PACT Workshop 2010 St. Paul, MN

# Adoptive Transfer of Regulatory T Cells in UCB Transplantation

**Claudio Brunstein**
**University of Minnesota**

## Definition

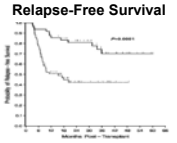
Tregs are a specialized subpopulation of T cells that act to suppress activation of the immune system and thereby maintain homeostasis and tolerance to self-antigens.

## The problem

- **Immune reconstitution**
- **GVHD after allogeneic transplantation**
- **Strategies used reduce GVHD**

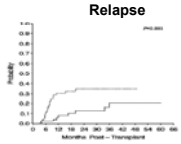
## Children who had a herpesvirus specific Ag response had better RFS and lower relapse rates

**Relapse-Free Survival**



| Variable | Hazard Ratio (95% CL) | P |
|---|---|---|
| Time to relapse or death | | |
| Gender | | |
| Female | 2.9 (1.6, 5.2) | .0003 |
| Male | 1 | |
| Remission status | | |
| Worse than CR2 | 2.4 (1.3, 4.4) | .004 |
| CR1 or CR2 | 1 | |
| Proliferative response | | |
| Negative | 3.6 (1.8, 7.0) | .0002 |
| Positive | 1 | |

**Relapse**



| Variable | Hazard Ratio (95% CL) | P |
|---|---|---|
| Time to relapse | | |
| Gender | | |
| Female | 6.6 (2.8, 15.5) | .0001 |
| Male | 1 | |
| Remission status | | |
| Worse than CR2 | 3.6 (1.6, 7.8) | .001 |
| CR1 or CR2 | 1 | |
| Proliferative response | | |
| Negative | 3.7 (1.6, 8.5) | .003 |
| Positive | 1 | |
| Performance status | | |
| ≤80 | 3.4 (1.4, 8.4) | .007 |
| >80 | 1 | |

Parkman et al BBMT 2006

## Patients with NO specific Ag response had a higher risk of infectious death

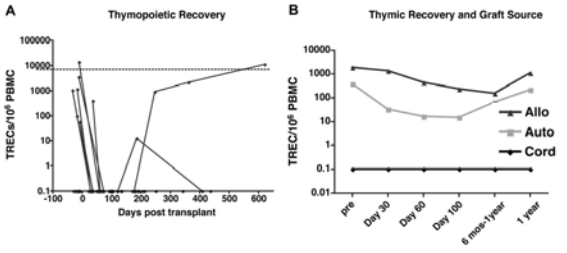
Table 4. *Causes of Death/Relapse by Antigen-Specific Proliferative Response Status**

| | Negative (n = 69) | Positive (n = 48) |
|---|---|---|
| Relapse | 21 | 8 |
| TRM | 4 | 0 |
| Acute GVHD | 1 | 1 |
| Chronic GVHD | 2 | 2 |
| Infection | 10 | 1 |
| Viral | 5 | 1 |
| Fungal | 3 | 0 |
| Bacterial | 1 | 0 |
| Other | 1 | 0 |
| Total (relapse or death) | 38 | 12 |

TRM indicates treatment-related mortality.
*Comparison of proportion of infectious deaths (10/69 versus 1/48) gives $P = 0.04$ when using 2-sided Fisher exact test.

Parkman et al BBMT 2006

## UCB recipients had poor thymic function even before transplantation, and it persisted post-transplantation

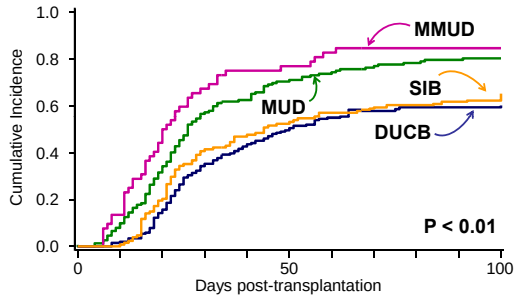



Komanduri et al. Blood 2007

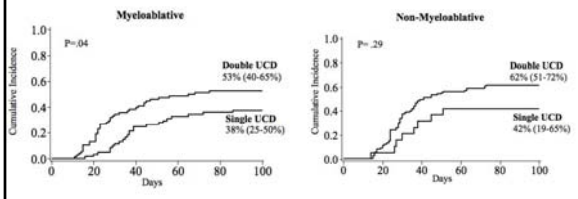
# The problem

- Immune Reconstitution
- **GVHD after allogeneic transplantation**
- Strategies used reduce GVHD

## Grade II-IV Acute GvHD by Donor Type




## Grade II-IV Acute GVHD
### Single versus Double UCB




*McMillan et al. Blood 2009*

# The problem

- Immune Reconstitution
- GVHD after allogeneic transplantation
- **Strategies used reduce GVHD**

# Strategies used reduce GVHD

- **Pharmacological post-transplant immunosuppression**
  - **Calcineurin inhibitors**
  - **Anti-metabolites**
  - **m-TOR inhibitors**
- **Ex vivo and in vivo T-cell depletion**
  - **Anti-T cell antibodies**
  - **Elutriation/rosetting**

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- **Adoptive transfer of Treg**

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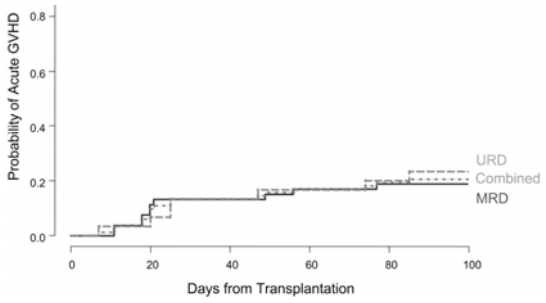
  - Anti-Tcell antibodies
  - Elutriation/rosetting
- Adoptive transfer of Treg

Extended follow-up of methotrexate-free immunosuppression using sirolimus and tacrolimus in related and unrelated donor peripheral blood stem cell transplantation

Corey Cutler,[1] Shuli Li,[2] Vincent T. Ho,[1] John Koreth,[1] Edwin Alyea,[1] Robert J. Soiffer,[1] and Joseph H. Antin[1]

[1]Medical Oncology and [2]Biostatistical Science and Computational Biology, Dana-Farber Cancer Institute, Boston, MA

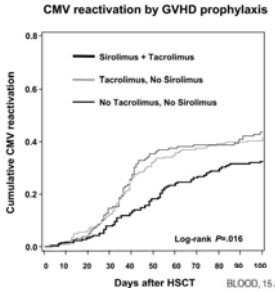



BLOOD, 1 APRIL 2007 • VOLUME 109, NUMBER 7

Sirolimus-based graft-versus-host disease prophylaxis protects against cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation: a cohort analysis

Francisco M. Marty,[1,3,4] Julie Bryar,[1] Sarah K. Browne,[1,4] Talya Schwarzberg,[1,4] Vincent T. Ho,[2,4] Ingrid V. Bassett,[1,4] John Koreth,[2,4] Edwin P. Alyea,[2,4] Robert J. Soiffer,[2,4] Corey S. Cutler,[2,4] Joseph H. Antin,[2,4] and Lindsey R. Baden[1,3,4]

[1]Division of Infectious Diseases, [2]Division of Medical Oncology, Brigham & Women's Hospital, [3]Dana-Farber Cancer Institute, [4]Harvard Medical School, Boston, MA




BLOOD, 15 JULY 2007 • VOLUME 110, NUMBER 2

# Strategies used reduce GVHD

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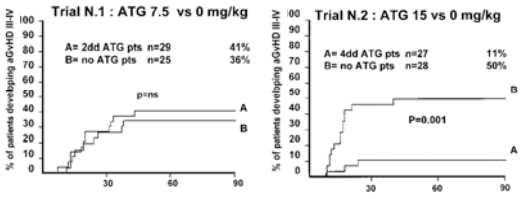
Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO)

Andrea Bacigalupo, Teresa Lamparelli, Paolo Bruzzi, Stefano Guidi, Paolo Emilio Alessandrino, Paolo di Bartolomeo, Rosa Oneto, Barbara Bruno, Mario Barbanti, Nicoletta Sacchi, Maria Teresa Van Lint, and Alberto Bosi for Gruppo Italiano Trapianti Midollo Osseo (GITMO)




BLOOD, 15 NOVEMBER 2001 • VOLUME 98, NUMBER 10

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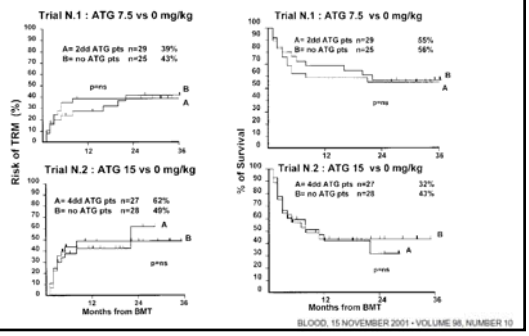


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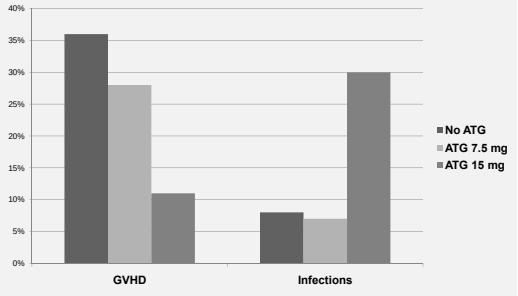


BLOOD, 15 NOVEMBER 2001 • VOLUME 98, NUMBER 10

# Strategies used reduce GVHD

- Pharmacological post-transplant immunosuppression
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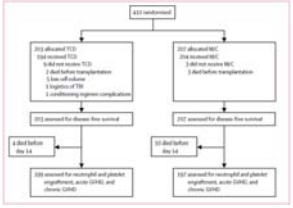
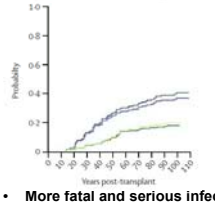
  - m-TOR inhibitors
- Ex vivo and in vivo T-cell depletion
  - Anti-Tcell antibodies
  - **Elutriation/rosetting**
- Adoptive transfer of Treg

Effect of graft-versus-host disease prophylaxis on 3-year disease-free survival in recipients of unrelated donor bone marrow (T-cell Depletion Trial): a multi-centre, randomised phase II–III trial

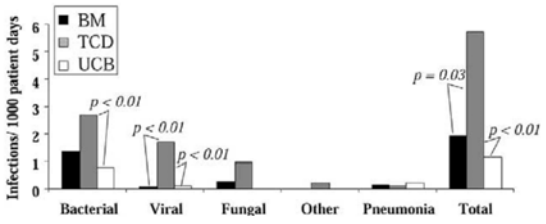
John E Wagner, John S Thompson, Shelly L Carter, Nancy A Kernan, for the members of the Unrelated Donor Marrow Transplantation Trial*






- **More fatal and serious infections**
- **More EBV PTLD**
- **More relapse in CML patients**

**The risk of serious infections between day +181 and +2 years, was significantly greater in recipients TCD grafts**




Barker et al BBMT 2005

## Summary

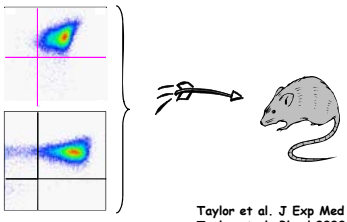
- Immune recovery of adults after UCBT is slow
- GVHD is a frequent complication of allogeneic HCT
- Strategies to reduce GVHD adversely impact immune recovery

## Strategies used reduce GVHD

- Pharmacological post-transplant immunosuppression
  - Calcineurin inhibitors
  - Anti-metabolites
  - m-TOR inhibitors
- Ex vivo and in vivo T-cell depletion
  - Anti-Tcell antibodies
  - Elutriation
- Adoptive transfer of Treg

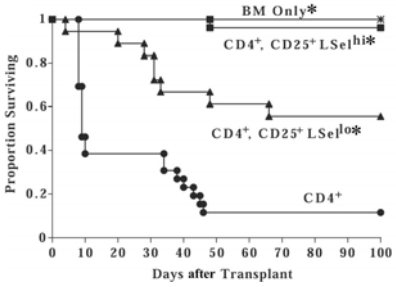
## Adoptive transfer of Tregs: pre-clinical development

## In murine models Treg cells enhance engraftment and prevent lethal GVHD



Taylor et al. J Exp Med 2001;11:1311
Taylor et al. Blood 2002;99:3493
Cohen et al. J Exp Med 2002;196:401
Hoffmann et al. J Exp Med 2002;196:389
Taylor et al. Blood 2004;104:3804

## LSel$^{hi}$, but not LSel$^{lo}$, Tregs protect against GVHD-associated mortality and morbidity

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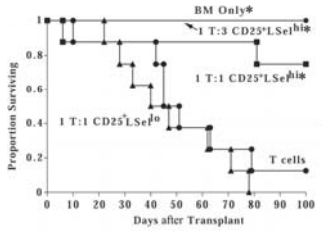
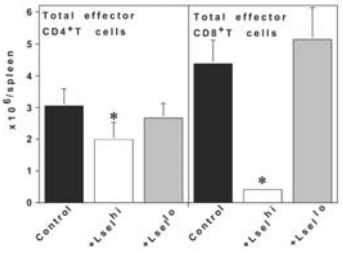


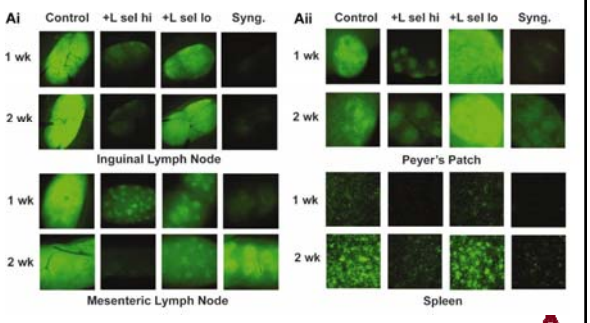

Taylor et al. Blood 2004;104:3804

## LSel$^{hi}$, but not LSel$^{lo}$, Tregs protect against GVHD-associated mortality and morbidity by interfering with expansion of effector T cells

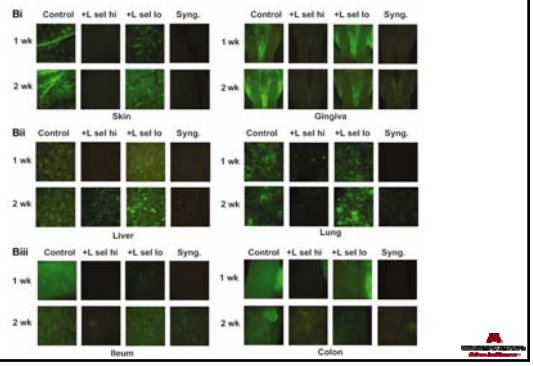



Taylor et al. Blood 2004;104:3804

## LSel$^{hi}$ inhibits effector T cells expansion in lymphoid organs



## LSel$^{hi}$ inhibits effector T cells expansion in GVHD target tissues




## Ex vivo expanded and activated host or donor-derived CD25+Sel$^{hi}$, but not Sel$^{lo}$, increases donor engraftment

| Donor | Host | Selectin | CD25+ origin | # Chimeric | % Donor |
|---|---|---|---|---|---|
| Exp 1 | | | | | |
| BALB/c | B6 | NA | NA | 0/10 | 0 |
| BALB/c | B6 | Sel$^{hi}$ | Donor | 10/10* | 87* |
| BALB/c | B6 | Sel$^{lo}$ | Donor | 3/10 | 3 |
| Exp 2 | | | | | |
| BALB/c | B6 | NA | NA | 0/10 | 0 |
| BALB/c | B6 | Sel$^{hi}$ | Donor | 9/9* | 70* |
| Exp 3 | | | | | |
| B6 | BALB/c | NA | NA | 0/8 | 0 |
| B6 | BALB/c | Sel$^{hi}$ | Host | 10/10* | 69* |
| B6 | BALB/c | Sel$^{lo}$ | Host | 0/8 | 0 |
| Exp 4 | | | | | |
| BALB/c | B6 $\beta_{2m}$-/- DNRII | NA | NA | 3/9 | 8 |
| BALB/c | B6 $\beta_{2m}$-/- DNRII | Sel$^{hi}$ | Donor | 7/9 | 37* |
| Exp 5 | | | | | |
| BALB/c | B6 $\beta_{2m}$-/- DNRII | NA | NA | 6/8 | 21 |
| BALB/c | B6 $\beta_{2m}$-/- DNRII | Sel$^{hi}$ | Donor | 9/9 | 74* |

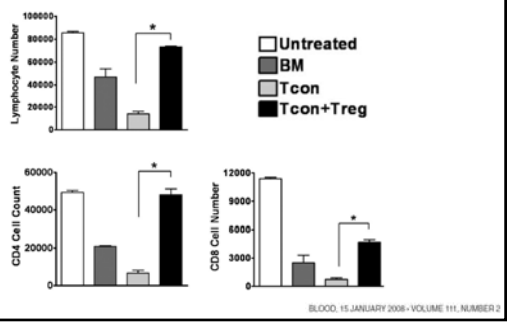
* p<0.02 COMPARED TO CONTROL

Taylor et al. Blood 2004;104:3804

## The impact of regulatory T cells on T-cell immunity following hematopoietic cell transplantation

Vu H. Nguyen,[1] Sumana Shashidhar,[1] Daisy S. Chang,[1] Lena Ho,[2] Neeraja Kambham,[3] Michael Bachmann,[4] Janice M. Brown,[1] and Robert S. Negrin[1]

[1]Department of Medicine, Division of Bone Marrow Transplantation; [2]Program in Immunology; [3]Department of Pathology; and [4]Department of Pediatrics, Stanford University, Stanford, CA



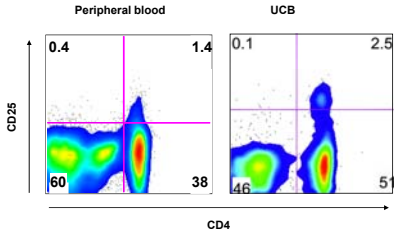

BLOOD, 15 JANUARY 2006 • VOLUME 111, NUMBER 2

## Summary

- In murine models Tregs were shown to:
  - Migrate priming lymphoid tissues
  - Migrate to GVHD target tissues
  - Inhibit GVHD
  - Improve engraftment
  - Improve T cell reconstitution

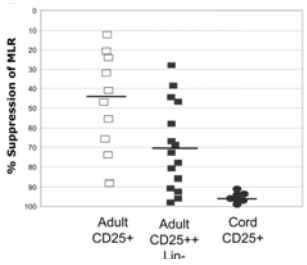
## Adoptive transfer of Tregs
## Why UCB?

## As compared to peripheral blood, in UCB Tregs are a more distinct cell population




Godfrey et al. Blood. 2005 15;105(2):750-8.
Godfrey et al. Blood. 2004 15;104(2):453-61.

## UCB-Derived Regulatory T Cells are highly and reproducibly suppressive




Godfrey et al. Blood. 2005 15;105(2):750-8.

## Regulatory T cell unit Expansion and Activation Culture

**CD25+ selection** → **Flask culture** → **Lot Release**

- Culture in X-VIVO 15
- Human AB serum 10% ,
- Anti-CD3/antiCD28-coated beads.
- Supplemented with IL-2 300 IU/mL

- Gram stain neg
- Endotoxin < 5 EU/kg
- Viability ≥ 70%
- CD4+/CD25+ ≥ 70%
- CD3+/CD8+ ≤ 10%
- Sterility neg
- Mycoplasma neg
- Bead count < 100/3 x$10^6$ cells

**Figure showing flow cytometry and phenotype pre and post separation and at the end of expansion**

## Adoptive transfer of UCB-Tregs:

## phase I clinical trial

Brunstein et al Blood 2010, in press

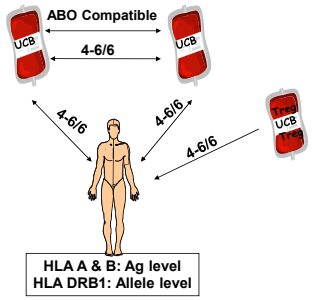
## Hypothesis

**We hypothesized that the infusion of ex vivo expanded and activated CD4+CD25+ Treg cells would protect patients from acute GVHD.**

**However, before we had to determine the safety of administration**

Brunstein et al Blood 2010, in press

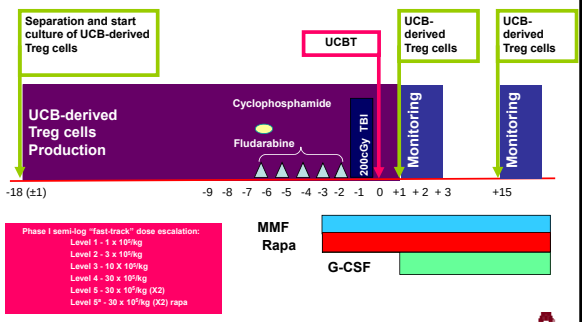
## Patient Eligibility




- Age > 12 y.o
- Heme malignancy
- Not eligible for myeloablation
- Patients must have 2 or 3 partially HLA matched UCB units.
- Treg unit ≥ $1.0 \times 10^7$/kg
- Graft units must be ABO matched

Brunstein et al Blood 2010, in press

## Phase I Treatment Plan
## UCB-derived Regulatory T Cells




Brunstein et al Blood 2010, in press

## Demographics

- 24 pts enrolled
- AUG 2007 and SEP 2009
- Median Age 52 years (r, 24-68 yrs)
- Median Weight 77 kg (r, 50-133 kg)
- Diagnosis
  - AML n=9
  - NHL n=7
  - ALL n=3
  - CLL n=3
  - Hodgkin's n=1
  - PLL n=1

Brunstein et al Blood 2010, in press

## Expansion and Function

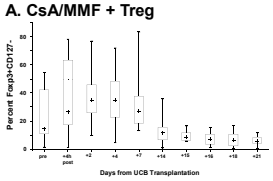
Brunstein et al Blood 2010, in press

# Infusional Toxicity

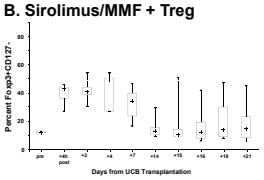
Brunstein et al Blood 2010, in press

**The kinetics of detection of CD4+ cells that were CD127- FoxP3+ in the peripheral blood were similar for patients receiving CsA and sirolimus based immunosuppression**

**A. CsA/MMF + Treg**




**B. Sirolimus/MMF + Treg**



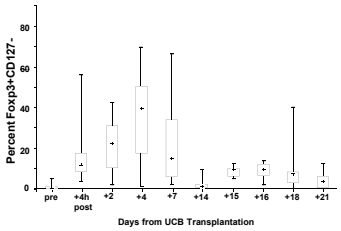

Note that Treg detection here inlcuded any Treg that were derived from patient, graft UCB units and expansion product.

Brunstein et al Blood 2010, in press

**Immune monitoring figure**

Immune monitoring figure

## Kinetics of detection of expansion product-derived CD4+ cell that were CD127-FoxP3+ in patients' peripheral blood




Brunstein et al Blood 2010, in press

## Summary

- UCB-derived Tregs can be consistently expanded to provide doses up to 3x10e6 (x2)
- UCB-derived Tregs are highly suppressive
- Infusional toxicity after Treg infusion is mild
- UCB-derived Tregs were detectable up to 14 days post-infusion
- Cryopreserved cells provide lower circulating levels
- Long-term persistence in the peripheral blood of UCB-derived Tregs was NOT observed

Brunstein et al Blood 2010, in press

# Clinical Outcomes

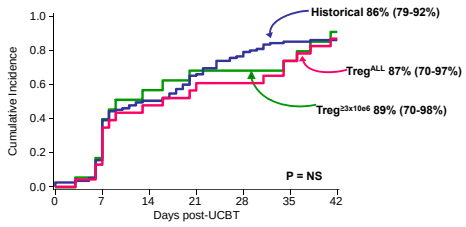
**Primary Endpoint: Safety**

**Major question: What happens when we add a 3rd UCB unit?**

**There are 3 groups:**
- **23 Treg patients = $Treg^{ALL}$**
- **18 Treg patients who received ≥ 3x10e6/kg = $Treg^{\geq 3x10e6}$**
- **108 historical controls = Historical**

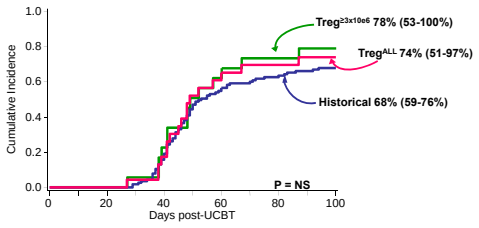
Brunstein et al Blood 2010, in press

# Sustained Donor Engraftment Historical Controls vs. Treg patients




Brunstein et al Blood 2010, in press

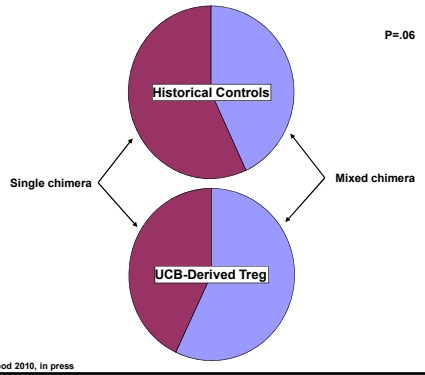
# Platelet recovery ≥ 50,000/μL at 100 days Historical Controls vs. Treg patients




Historical median: 46 (29-94) days
$Treg^{ALL}$ median: 46 (27-87) days
$Treg^{\geq 3x10e6}$ median: 48 (27-87) days

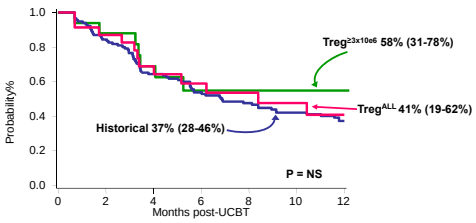
Brunstein et al Blood 2010, in press

## Prevalence of mixed donor chimerism in unseparated BM at day 21



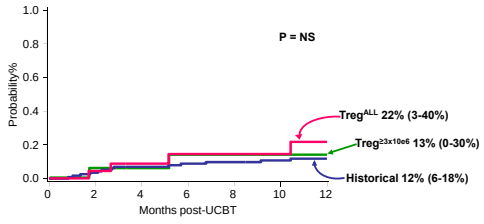

Brunstein et al Blood 2010, in press

## Disease Free Survival at 1 year Historical Controls vs. Treg patients



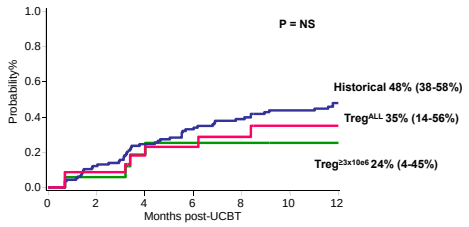

Brunstein et al Blood 2010, in press

## Non-relapse Mortality at 1 year Historical Controls vs. Treg patients



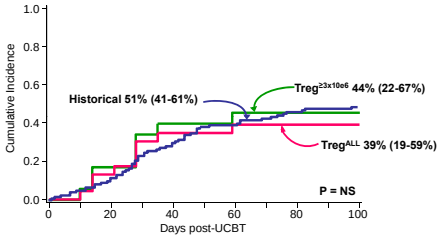

Brunstein et al Blood 2010, in press

## Relapse at 1 year
## Historical Controls vs. Treg patients




Brunstein et al Blood 2010, in press

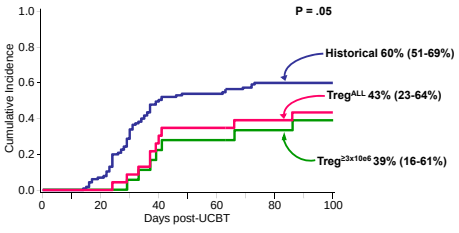
## Viral + Fungal Infections at 100 days
## Historical Controls vs. Treg patients




Historical median: 25 (0-88) days
Treg^ALL median: 28 (10-59) days
Treg^≥3x10e6 median: 28 (10-59) days

Brunstein et al Blood 2010, in press

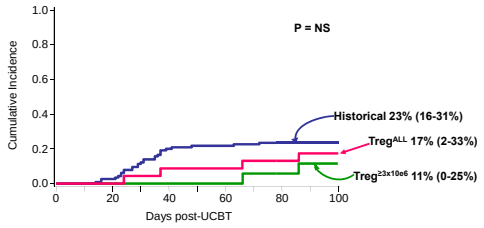
## Grade II-IV Acute GVHD
## Historical Controls vs. Treg patients




Historical median: 30 (14-73) days
Treg^ALL median: 38 (24-86) days
Treg^≥3x10e6 median: 39 (29-86) days

Brunstein et al Blood 2010, in press

## Grade III-IV Acute GVHD Historical Controls vs. Treg patients




Historical median: 30 (14-72) days
$Treg^{ALL}$ median: 52 (24-86) days
$Treg^{\geq 3x10e6}$ median: 76 (66-86) days

Brunstein et al Blood 2010, in press

## Absolute CD4 after Double UCBT

## Summary

- Clinical outcomes were not adversely impacted by the infusion of ex vivo expanded UCB-derived Treg
- The reduction in the incidence of acute GVHD observed when comparing to that of historical controls is promising

## Future Directions

- Further dose escalation
- Off-the-shelf product
- Randomized clinical trial
- Effect of Tregs in the absence of pharmachological immunosuppression

## Further dose escalation

- We did not reach MTD
- Current expansion methology limited cell dose that could be given
- Restimulation
  - Anti-CD3/CD28 beads
  - aAPC
- In vivo expansion

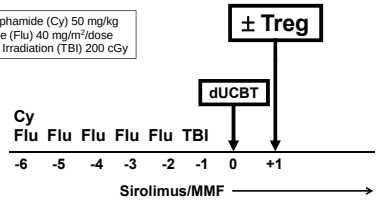
**Expanding nTreg with cell-based aAPC (KT64/86) increases expansion and decreases contamination with potentially cytotoxic IFNg secreting cells**

# Off-the-shelf product

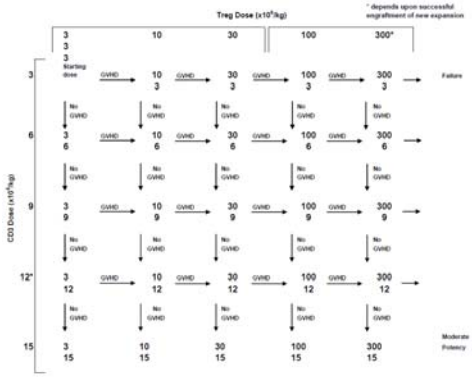
- Would not require lead time for expansion culture
- Would allow studies for the treatment of GVHD
- Would perhaps allows studies in auto-immune diseases

## Outline Randomized Clinical Trial of UCB-derived Regulatory T Cells in Nonmyeloablative UCB Transplantation

Cyclophosphamide (Cy) 50 mg/kg
Fludarabine (Flu) 40 mg/m$^2$/dose
Total Body Irradiation (TBI) 200 cGy




## Effect of Tregs in the absence of pharmacological immunosuppression



# Acknowledgements


**Treg Research Team**
Bruce R. Blazar
Qing Cao
Julie Curtsinger
Keli L. Hippen
Carl H. June
Bruce L. Levine
David H. McKenna
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Jeffrey S. Miller
James L Riley
Darin Sumstad
John E. Wagner

**U of M Adult BMT**
Mukta Aurora
Veronica Bachanova
Linda Burns
Sarah Cooley
Dan Kaufman
Navneet Majhail
Brian McClune
Philip McGlave
Jeffrey Miller
Arne Slungaard
Marcie Tomblyn
Greg Vercelotti
Erica Warlick
Dan Weisdorf

**CETI**
Jill Aughey
Anne Hopper
Elizabeth Kerr
Judy Witte

**MCT**
Diane Kadidlo

**Stats**
Todd Defor

**CTO**
Marilee Larkin

**BMT Office**
Tim Krepski
Kathy French
Nacy Konstantinides

Andromachi Scaravadou
Pablo Rubinstein

NCI PPG
P01 CA065493
Phil McGlave (P.I.)