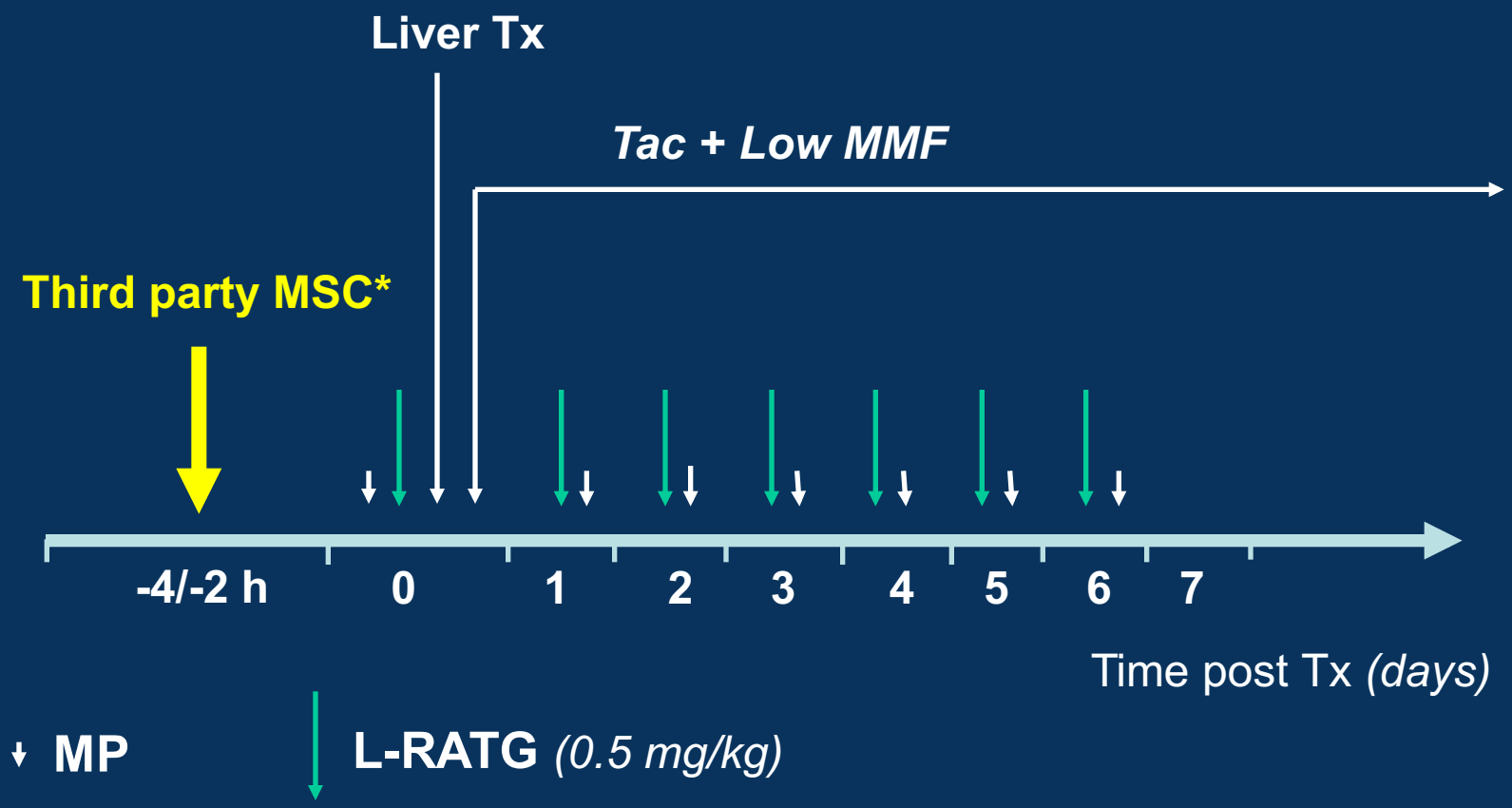
Bergamo

- IRCCS, Mario Negri Institute
  (coordinator/immunomonitoring)
- Azienda Ospedaliera Papa Giovanni XXIII
  - U.O. Chirurgia III
    (Liver Tx)
  - U.O. Ematologia
    (Bone marrow explants)
  - Laboratorio Terapie Cellulari "G. Lanzani"
    (MSC preparation according to European GMP)

Bologna

- Azienda Ospedaliera Policlinico S. Orsola-Malpighi
  - U.O. Chirurgia Generale e Trapianto
    (Liver Tx)

Phase 1: A pilot explorative study



* Dose: 1-2 x 10^6/kg i.v.

Group 1: MSC (n = 10)
Group 2: Control (n = 10)

- *safety*
- *immuno-mechanisms*

Research Article

EASL | JOURNAL OF HEPATOLOGY

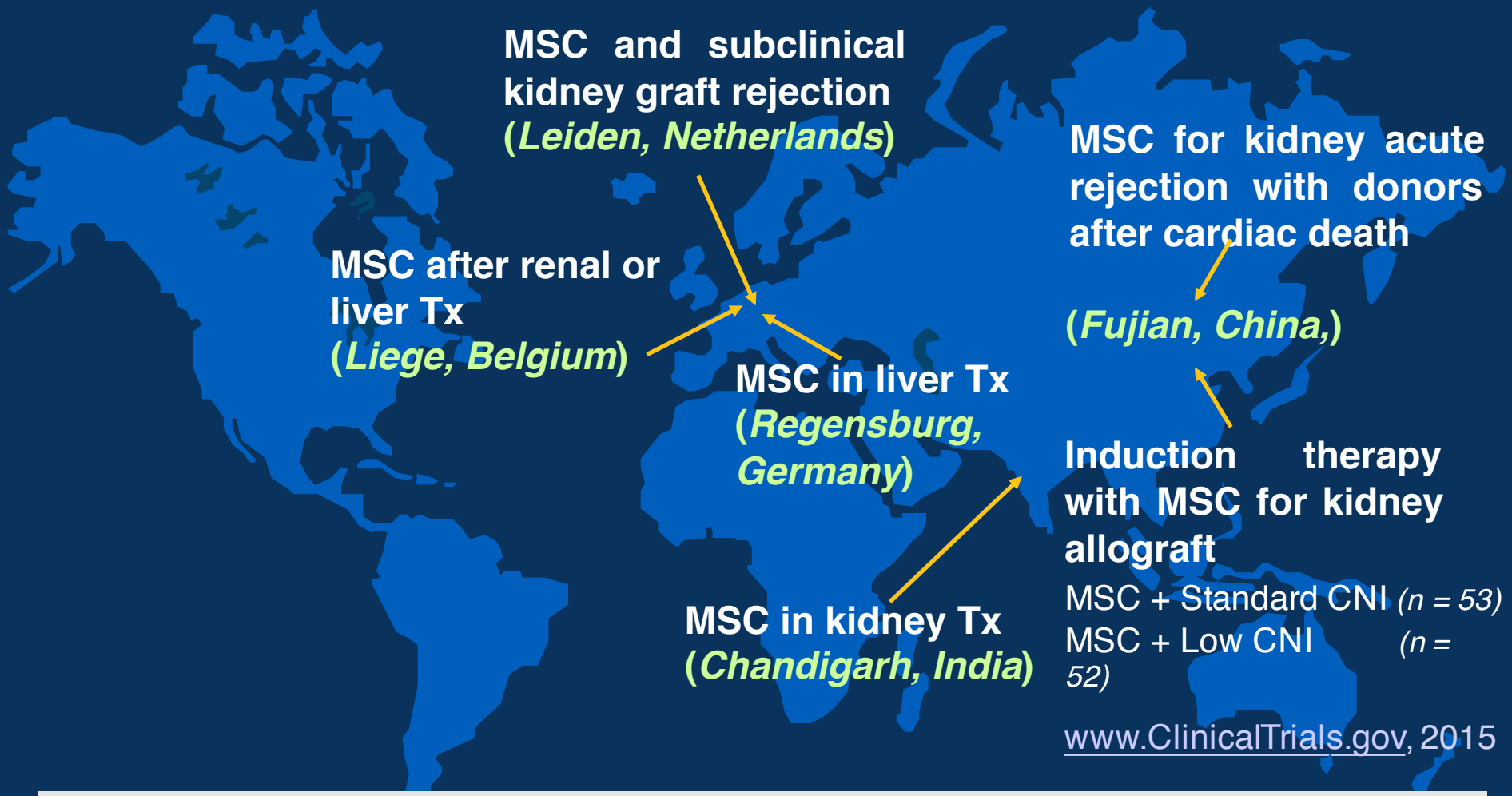
Infusion of mesenchymal stromal cells after deceased liver transplantation: A phase I–II, open-label, clinical study

Olivier Detry[1,2,*], Morgan Vandermeulen[1,2], Marie-Hélène Delbouille[1], Joan Somja[3], Noella Bletard[3], Alexandra Briquet[4], Chantal Lechanteur[4], Olivier Giet[4], Etienne Baudoux[4], Muriel Hannon[5], Frederic Baron[5,6], Yves Beguin[5,6]

[1]*Department of Abdominal Surgery and Transplantation, CHU Liege, University of Liege, (CHU ULg), Belgium;* [2]*Mesenchymal stromal cell In Solid Organ Transplantation (MISOT) consortium*[1]; [3]*Department of Pathology, CHU Liege, University of Liege, (CHU ULg), Belgium;* [4]*Laboratory of Cell and Gene Therapy (LTCG), CHU Liege, University of Liege, (CHU ULg), Belgium;* [5]*Interdisciplinary Cluster for Applied Genoproteomics (GIGA)-I3-haematology, University of Liege, Belgium;* [6]*Department of Haematology, CHU Liege, University of Liege, (CHU ULg), Belgium*

- MSC were infused on 3 $\pm$ 2 days post liver transplant
- No side effect of MSC infusion
- The immunosuppression weaning in MSC recipients was not successful

REGISTERED CLINICAL TRIALS OF MSCs IN SOLID ORGAN TRANSPLANTATION



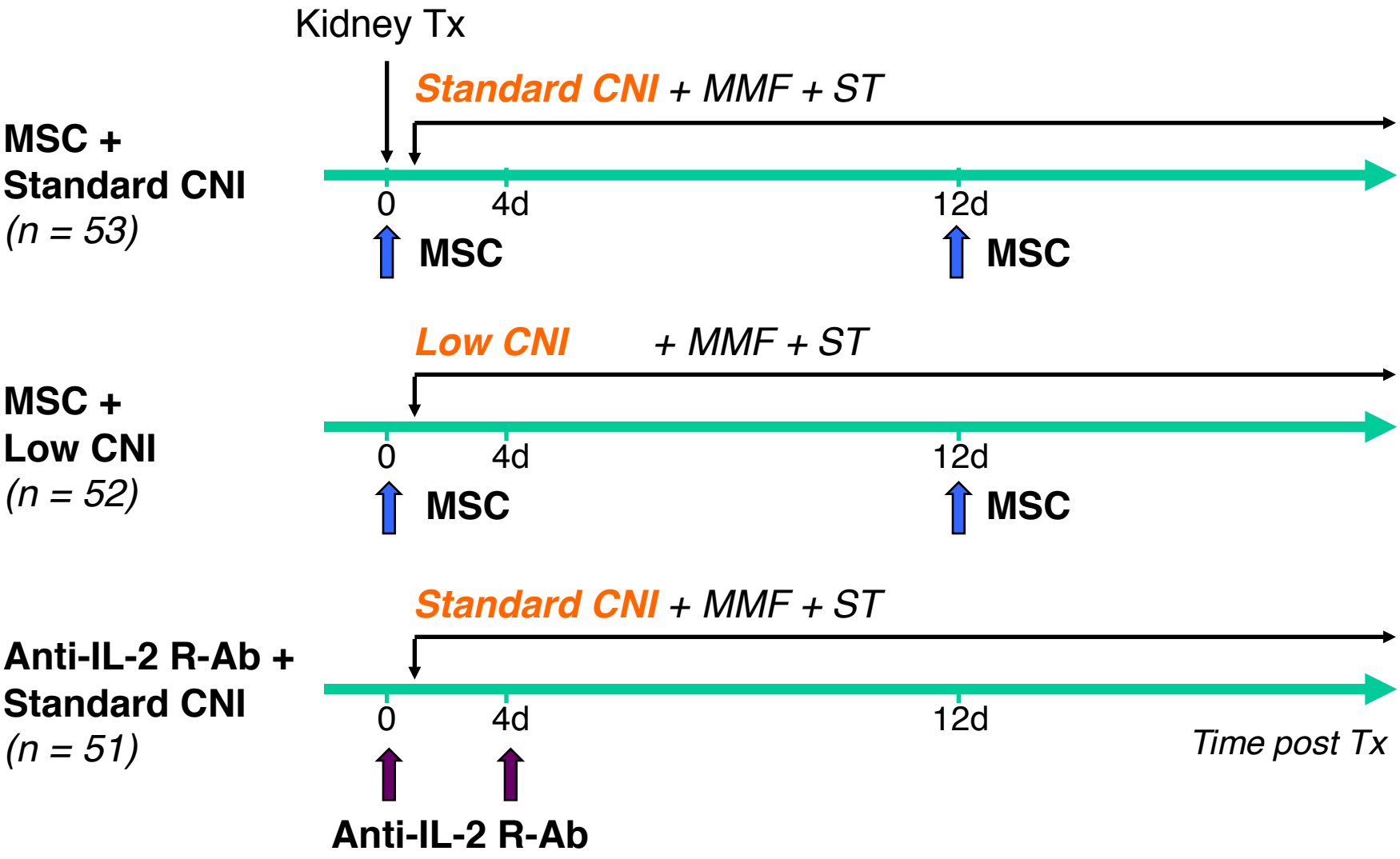
MSC groups *n = 105*
Control groups *n = 51*

Endpoint: acute graft rejection

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


AUGUST 7, 2006
TIME
THE TRUTH ABOUT
STEM CELLS
THE HOPE, THE HYPE AND WHAT IT MEANS FOR YOU
Adult bone marrow stem cell
www.time.com AOL Keyword: TIME

INDUCTION THERAPY WITH AUTOLOGOUS MESENCHYMAL STEM CELLS IN LIVING-RELATED KIDNEY TRANSPLANT

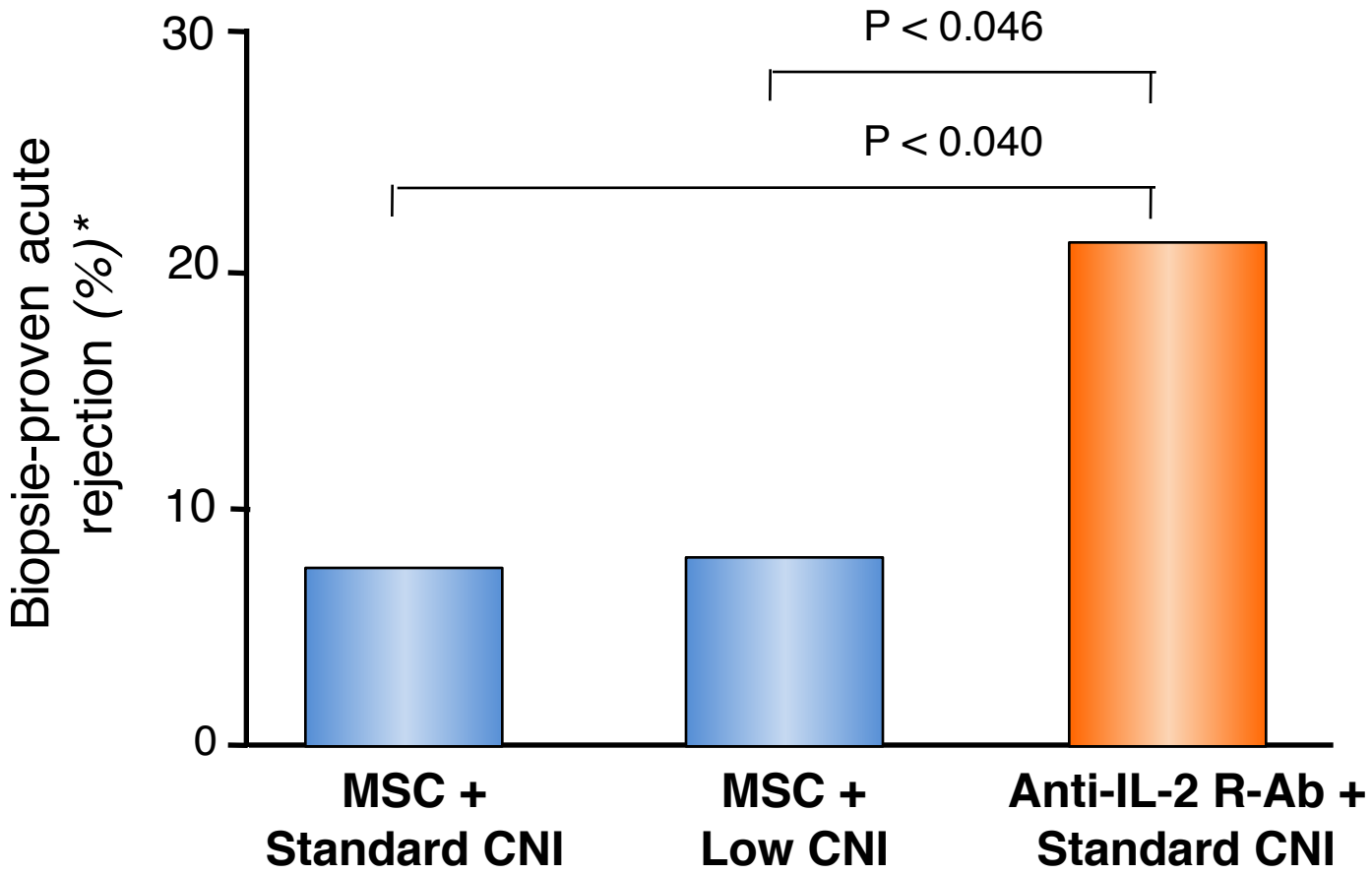


MSC: Bone Marrow MSC *(1-2 x 10^6/kg)*

Anti-IL-2 R-Ab

Tan et al., *J Am Med Assoc*, 2012

LOWER FREQUENCY OF ACUTE REJECTION EPISODES IN MSC-TREATED PATIENTS THAN IN THOSE GIVEN INDUCTION THERAPY WITH ANTI-IL-2 R-Ab



* At 6 months

Tan et al., *J Am Med Assoc,* 2012

NO DIFFERENCE IN ACUTE REJECTION INCIDENCE AMONG GROUPS AT 12 MONTHS POST TRANSPLANTATION

	MSC + Standard CNI	*MSC + Low-dose CNI*	*Anti-IL2R Ab + Standard CNI*
Acute rejection (%)			
At 6 months post-Tx	7.5 *	7.7 **	21.6
At 12 months post-Tx	15.1	17.3	25.5

*p<0.040, **p<0.046 vs. Anti-IL2R Ab + standard CNI

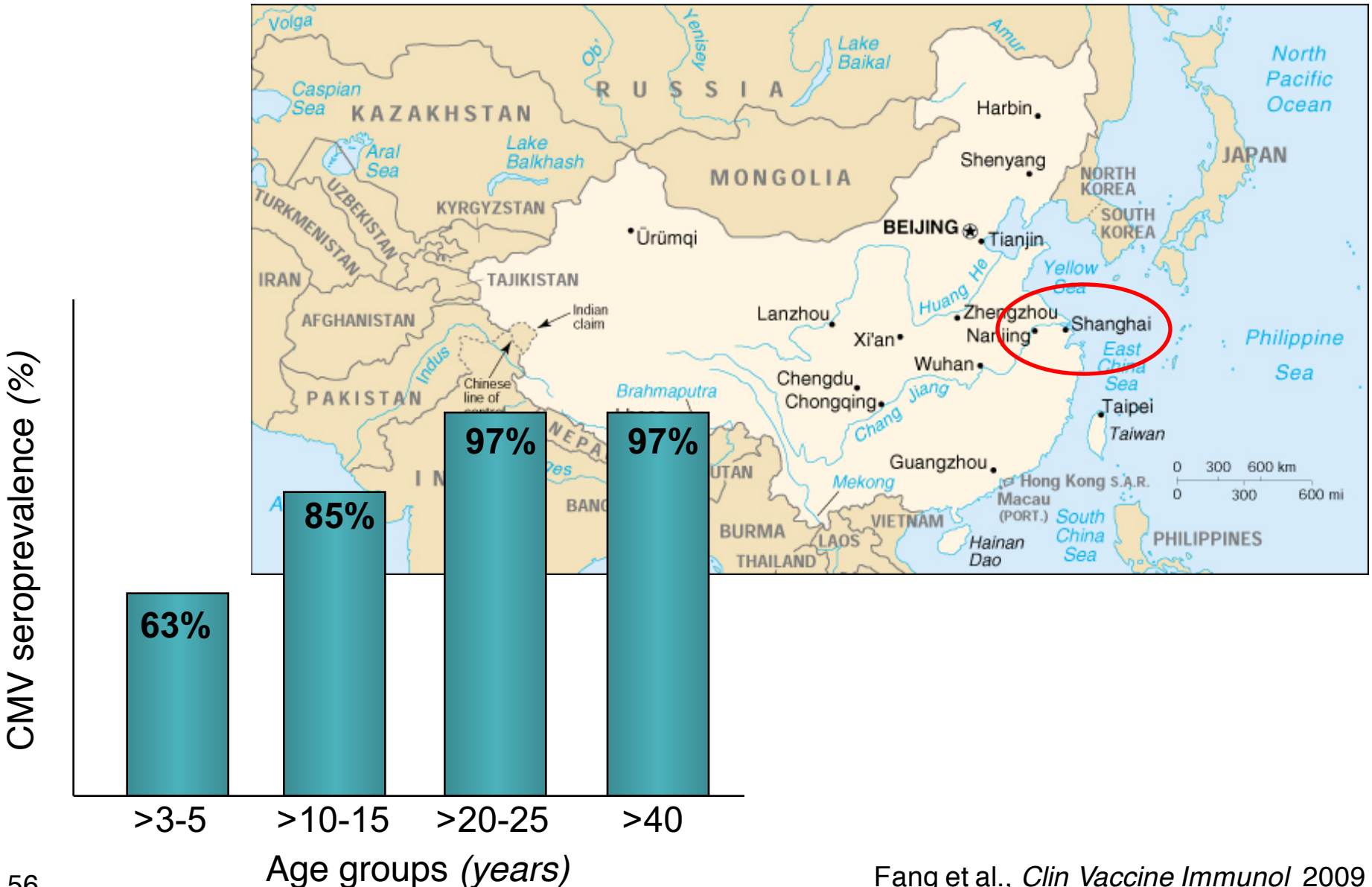
Tan et al., *JAMA,* 2012

CMV DONOR/RECIPIENT STATUS

	MSC + Standard CNI *(n=53)*	MSC + Low-dose CNI *(n=52)*	Anti-IL2R Ab + Standard CNI *(n=51)*
CMV status			
D+/R-	1	2	2
D-/R-	52	50	49

Tan et al., *JAMA*, 2012

A CMV SEROSURVEY ON 8,190 SERUM SAMPLES IN SHANGHAI *(June 2006 - May 2008)*



Fang et al., *Clin Vaccine Immunol* 2009

TAN'S STUDY COHORT

CMV negative Tx recipients	156
Age range (yrs)	34-40
Estimated screened individuals on waiting list with CMV negative living donors *	**5033**

* *Based on 3% CMV negative individuals in the Eastern China population (Shanghai) of comparable age range (Fang et al., Clin Vaccine Immunol 2009)*

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Giuseppe Remuzzi, M.D.

Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

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