Alessandro Rambaldi: MSC and other cell-based therapies for aGvHD

UNIVERSITÀ DEGLI STUDI DI MILANO

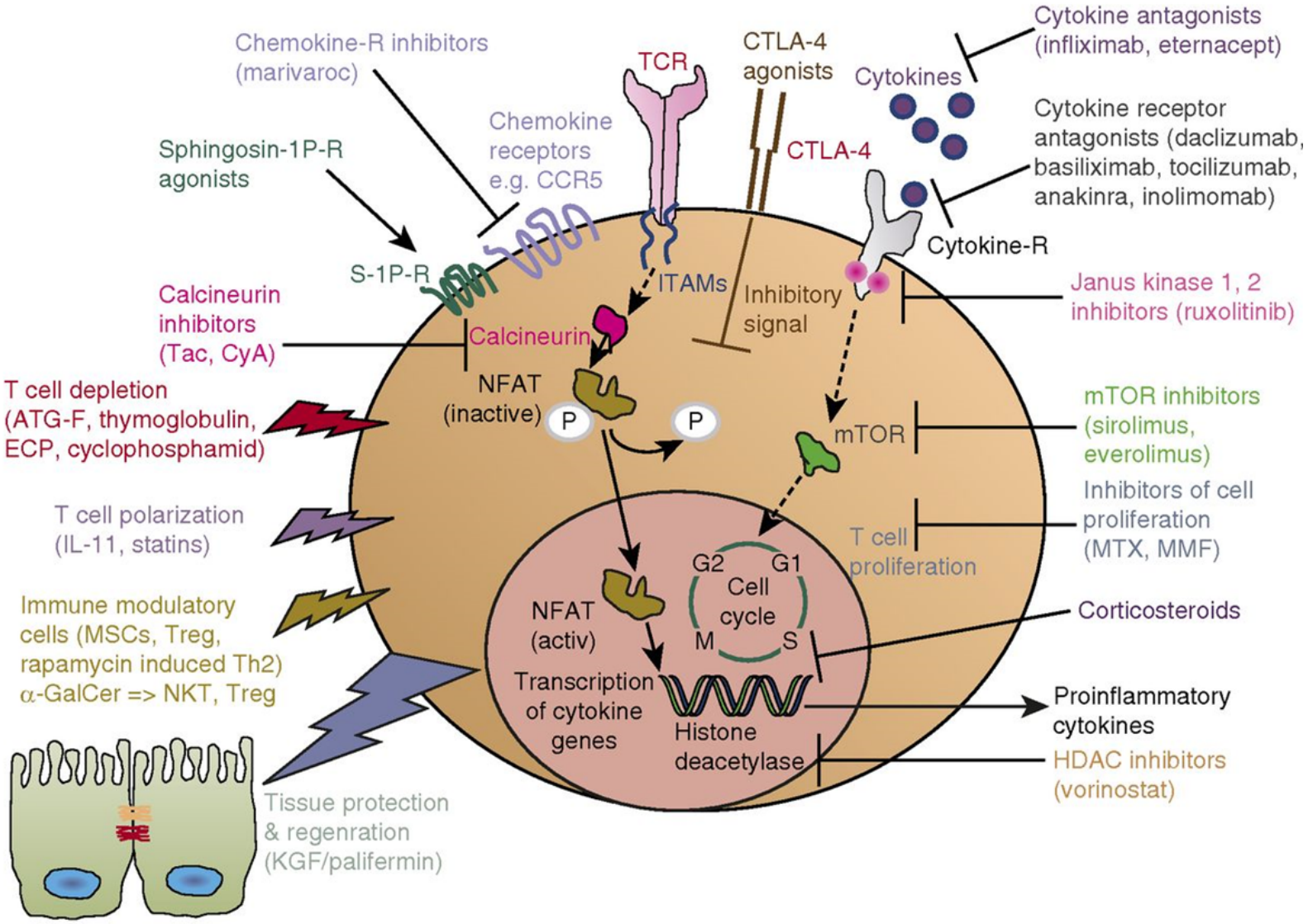
Azienda Ospedaliera Papa Giovanni XXIII Bergamo

Overview

1. **Clinical use of T regulatory T cells (Tregs) for GvHD**
   - **drug mediated *in vivo* expansion of Tregs**
   - ***ex vivo* selection and/or expansion of Tregs**

2. **Clinical use of Mesenchymal Stromal Cells (MSCs)**
   - **the available results (and their limits)**
   - **the ongoing trial with UCB derived MSCs**

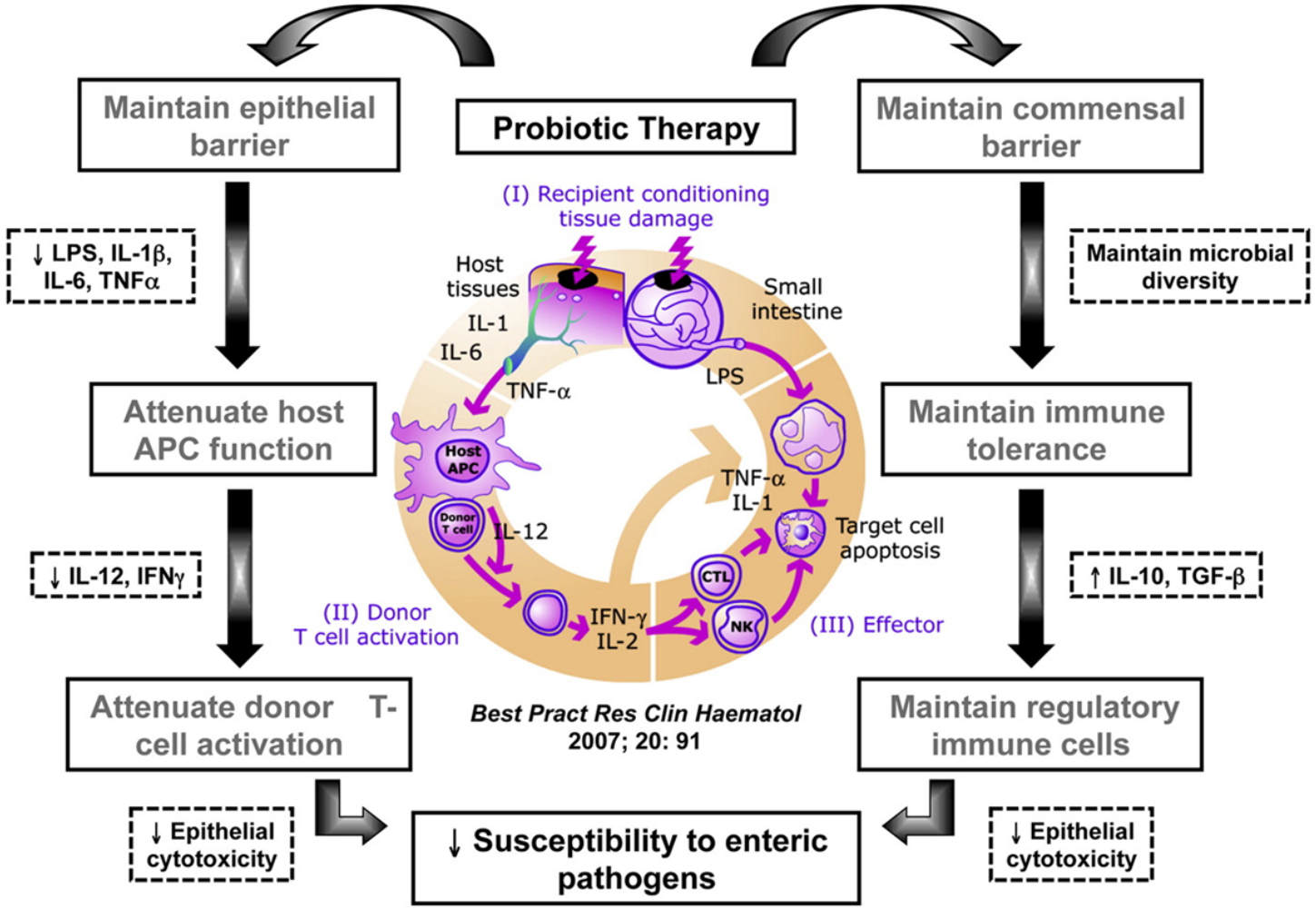
The Role of T Lymphocytes in the Biology and Treatment of aGvHD



aGVHD, acute GVHD

Zeiser R, et al. *Blood*. 2016;127(25):3117-3126.

Transplant-Related Influences on the Intestinal Microbiota



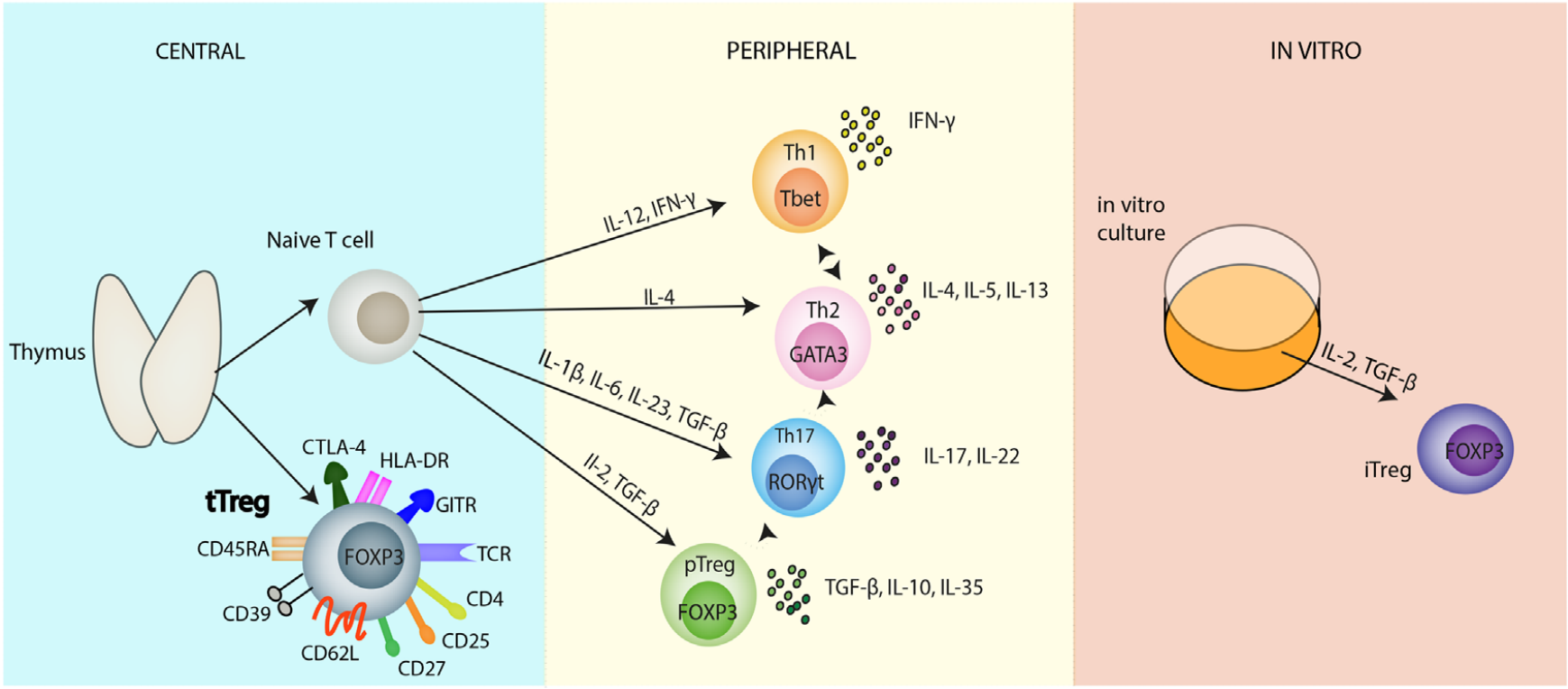
Docampo MD, et al. *Biol Blood Marrow Transplant*. 2015;21(8):1360-1366.

Regulatory T cell populations

thymus-derived natural Tregs (tTregs)

peripherally induced Tregs (pTregs)

in vitro generated Tregs iTregs



2. at least two well-defined populations of pTregs;

- Th3, identified from their role in oral tolerance through the secretion of TGF-β
- Tr1, by their role in preventing autoimmune colitis and ability to secrete IL-10

Safinia *Front. Immunol. 6:438.2015*

Clinical studies with Tregs

- ***Indirect evidence for a role of Tregs on GvHD***

High proportions of regulatory T cells in PBSC grafts predict improved survival after allogeneic hematopoietic SCT

- prospective study of 94 adult allogeneic PBSC transplants
- the median Tregs (CD3+CD4+CD25+FOXP3+CD127dim/ –) dose transplanted was 4.7×10^6/kg, with Tregs accounting for a median of 2.96% of CD4+ T cells
- Patients transplanted with grafts containing a Treg/CD4+ T-cell ratio above the median had a 3-year overall survival of 75%, compared with 49%

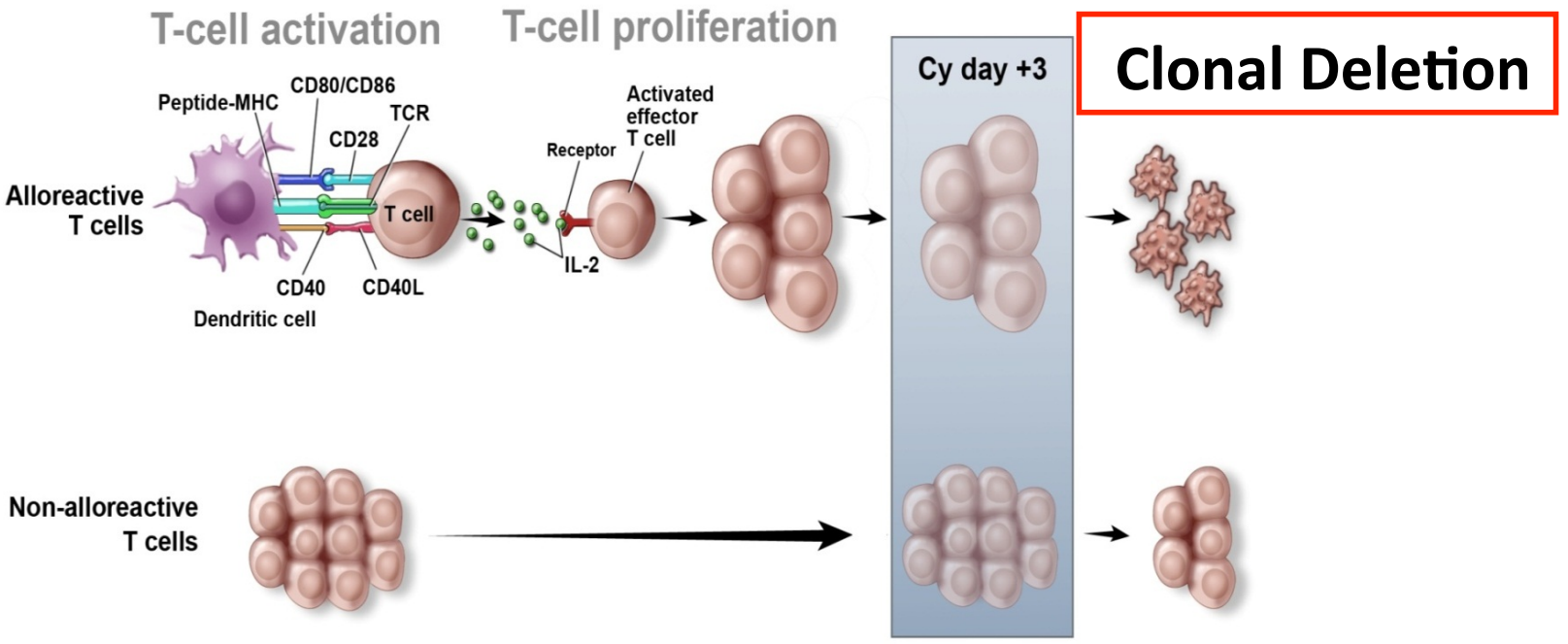
Danby, RD.: Bone Marrow Transplantation (2015), 1–9

Clinical studies with Tregs

- ***Drug induced, in vivo expansion of Tregs***

High dose, post transplantation cyclophosphamide to promote graft-host tolerance after allogeneic hematopoietic stem cell transplantation

Alloreactive T-cells: Direct recognition of foreign MHC of haploidentical recipient



Leo Luznik · Ephraim J. Fuchs
Immunol Res (2010) 47:65–77

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

TRANSPLANTATION

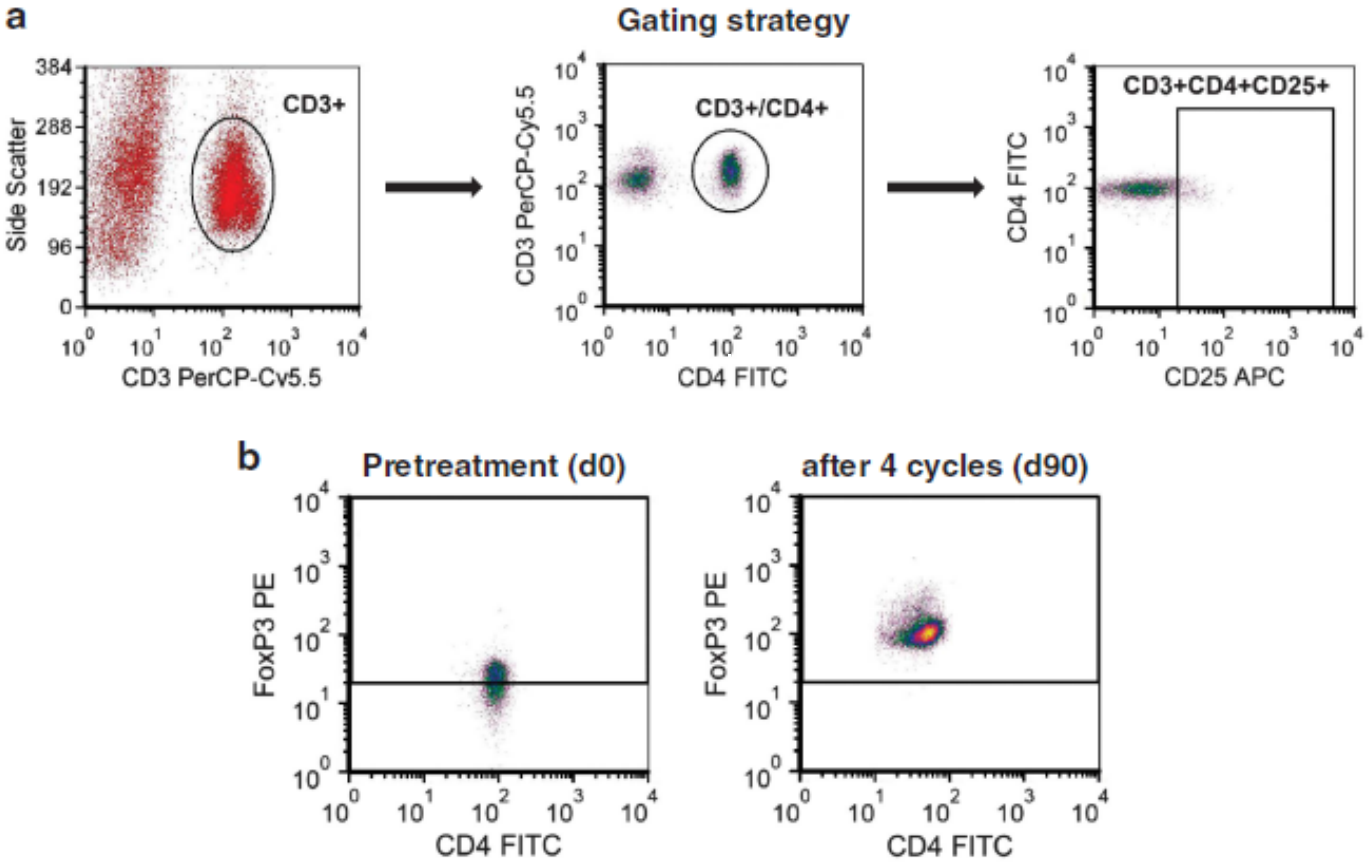
Donor CD4$^+$ Foxp3$^+$ regulatory T cells are necessary for posttransplantation cyclophosphamide-mediated protection against GVHD in mice

Sudipto Ganguly,[1] Duncan B. Ross,[2] Angela Panoskaltsis-Mortari,[3] Christopher G. Kanakry,[1] Bruce R. Blazar,[3] Robert B. Levy,[2] and Leo Luznik[1]

- PTCy treatment was associated with recovery of epigenetically stable and suppressive donor thymus derived Tregs in secondary lymphoid organs
- infusing Tregs-depleted grafts abrogated the GVHD-prophylactic activity of PTCy
- The efficacy of post-transplantation cyclophosphamide (PTCy) against GVHD is dependent on donor CD4+ Foxp31 Tregs

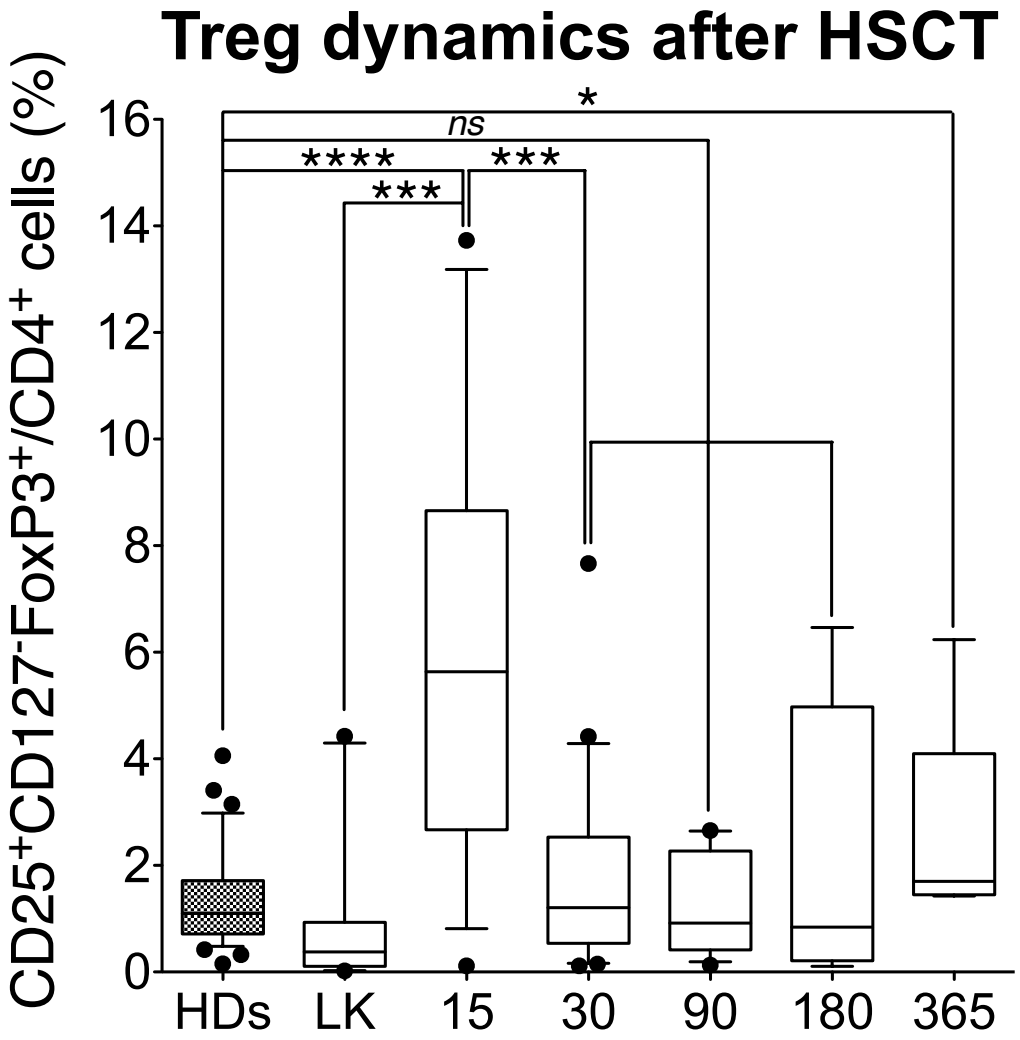
Ganguly, S Blood 2014;124(13):2131-2141)

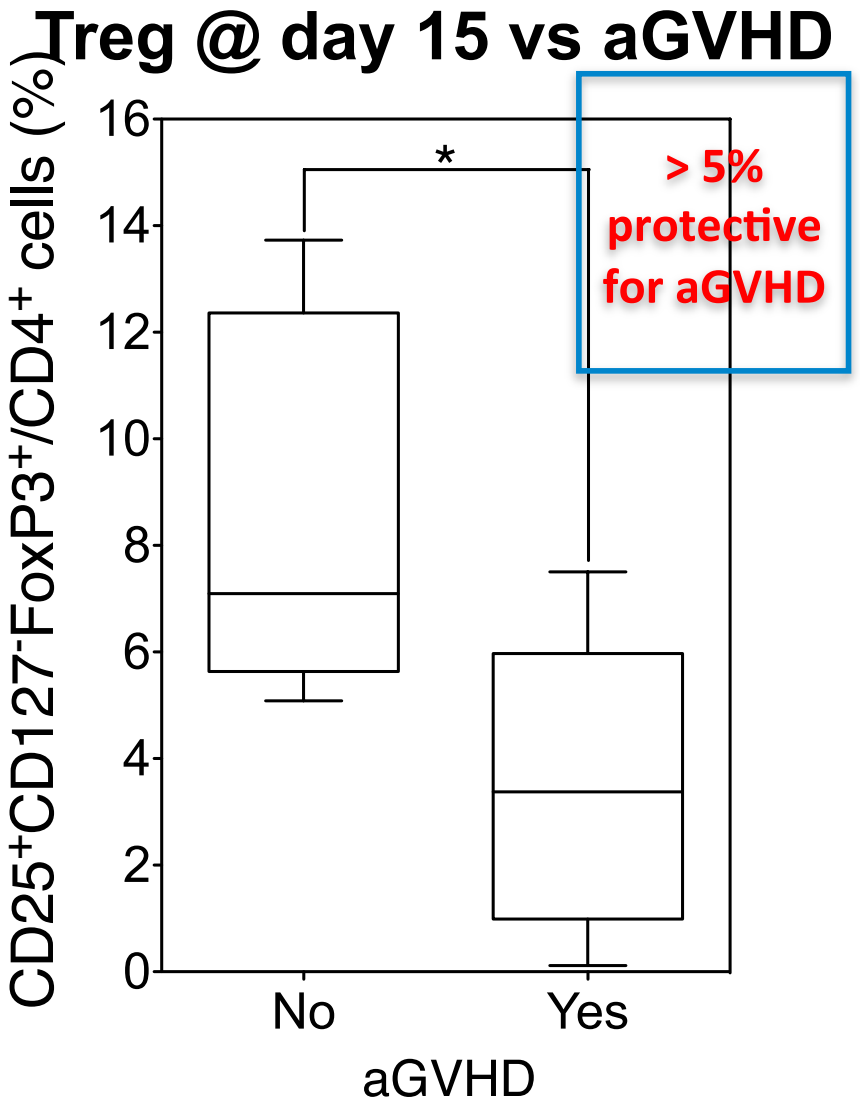
5-Aza has the capacity to increase Tregs



Schroeder T et al.: Leukemia, 27: 1910-13, 2013

TREG DYNAMICS

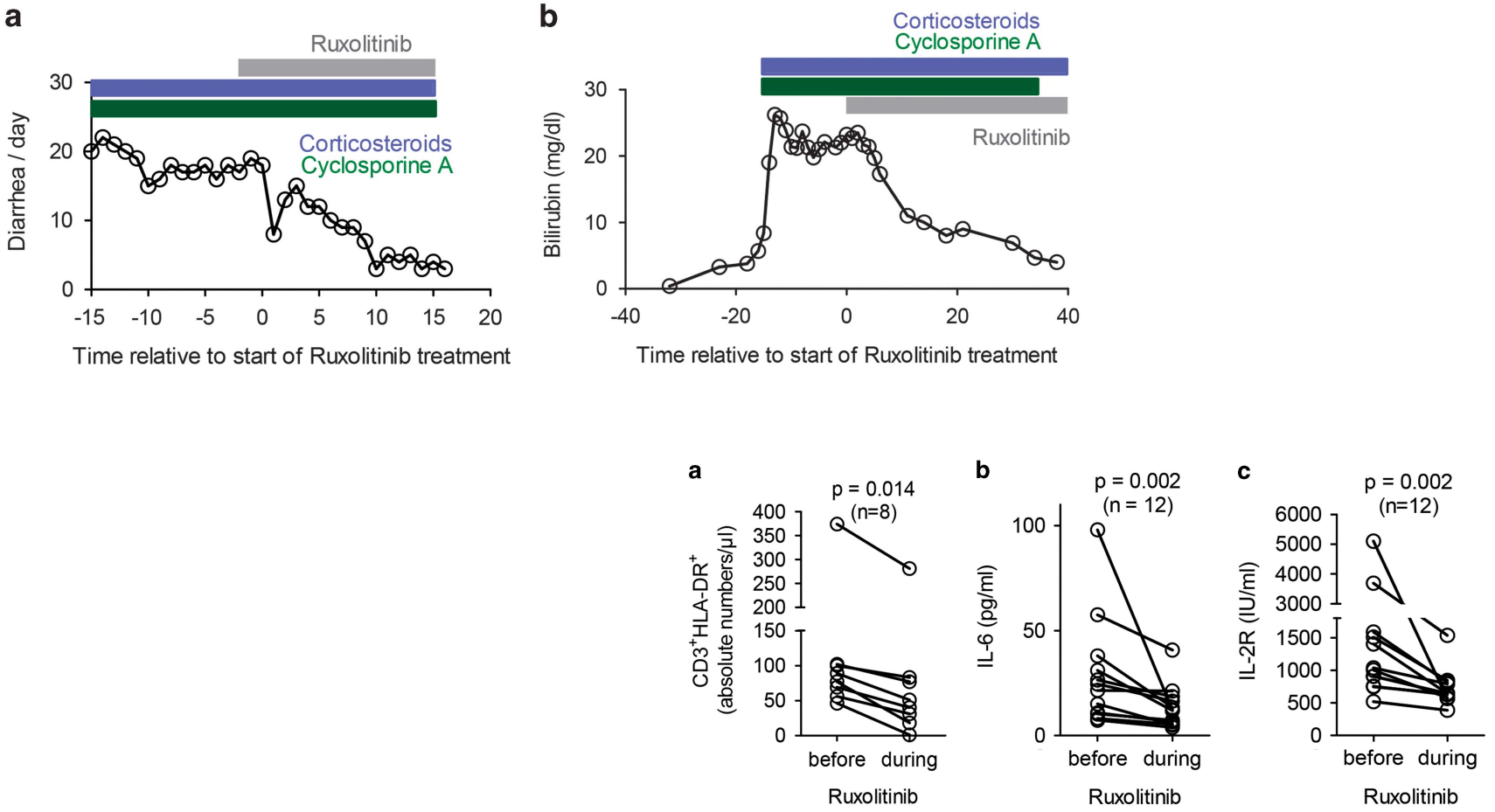




By courtesy of Prof F. Ciceri

ORIGINAL ARTICLE

Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey



Zeiser, R. Leukemia (2015) 29, 2062–2068

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 1, 2011 VOL. 365 NO. 22

Interleukin-2 and Regulatory T Cells in Graft-versus-Host Disease

- observational cohort study, in patients with chronic GVHD refractory to glucocorticoid therapy

- daily low-dose subcutaneous IL-2 (0.3×10^6, 1×10^6, or 3×10^6 IU per square meter of body-surface area) for 8 weeks

- the end points: safety and clinical and immunologic response

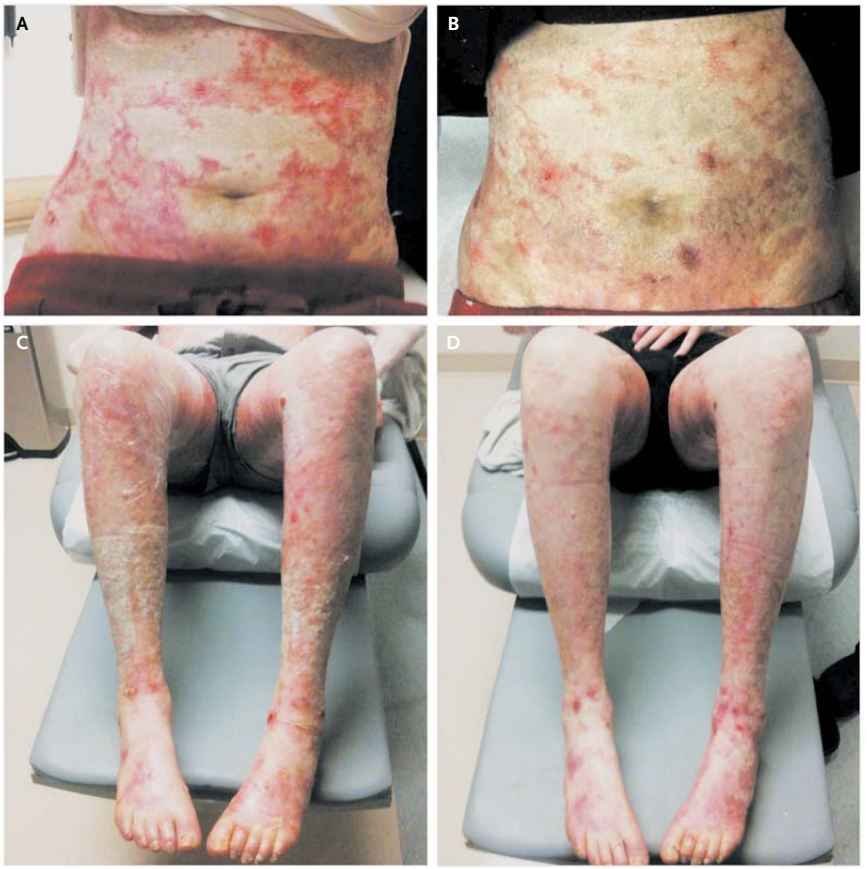
Outcomes	
Patients who could be evaluated	23
Partial response	12
Stable disease‡	11
Progression of disease	0

Koreth J. N Engl J Med 2011;365:2055-66

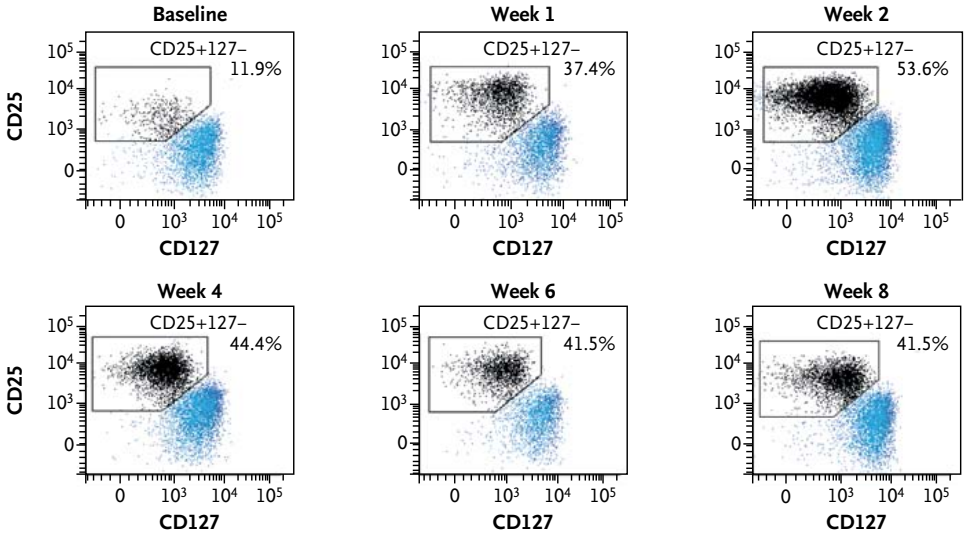
Clinical and Regulatory T (Treg) Cell Responses to 8 Weeks of Daily Low-Dose Interleukin-2

Before

After



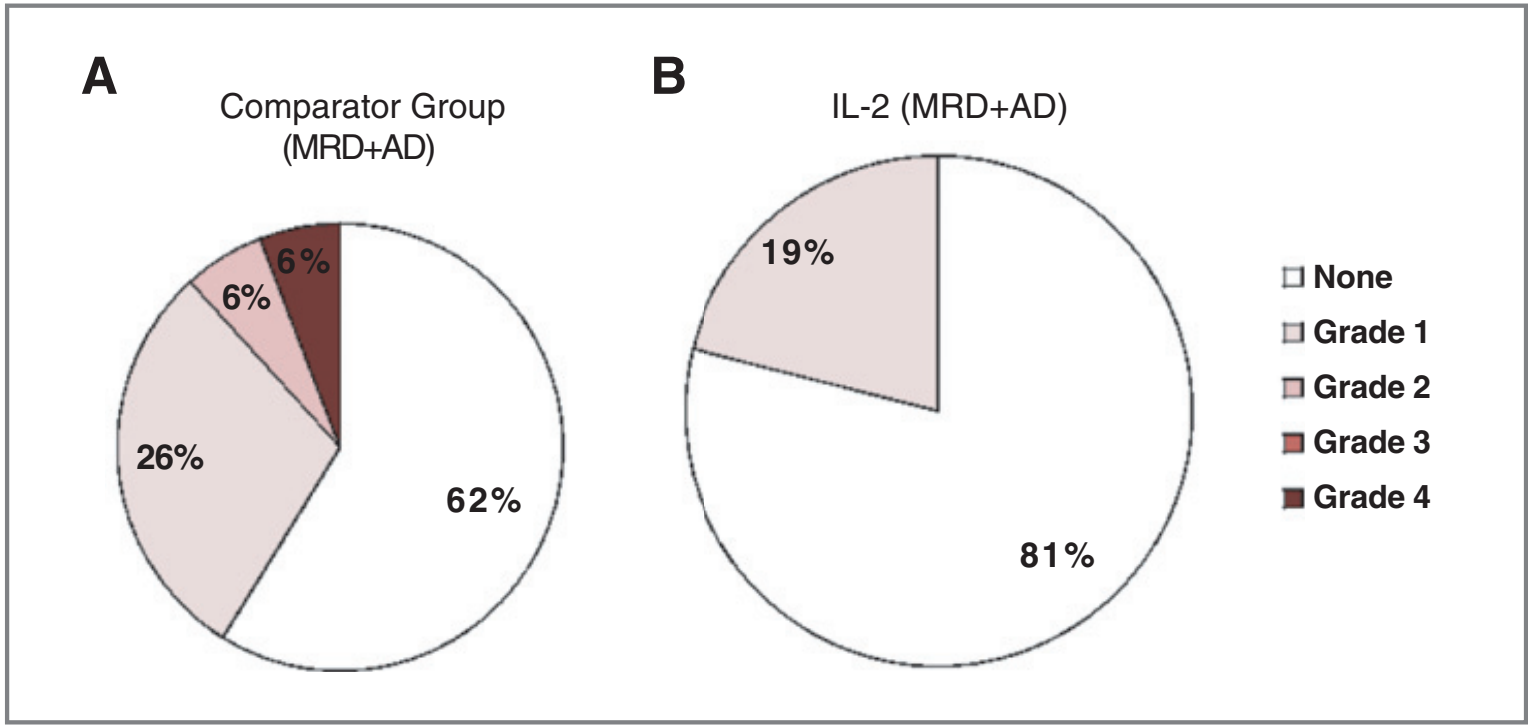
In vivo expansion of Tregs



Koreth J. N Engl J Med 2011;365:2055-66

Clinical Cancer Research

Ultra Low-Dose IL-2 for GVHD Prophylaxis after Allogeneic Hematopoietic Stem Cell Transplantation Mediates Expansion of Regulatory T Cells without Diminishing Antiviral and Antileukemic Activity



Incidence of aGVHD in patients who who did not receive ULD IL-2 (A) versus patients who did receive ULD IL-2

Kennedy-Nasser, AA. : Clin Cancer Res; 20(8); 2215–25.

Drug induced i*n vivo* expansion of Tregs

Author	Setting	patients	Intervention and doses	Main results
Koreth 2011 [38]	Treatment of steroid refractory chronic GvHD	29	Low-dose subcutaneous interleukin-2 ($0.3x10^6$, $1x10^6$, or $3x10^6$ IU per m^2 BSA) for 8 weeks.	The maximum tolerated dose of interleukin-2 was $1x10^6$ IU/ m^2. Administration was associated with Treg cell expansion in vivo and improvement of chronic GVHD in 12 of 23 evaluable patients
Koreth 2016 [39]	Patients with steroid-refractory chronic GvHD	35	Daily IL-2 (1x106IU/m2/d) for 12 weeks	20 of 33 (61%) evaluable patients had clinical responses. Compared with pre-treatment levels, Treg and NK-cell counts rose more than 5-fold and 4-fold respectively
Kennedy-Nasser 2014 [40]	GvHD prophylaxis in pediatric patients	16	Ultra low-dose IL-2 injections (100,000-200,000 IU/m^2 x3 per week)	No IL-2 patients developed grade 2-4 acute GvHD, compared with 4 of 33 (12%) of the comparator group. Among IL-2 recipients *in vivo* expansion of Tregs was observed
Peccatori 2005 [43]	GvHD prophylaxis in haplo transplant using PBSC grafts	121	Sirolimus based, calcineurin-inhibitor-free prophylaxis of GvHD	T cell reconstitution was rapid and skewed toward Tregs. The occurrence and severity of GvHD was negatively correlated with Tregs frequency.
Cieri 2015 [44]	GVHD prophylaxis in haploidentical using peripheral blood stem cells grafts	40	Post-transplant Cy and sirolimus-based GvHD prophylaxis (Sir-PTCy)	Grade II to IV and III-IV acute GVHD were 15% and 7.5%, respectively. The 1-year cumulative incidence of chronic GVHD was 20%. The number of circulating regulatory T cells at day 15 after HSCT were predictive of subsequent GVHD occurrence
Goodyear 2012 [49]	5-Aza administration after reduced intensity alloHSCT for AML	27	Monthly courses of 5-Aza after after reduced intensity alloHSCT	5-Aza after transplantation was well tolerated with a low incidence of GvHD. 5-Aza increased the number of Tregs within the first 3 months
Schroeder 2013 [51]	Aza and DLI administration as salvage therapy for relapse after alloHSCT	13	Aza 100mg/m^2 /day on days 1-5 or 75 mg/m^2/day on days 1-7 every 28 days and DLI after every second Aza cycle	After 4 Aza cycles was observed an increase in the absolute number of Tregs, especially in patients relapsing early after alloHSCT. A relatively low rate and mild presentation of GvHD despite a dose-escalating DLI schedule was reported
Choi 2014 and 2015 [54, 55]	Prevention of GvHD after alloHSCT	50	Vorinostat (100 mg or 200 mg, twice a day) combined with standard immunoprophylaxis for GvHD	Grade 2-4 acute GvHD by day 100 was lower than expected 22% (95% CI 13-36). Vorinostat enhances Tregs after allo-HSCT
Zeiser 2015 [56]	Treatment of steroid-refractory acute and chronic GvHD	95	Ruxolitinib, as an add-on immunosuppression therapy, at a dose of 5–10 mg orally twice daily	The overall response rate was 81.5% and 85.4% for acute GvHD and cGvHD, respectively. The rate of GvHD-relapse was 7% and 6% for aGvHD and cGvHD, respectively

Lussana, F et al.: BMT, in press

Ex vivo selection (and expansion) of T-regs: from the laboratory to clinical application (questions to be answered)

- Which population of Tregs is the most effective?
- What is the best method for Tregs isolation?
- Can Tregs be expanded in vitro? Do Tregs need to be antigen-specific?
- Warning: can in vitro selected Tregs be reprogrammed in vivo to pro-inflammatory cells?

Bead-enrichment protocols

- Several GMP-compliant Tregs isolation protocols have been published [1,2]

- The level of purity of the Tregs resulting from bead enrichment was 40-60%.

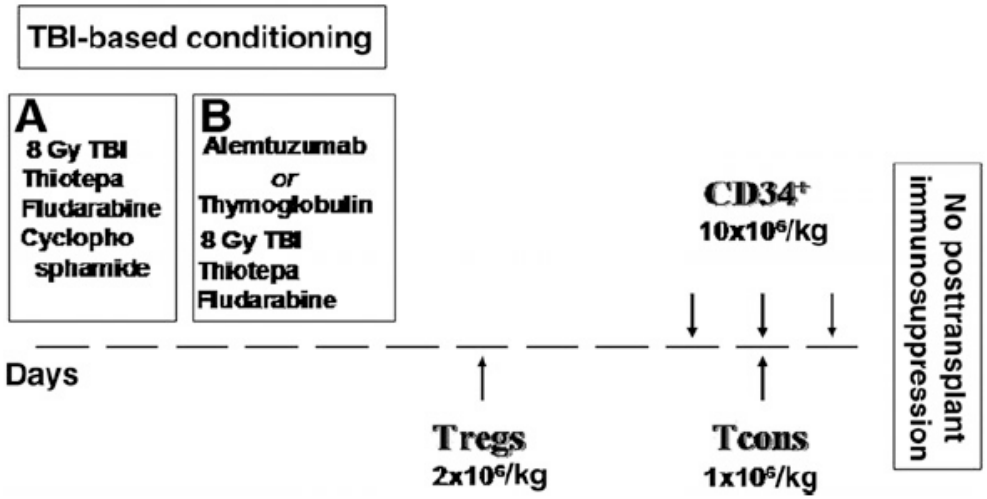
- These cells are more heterogeneous than those described by Edinger's group!

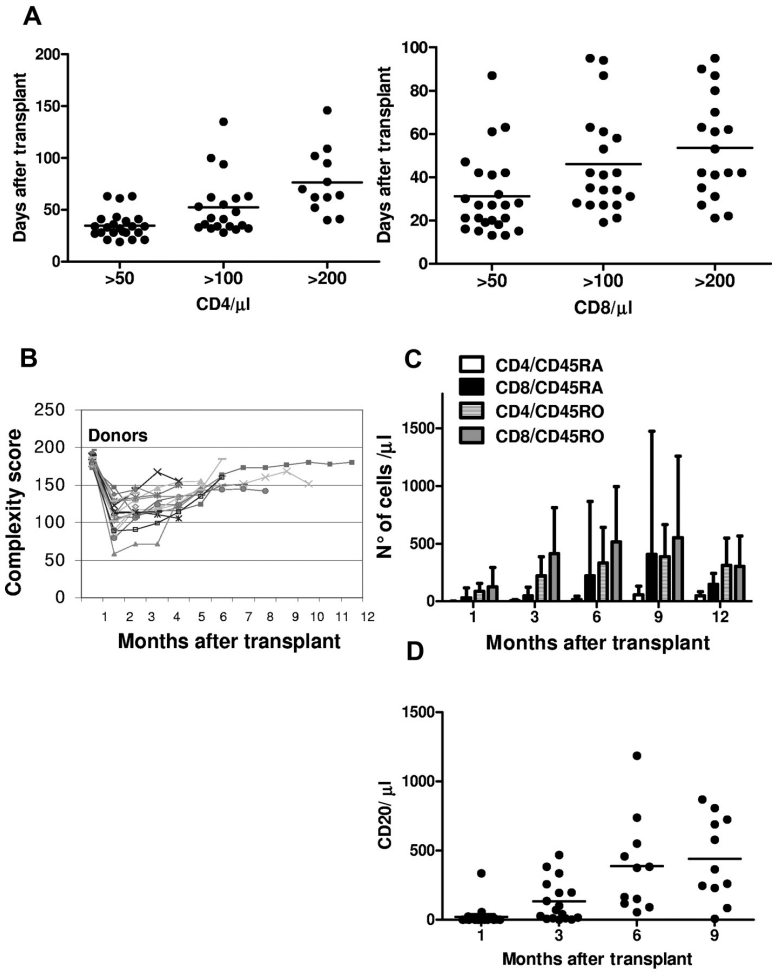
- Purity is obviously important for translating Tregs cell therapy to the clinic, as contaminating conventional T cells could contribute to rejection or GvHD

1) Hoffmann P, et al. Biol Blood Marrow Transplant 2006;12:267–274.

2) Peters JH, et al. PLoS ONE 2008;3: e2233.

The clinical use of Tregs after HaploHSCT





Mauro Di Ianni et al. Blood 2011;117:3921-3928

blood

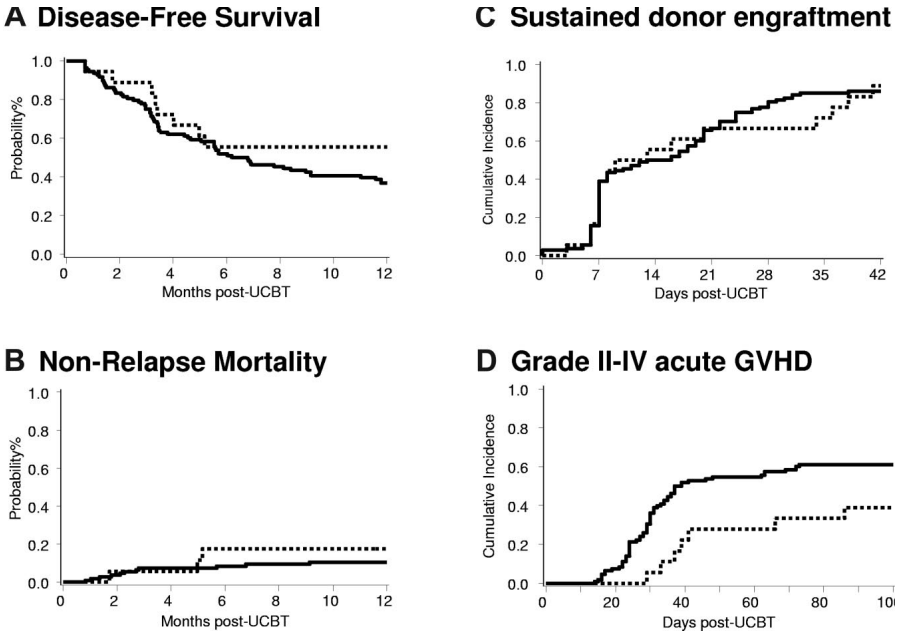
Clinical studies with Tregs

- ***Can Tregs be expanded in vitro?***

Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics

Claudio G. Brunstein,[1,2] Jeffrey S. Miller,[1,2] Qing Cao,[1] David H. McKenna,[3] Keli L. Hippen,[1,4] Julie Curtsinger,[1,5] Todd DeFor,[1] Bruce L. Levine,[6] Carl H. June,[6] Pablo Rubinstein,[7] Philip B. McGlave,[1,2] Bruce R. Blazar,[1,4] and John E. Wagner[1,4]

- CD4 CD25 FoxP3 (Tregs) enrichment from cryopreserved UCB
- 18 day expansion culture with anti-CD3/anti-CD28 antibody-coated beads and IL-2
- Patients received a dose of 0.1-30 x 10^5UCB Tregs/kg after double UCB transplant
- UCB Treg could be detected for 14 days, with the greatest proportion of circulating CD4 CD127 FoxP3 cells observed on day 2



blood

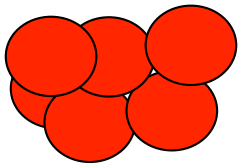
Brunstein C, A. Blood 2011;117(3): 1061-1070

Adoptive cellular therapy with Tregs

Author	Setting	Total patients	Intervention and Tregs doses	Main results
Brunstein 2011 [26]	Prevention of GvHD after double-UCB transplantation	23	Infusion immediately after transplantation of ex vivo expanded UCB-derived natural regulatory T cells (nTregs) (average 64% FOXP3+ after expansion	Reduced incidence of acute GvHD relative to historical controls. Similar incidence of opportunistic infections or relapse.
Edinger and Hoffman 2011 [33]	Patients with high risk of leukemia relapse after alloHSCT	9	Infusion of freshly isolated donor Treg. Up to 5 x10^6 cells per kg (>50% FOXP3+). After an observation period of 8 weeks, additional Tcons cells were administered at the same dose to promote GvL activity	No Treg transfusion-related adverse events were observed despite the absence of pharmacologic immunosuppression. Neither GvHD nor opportunistic infections or early disease relapses occurred after Treg transfusion.
Di Ianni 2011 [18] **and Martelli 2014**[19]	Improving the quality of immune reconstitution after haploidentical ex vivo T-cell depleted tranplantation	43	Infusion of donor CD4/CD25+ Tregs, followed by an inoculum of Tcons and positively immunoselected CD34 + cells. Patients did not receive any prophylactic immunosuppression.	Effective not only in improving the immune reconstitution but it was also associated to a low incidence of leukemia and GvHD prevention. NRM remained significantly high
Trzonkowski 2009 [35]	Patients with chronic GvHD and resistant acute GvHD	2	Infusion of in vitro expanded donor Treg (90% FOXP3+, dose of 1x10^5/Kg for patient with chronic GvHD and (3x10^6/Kg for patient with resistant acute GvHD	Contributed to amelioration of chronic GvHD and permitted to reduce immunosuppressive drugs. In contrast, for resistant acute GvHD no benefit was observed
Brunstein 2016 [27]	Prevention of GvHD after double-UCB transplantation	11	Treg doses from 3-100x10^6 Treg/kg	Tregs were safe and resulted in low risk of acute and chronic GvHD.

Lussana, F et al.: BMT, in press

Adoptive immunotherapy with Tregs: expanding the pool of eligible patients



Tregs isolation:
magnetic beads or GMP sorter

Option 1: Isolation and infusion

Pros: easy separation from apheresis
Cons: maximum 3-4 x10^6/kg

Option 2: Isolation, expansion (with anti-CD3/CD28 +IL2+ Rapamycin) and infusion

Pros: up to 20x10^6/kg
Cons: GMP facilities

Indications

Prevent GvHD in Haplo-HSCT for elderly/unfit patients

Treat early post transplant relapses (Treg-protected DLI)

Treat chronic GvHD

Lussana F et al.: BMT in press

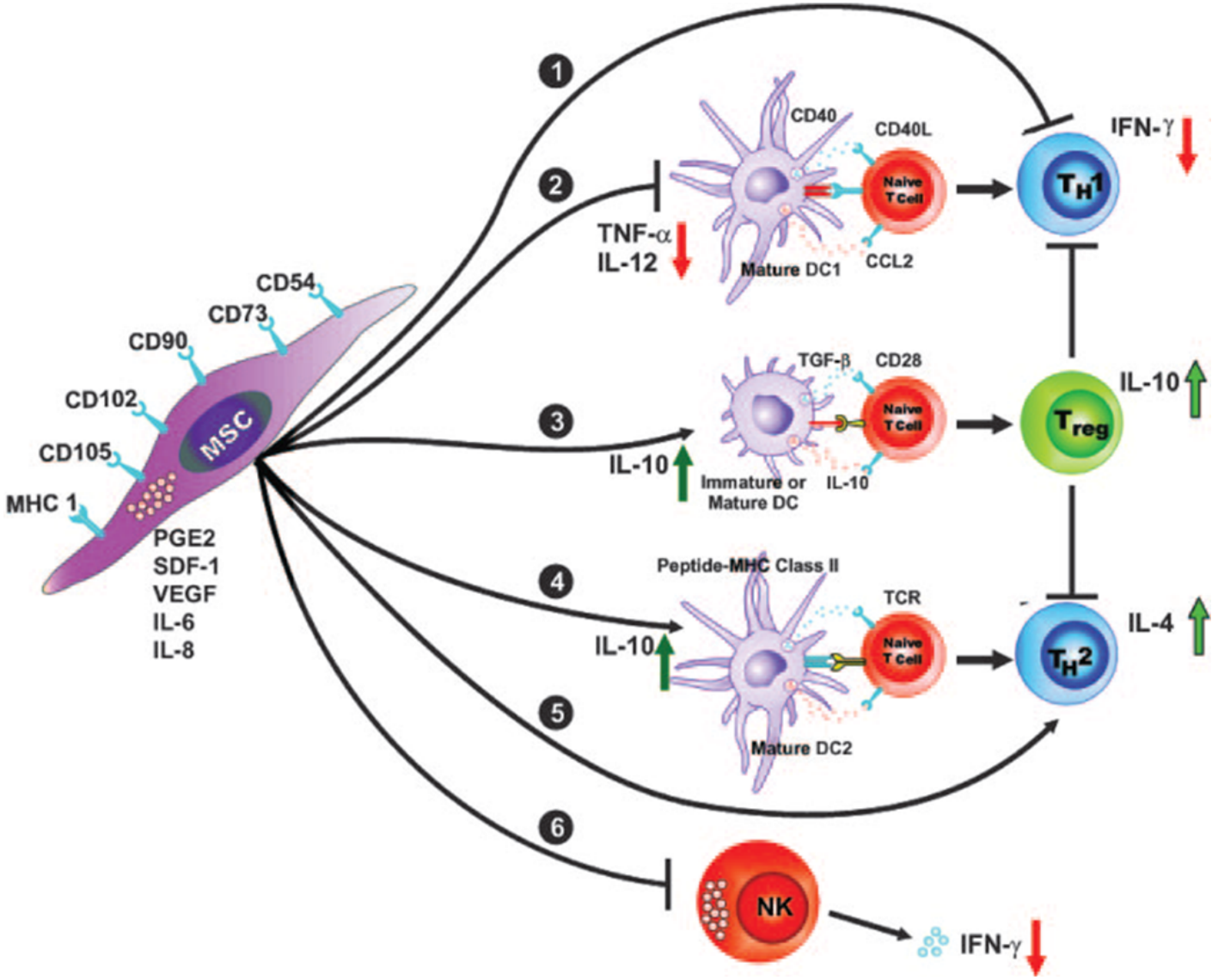
Warning about adoptive T cell therapy with T-regs

- Can T-regs, lose Foxp3 and transform into pathogenic cells under inflammatory conditions in vivo?

- The possibility of them converting into pathogenic effector T cells could be a critical threat to the host in the context of autoimmunity

Zhou X, .: Plasticity of CD4(+) FoxP3(+) T cells. Curr. Opin. Immunol. 2009;21:281–5.

The Role of MSCs in the Biology of GVHD



Aggarwal S, et al. *Blood*. 2005;105(4):1815-1822.

Clinical results MSC for treatment of grade III-IV aGvHD

Author	Year	No pts	Preparation	Response CR/PR/Other	Outcome Alive/Dead
Le Blanc	2004	1	Custom	1	1/0
Ringden	2006	8	Custom	5/0/3	5/3
Prasad	2007	12	Custom	6/6/0	6/6
Fang	2007	6	Custom	5/0/1	4/2
Mulle	2008	2	Custom	0/0/2	1/1
LeBlanc	2008	55	Custom	30/9/16	21/34
VonBonin	2009	13	Custom	1/1/11	4/9
Muroi	2009	2	Custom	0/0/2	0/2
Kebriaei	2009	31	Custom	24/5/2	22/9
Osiris Thera	2009	260	Industrial	CR 40%	NR
Osiris Thera	2009	192	Industrial	NR	NR

- Adapted from Sato et al.: J Clin Exp Hematol, 2010

Outcomes after MSCs therapy

- Sample size: 40 patients (adults/children 25/15)
- Evaluation of response: At day +28 after the last MSC infusion

- Treatment response:

Adults	CR 16%	CR+PR 68%
Children	CR 47%	CR+PR 67%

- Median follow up from last MSC infusion: 250 (30-1066) days
- Deaths = 17
  - Relapse = 3
  - NRM = 14

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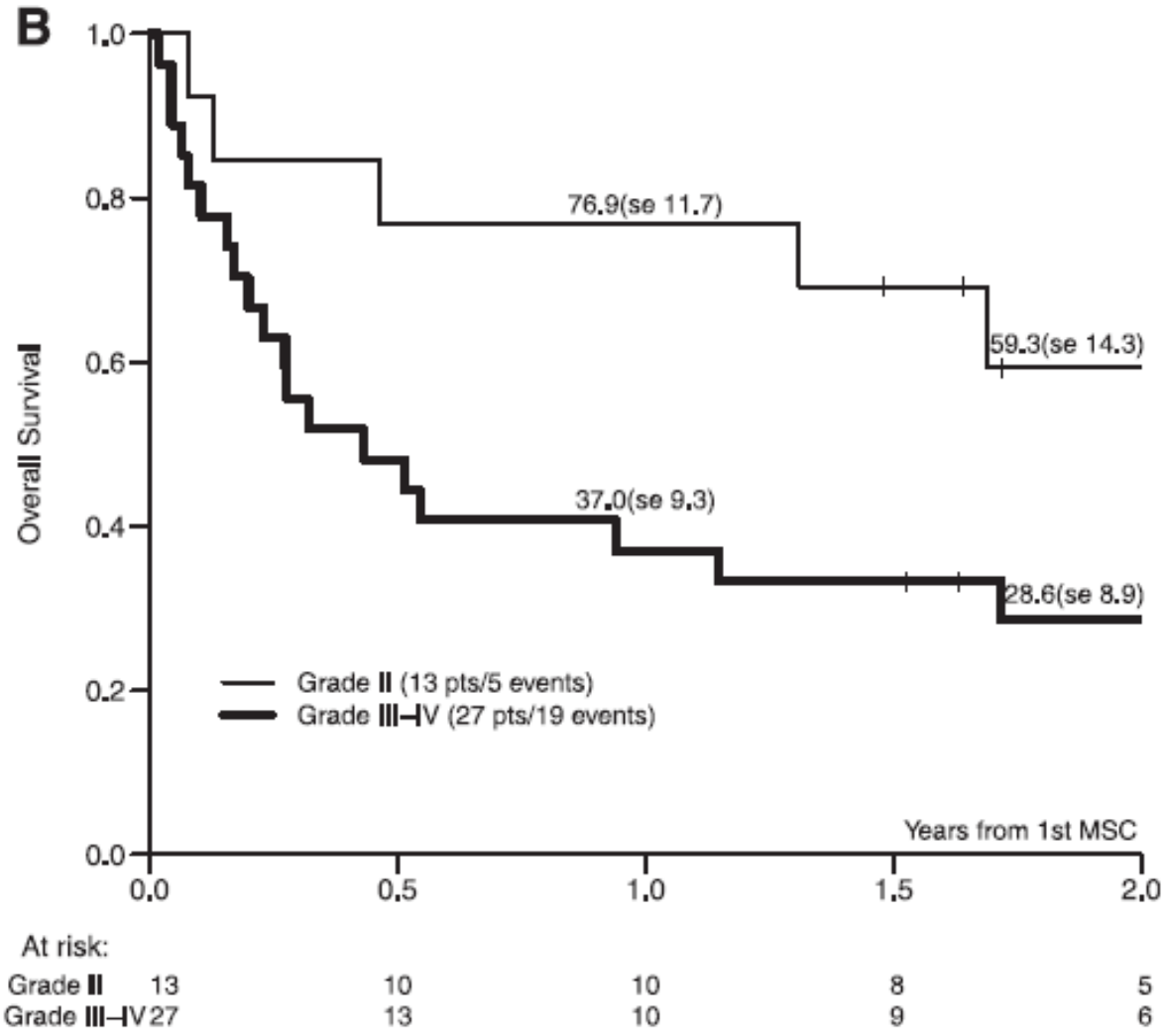
M. Introna et al. / Biol Blood Marrow Transplant 20 (2014) 375-381

Survival according to GVHD grade



M. Introna et al. / Biol Blood Marrow Transplant 20 (2014) 375-381

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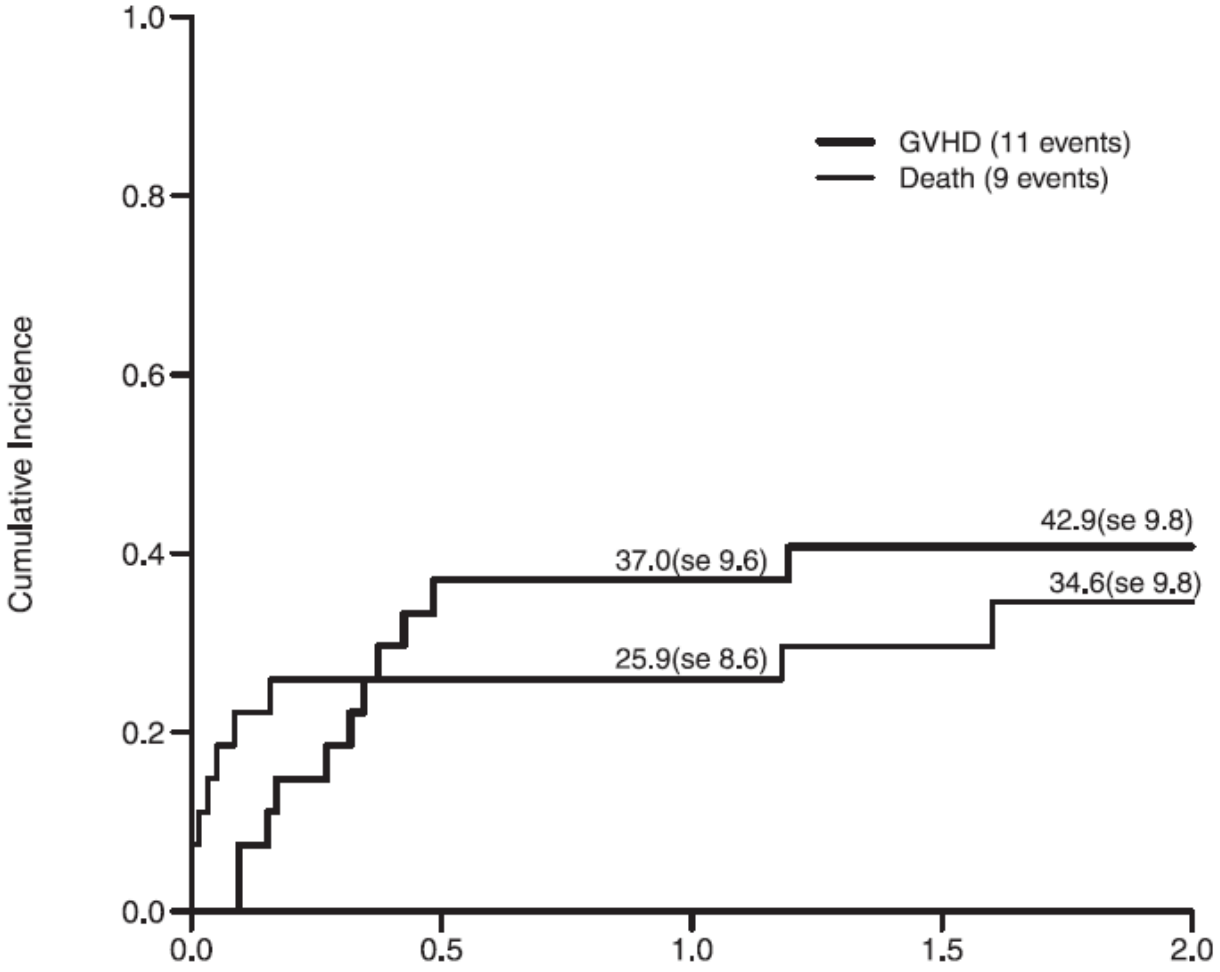
Cumulative incidence of GVHD and death post response, for patients who responded to MSC



M. Introna et al. / Biol Blood Marrow Transplant 20 (2014) 375-381

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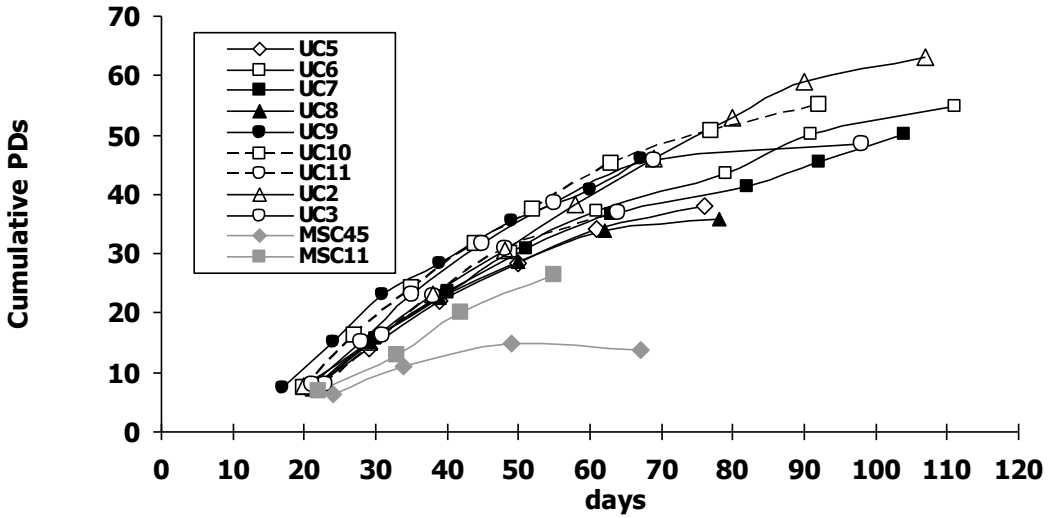
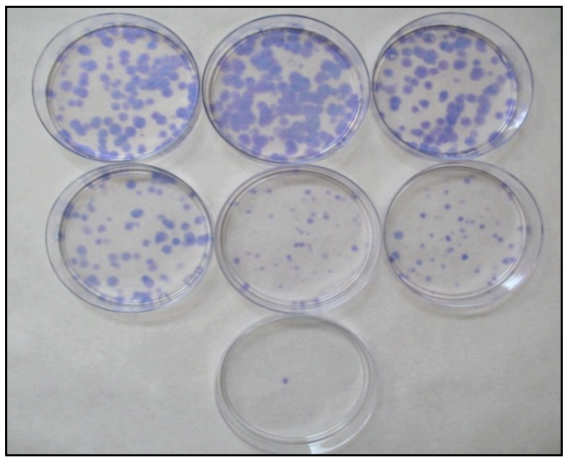
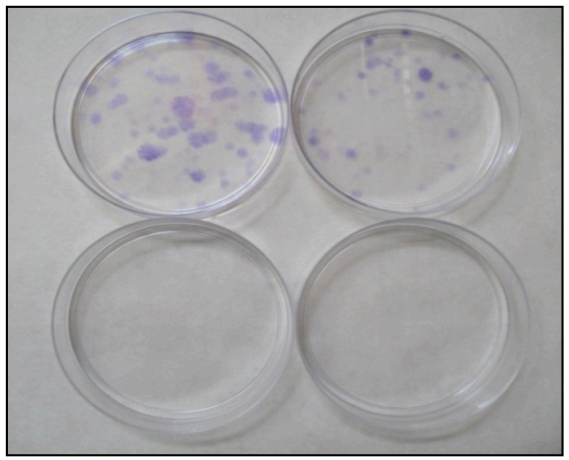
The umbilical cord wall as an alternative source of MSCs

- MSC obtained have comparable phenotype and immunosuppression activity to BM derived MSC
- Easily accessible, sterile and abundant source
- Much more abundant content of CFU-F precursors and very high yields





UC10



MSC11

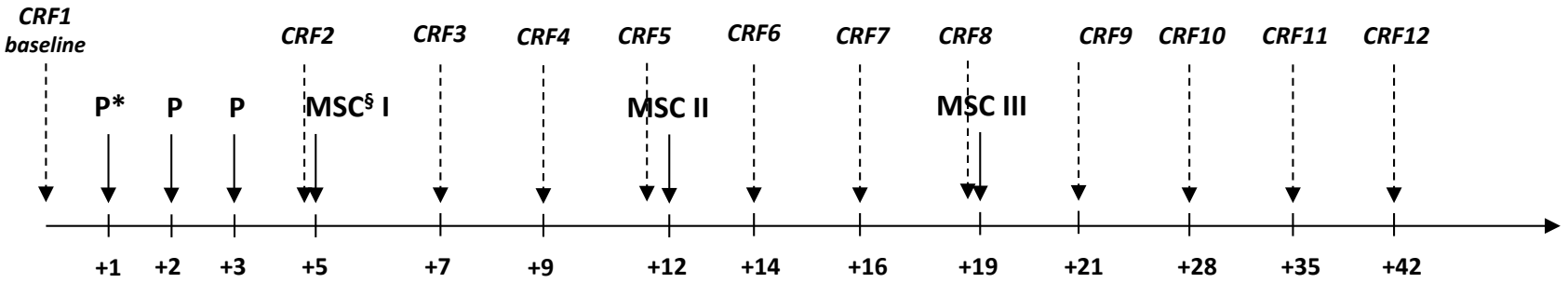
Capelli C et al.: Cytotherapy 2011;13:786-801

The umbilical cord wall as an alternative source of MSCs

UMBILICAL CORD DERIVED MESENCHYMAL STROMAL CELLS (UC-MSC) FOR THE TREATMENT OF SEVERE (GRADE III-IV) STEROID-RESISTANT GRAFT VERSUS HOST DISEASE: A PHASE I/II TRIAL

EudraCT number 2012-000582-21

ClinicalTrials.Gov Identifier NCT02032446



*** P = pentostatin, dose 1 mg/m^2**

§ MSC doses:

a) 3 patients → 3 infusions of 1x10^6 cells /kg

b) 3 patients → 3 infusions of 2x10^6 cells /kg

c) 3 patients → 3 infusions of 3x10^6 cells /kg

Umbilical Cord Derived Mesenchymal Stromal Cells (UC-MSC) for The Treatment of Severe (Grade III-IV) Steroid-Resistant GvHD. A Phase I/II Trial
EudraCT Number 2012-000582-21

Patients Enrolled	**15**
Patients with aGvHD	
(overall max grade 3)	**5**
(overall max grade 4)	**9**
overlap syndrome	**1**
CR at day +28 from last MSC infusion	**4**
Death	**9**
In Follow-up	**4**
Out of Study	**1**
Lost to f-up	**1**

Unmet clinical needs of patients with steroid resistant aGvHD

Poor efficacy and inconsistent (not reproducible) results of treatment

Poor quality of life

High rate of infectious complications

High rate of GvHD and disease relapse

Challenges for clinical investigation

1. CR/PR rate does not support the choice of any specific agent for steroid refractory GVHD

2. Define a consensus on the time to determine best response to individual therapies

3. Comparative data to demonstrate superior efficacy for any particular agent over others

4. Define the most appropriate efficacy endpoints in comparative trials

5. Careful evaluation of toxicity, infectious complications, and relapse

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AIL ASSOCIAZIONE ITALIANA CONTRO LE LEUCEMIE-LINFOMI E MIELOMA ONLUS

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