Transplantation Tolerance Through Therapeutic Cell Transfer: Where Do We Stand?

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I have financial relationship(s) with:
Talaris/Regenerex– Grant Support
Veloxis – Speakers Bureau/Grant Support
TRACT Therapeutics - Founder

AND

My presentation includes discussion of the investigational use of FCRx, a cell based therapy being developed by Regenerex LLC/Talaris, and TregCel, a cell therapy being developed by TRACT Therapeutics

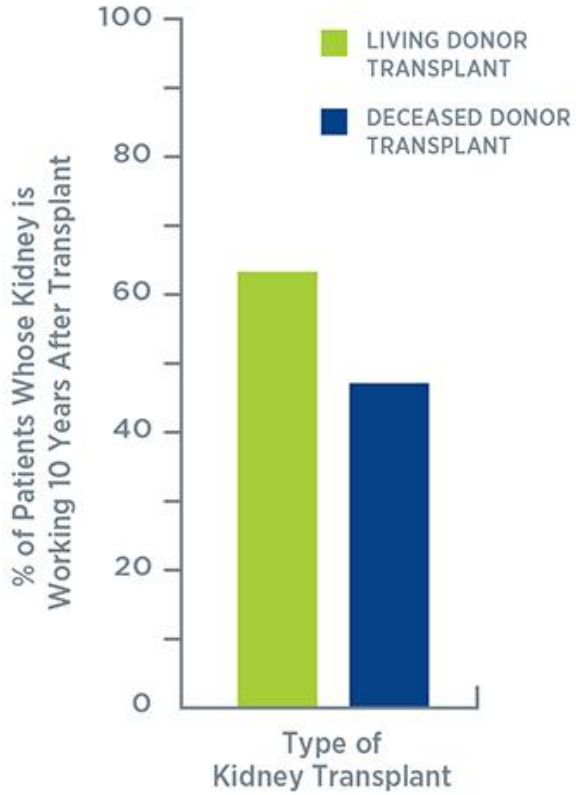
Tolerance

A state of fully functional graft in the absence of immunosuppressive treatment.

Allograft Survival without the need for drug-based immunosuppression in the absence of a deleterious allogeneic immune response

WHY?

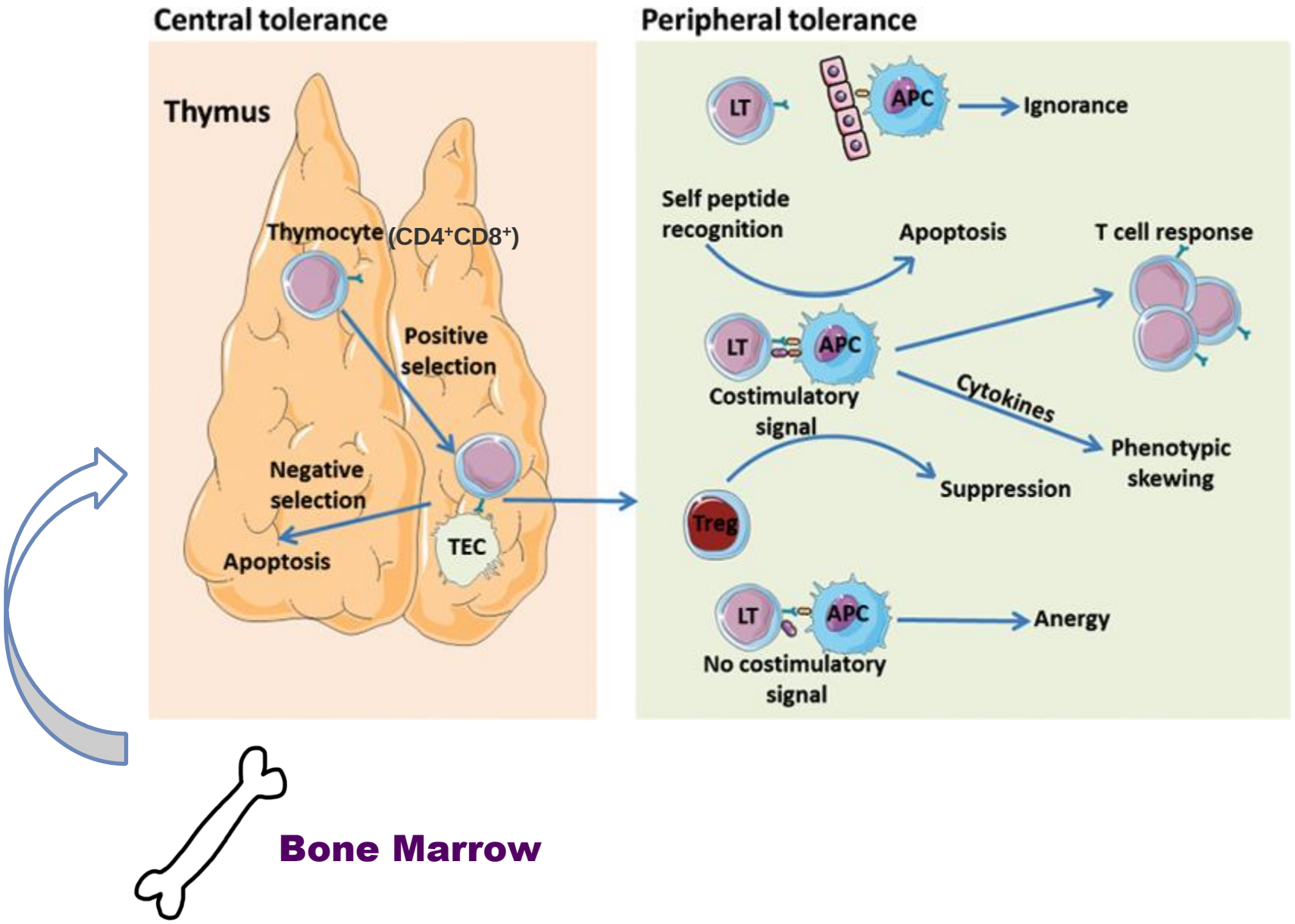
10 Years Graft Survival after Kidney Transplant
Living Vs. Deceased donor



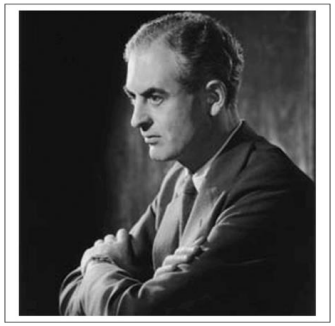
Why is the pursuit of tolerance so compelling?

- Better control of the immune system: potential for "one organ transplant for life"…
- Financial Costs
- Compliance … pediatric patients

- Better long term patient survival if IS can be discontinued


Central tolerance
Peripheral tolerance
Thymus
Thymocyte (CD4+CD8+)
Positive selection
Negative selection
Apoptosis
TEC
LT
APC
Ignorance
Self peptide recognition
Apoptosis
T cell response
Costimulatory signal
Cytokines
Phenotypic skewing
Suppression
Treg
No costimulatory signal
Anergy
Bone Marrow

Billingham, Brent and Medawar



In 1953 published on actively acquired tolerance to foreign cells in *Nature*:

Used neonatal injections of donor hematopoietic and lymphoid cells.

The injected mice developed sustained chimerism, defined as persistence of donor hematopoietic cells in the recipient

Adult mice failed to reject skin grafts from the donor strain while rejecting third-party skin grafts . Loss of chimerism resulted in the loss of immune tolerance.

Relevant questions regarding chimerism and tolerance

Is establishment of durable chimerism sufficient to achieve clinical transplantation tolerance?

Is establishment of durable chimerism necessary to achieve clinical transplantation tolerance?

Does the end justify the means?

Can we identify biomarkers in chimeric, tolerant subjects that would predict operational tolerance in others?

Fourth International Workshop For Clinical Tolerance

September 5-6th , 2019
University of Pittsburgh

Northwestern Medicine™

Center / Entity	Organ	HLA	Protocols	n
MGH	Kidney	Match *	Full / mixed chimerism (myeloma / kidney)	10
	Kidney	Mismatch *	Mixed (transient) chimerism	12
Stanford	Kidney	Match Φ	Mixed chimerism	29
	Kidney	Mismatch *	Mixed chimerism	19
CIRM (Stanford & Northwestern)	Kidney	Mismatch ¥	DHSC & Recipient Regulatory T cells (mixed chimerism)	22
Northwestern & Duke	Kidney	Match*	Alemtuzumab and donor HSC infusion	20
	Kidney	Mismatch *	Durable chimerism (FCRx)	37
	Kidney	Mismatch *	Regulatory T cells (TRACT)	9
	Liver	Mismatch *	TAC → SRL monotherapy → withdrawal	
Johns Hopkins	Kidney	Mismatch	Full chimerism (FCRx)	1
UCSF	kidney	Mismatch Φ	Regulatory T cells	3
	Liver	Mismatch Φ	Alloantigen-Specific Tregs (ARTEMIS)	18
The One Study	Kidney	Mismatch	Donor-Alloantigen-Reactive Regulatory T Cells (UCSF)	6
	Kidney	Mismatch	Autologous Tolerogenic Dendritic Cells	11
	Kidney	Mismatch	Donor-derived Regulatory Macrophage	8
	Kidney	Mismatch Φ	Regulatory T cells (UK)	15
	Kidney	Mismatch Φ	Regulatory T cells (Germany)	9
	Kidney	Mismatch Φ	Regulatory T cells With Belatacept (Boston)	8
Kings College (UK)	Liver	Mismatch	Regulatory T cells ((ThRIL)	9
IRCCS; Italy	Kidney	Mismatch Φ	Mesenchymal stromal cells	4+
Pittsburgh	Liver	Mismatch	Regulatory dendritic cells	12
Sam Sang University, (South Korea)	Kidney	Mismatch Φ	Mixed chimerism	9
Hokkaido University	Liver	Mismatch Φ	Regulatory T cells (Tregs)	10
UHN, Toronto, Canada	Liver	Mismatch	Autologous Hematopoietic Stem Cells	5
Talaris	Kidney	Mismatch ¥	Full chimerism (FCRx) - multicenter	120
TRACT Inc.	Kidney	Mismatch	Regulatory T cells (TRACT)	120
MEDEOR	Kidney	Match	Mixed Chimerism	

Cell Therapies being considered for Tolerance Induction

HSC to induce chimerism

HSC to induce immunomodulation

Regulatory T cells

Dendritic cells (DC)

Mesenchymal Stem Cells (MSC)

Apoptotic Cell Delivery (ECDI, ECP)

? Combination of cell types (HSC + Treg)

? Single vs multiple infusions

Mechanisms of Cellular Immunological tolerance

- Ignorance (Antigen not recognized):

  Questionable relevance in transplantation

- Suppression / Regulation:
- Anergy:
- Exhaustion:
- Senescence:
- Deletion of Reactive Clones:

These alloimmune tolerance pathways can be assessed phenotypically.

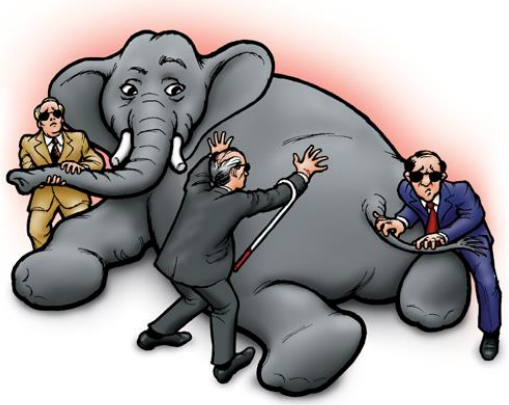
Operational Tolerance in Solid Organ Transplant Recipients

Deliberate IS withdrawal versus “Russian Roulette” (patient noncompliance)

Trials of IS withdrawal somewhat successful in liver transplant recipients – tolerogenic effect of the liver allograft? Has not been translatable to other solid organs

Operational tolerance as a dynamic process based upon immune regulation versus elimination of alloreactivity (clonal deletion).

Identifying Transplant Recipients with Operational Tolerance



- Functional assays: donor specific hyporesponsiveness – MLR, Elispot
- Signatures of tolerance: proteomics, genomics, immunophenotypic analyses
- Retrospective data in very few subjects – no prospective validation
- Little confirmation with histology in the allograft
- Stability of signature over time?
- Prospective trials currently being planned (Immune Tolerance Network, CTOT)

Research article Related Commentary, page 1803

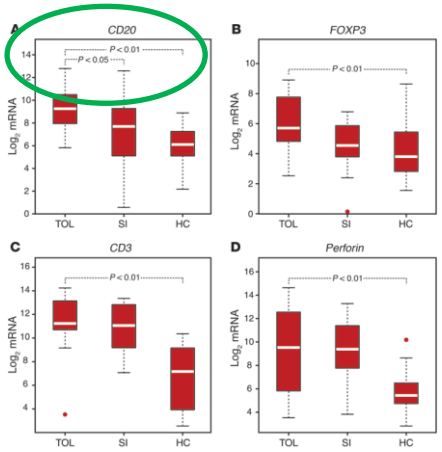
Identification of a B cell signature associated with renal transplant tolerance in humans

Kenneth A. Newell,[1] Adam Asare,[2,3] Allan D. Kirk,[1] Trang D. Gisler,[2,3] Kasia Bourcier,[2,3] Manikkam Suthanthiran,[4] William J. Burlingham,[5] William H. Marks,[6] Ignacio Sanz,[7] Robert I. Lechler,[8,9] Maria P. Hernandez-Fuentes,[8,9] Laurence A. Turka,[3,10] and Vicki L. Seyfert-Margolis,[3,11] for the Immune Tolerance Network ST507 Study Group

[1]Emory University, Atlanta, Georgia, USA. [2]University of California, San Francisco, California, USA. [3]Immune Tolerance Network, Bethesda, Maryland, USA (www.immnunetolerance.org). [4]Cornell University Medical Center, New York, New York, USA. [5]University of Wisconsin, Madison, Wisconsin, USA. [6]Swedish Medical Center, Seattle, Washington, USA. [7]University of Rochester, Rochester, New York, USA. [8]MRC Centre for Transplantation, King's College, London, United Kingdom. [9]Indices of Tolerance EU consortium (www.transplant-tolerance.org.uk). [10]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA. [11]Food and Drug Administration, Silver Spring, Maryland, USA.

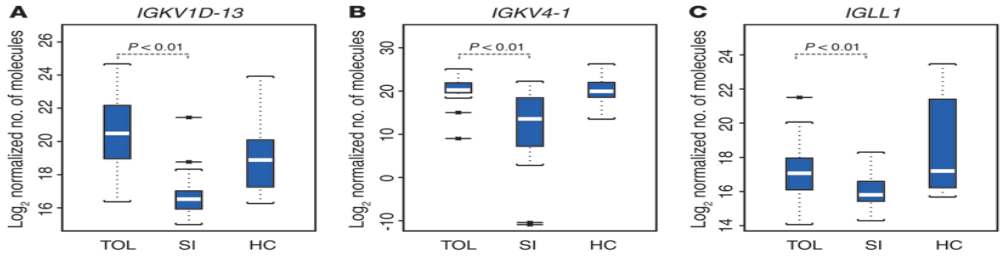
To identify immune parameters that would discriminate tolerant from subjects with stable allograft function while on immunosuppression.

Newell et al. *J Clin Invest.* 2010 Jun;120(6):1836-47.



Gene expression profile of urinary cell sediments

3 genes found to have the best predictive value



Newell et al. *J Clin Invest.* 2010 Jun;120(6):1836-47.

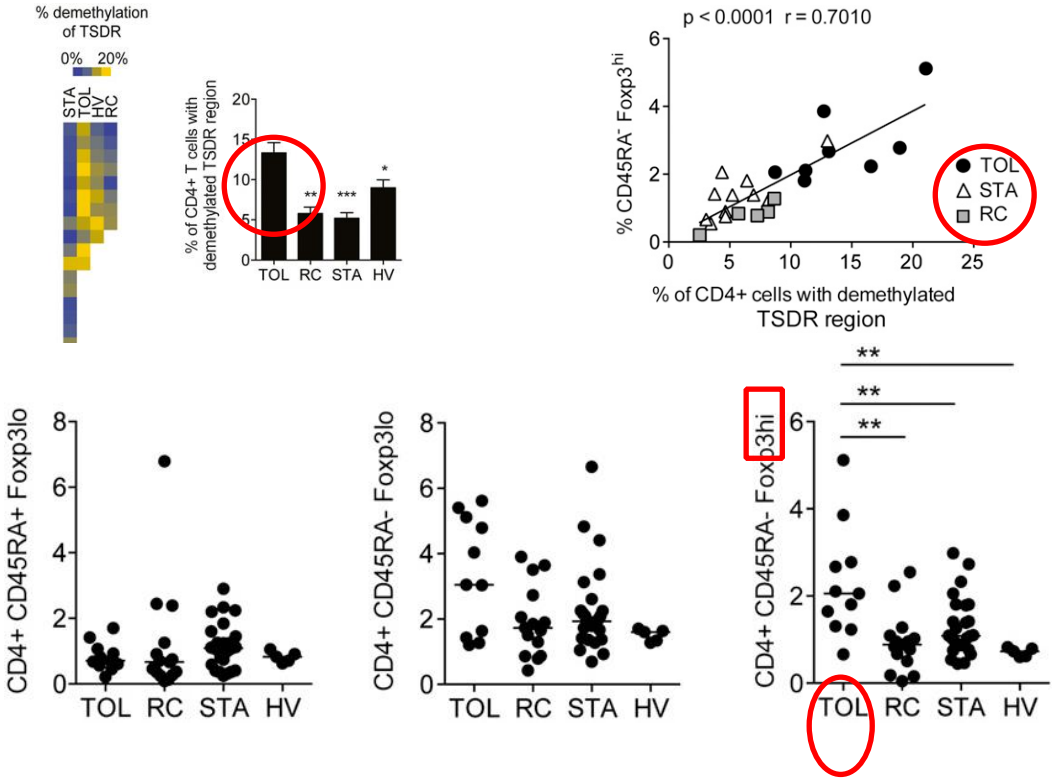
BASIC RESEARCH www.jasn.org

Central Role of CD45RA^{-} Foxp3^{hi} Memory Regulatory T Cells in Clinical Kidney Transplantation Tolerance

Faouzi Braza,[*†] Emilie Dugast,[†‡] Ivo Panov,[§‖] Chloé Paul,[†‡] Katrin Vogt,[§] Annaick Pallier,[†‡] Mélanie Chesneau,[*†] Daniel Baron,[*†] Pierrick Guerif,[‡] Hong Lei,[§‖] David-Axel Laplaud,[†‡] Hans-Dieter Volk,[§‖] Nicolas Degauque,[†‡] Magali Giral,[†‡] Jean-Paul Soulillou,[†‡] Birgit Sawitzki,[§‖] and Sophie Brouard[†‡]

*Faculty of Medicine, University of Nantes, Nantes, France; [†]French Institute of Health and Medical Research Unit 1064, Research Institute on Urology, Nephrology, and Transplantation, and [‡]Biotherapy Clinical Investigation Center, Hôtel Dieu University Hospital, Nantes, France; and [§]Institute of Medical Immunology and [‖]Berlin Brandenburg Center for Regenerative Therapies, Charité Medical University, Berlin, Germany

Braza et al. *J Am Soc Nephrol.* 2015.


% demethylation of TSDR
0%
20%
STA
TOL
HV
RC
% of CD4+ T cells with demethylated TSDR region
TOL
RC
STA
HV
p < 0.0001 r = 0.7010
% CD45RA- Foxp3hi
% of CD4+ cells with demethylated TSDR region
TOL
STA
RC
CD4+ CD45RA+ Foxp3lo
CD4+ CD45RA- Foxp3lo
CD4+ CD45RA- Foxp3hi

Clinical tolerance trials
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- Simultaneous kidney/HSC in HLA mismatched related and unrelated recipients (FCRx)
- Sequential kidney/HSC in HLA-matched related recipients
- Adoptive therapy with Treg adoptive cell transfer (TRACT) in living donor kidney transplant recipients (Phase 1)

Clinical Protocol:
Infusion of CD34+ donor hematopoietic stem cells (DHSC) postoperatively

MYCOPHENOLATE (MMF)

TACROLIMUS | SIROLIMUS (SRL) ← No IS Drugs →

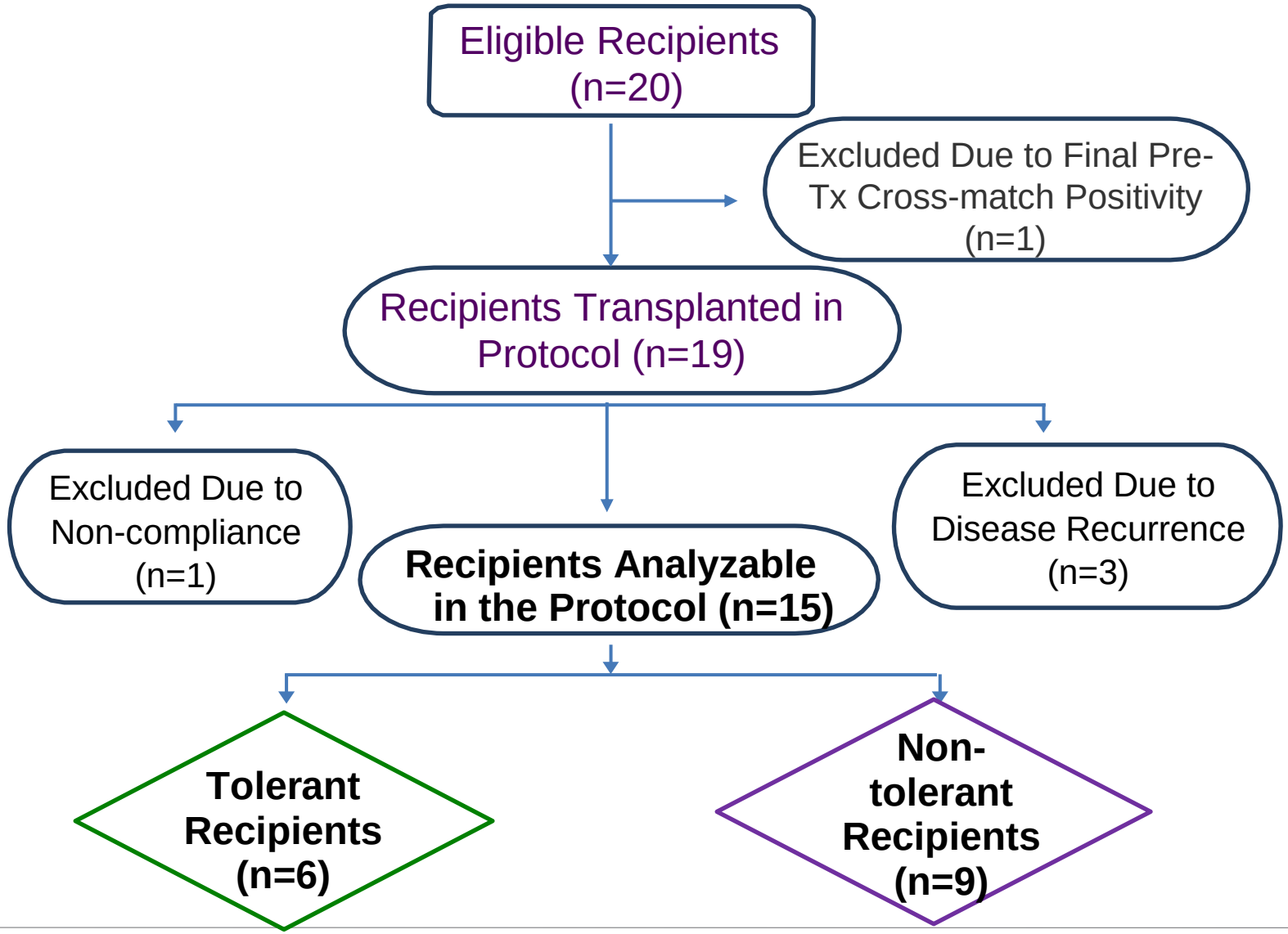
Tx, AL (0)
AL (4)
DHSC #1 (BM) (5)
DHSC #2 (PB) (2)
DHSC #3 (PB) (3)
DHSC #4 (PB) (6)
Biopsy (9)
Biopsy (12)
Biopsy (18)
Biopsy (24)
Biopsy (36)
Biopsy (48)
Biopsy (60)

Days: 0, 4, 5

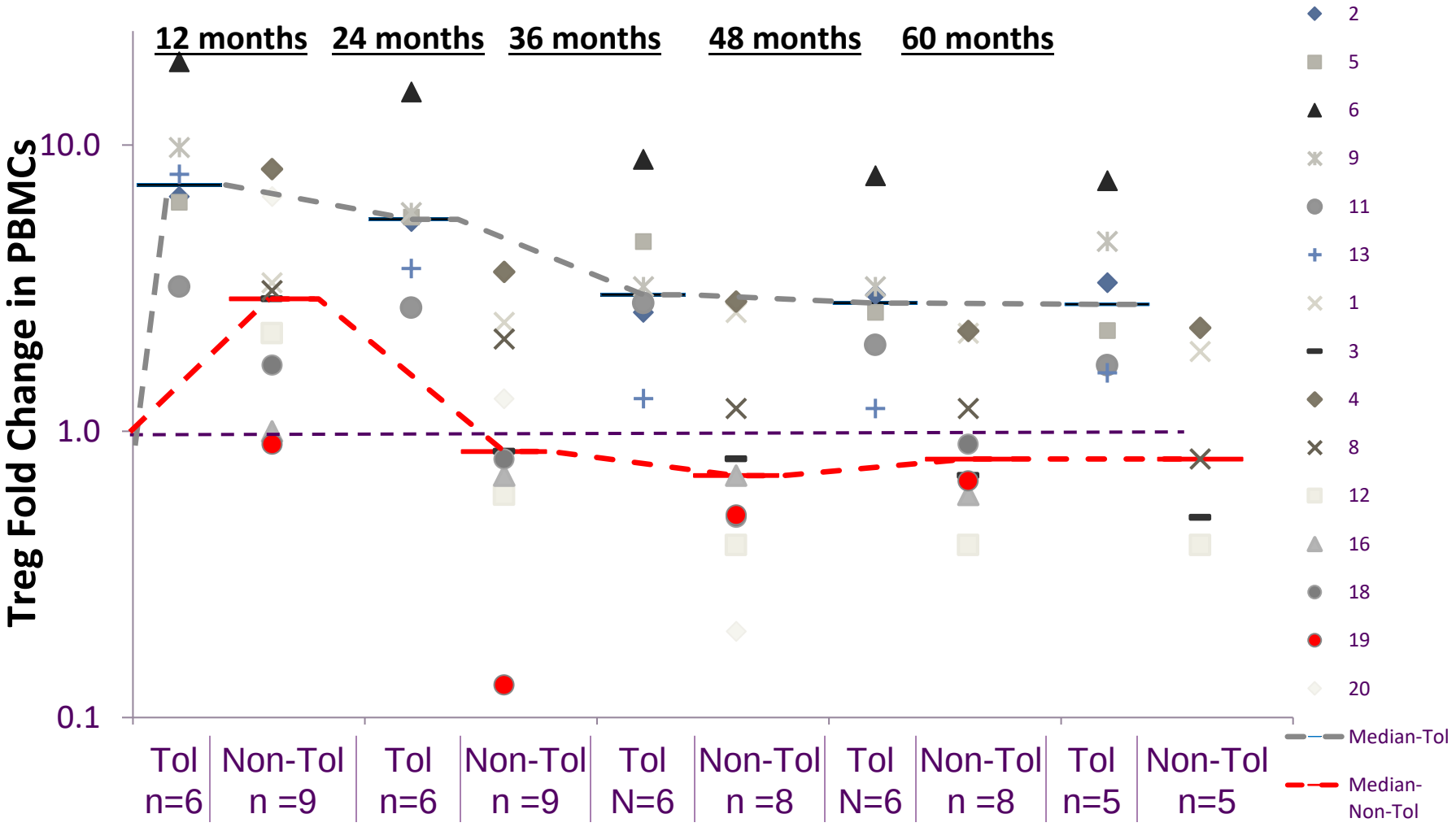
Months Post-op: 2, 3, 6, 9, 12, 18, 24, 36, 48, 60

If biopsy negative one year off IS - designated as TolI

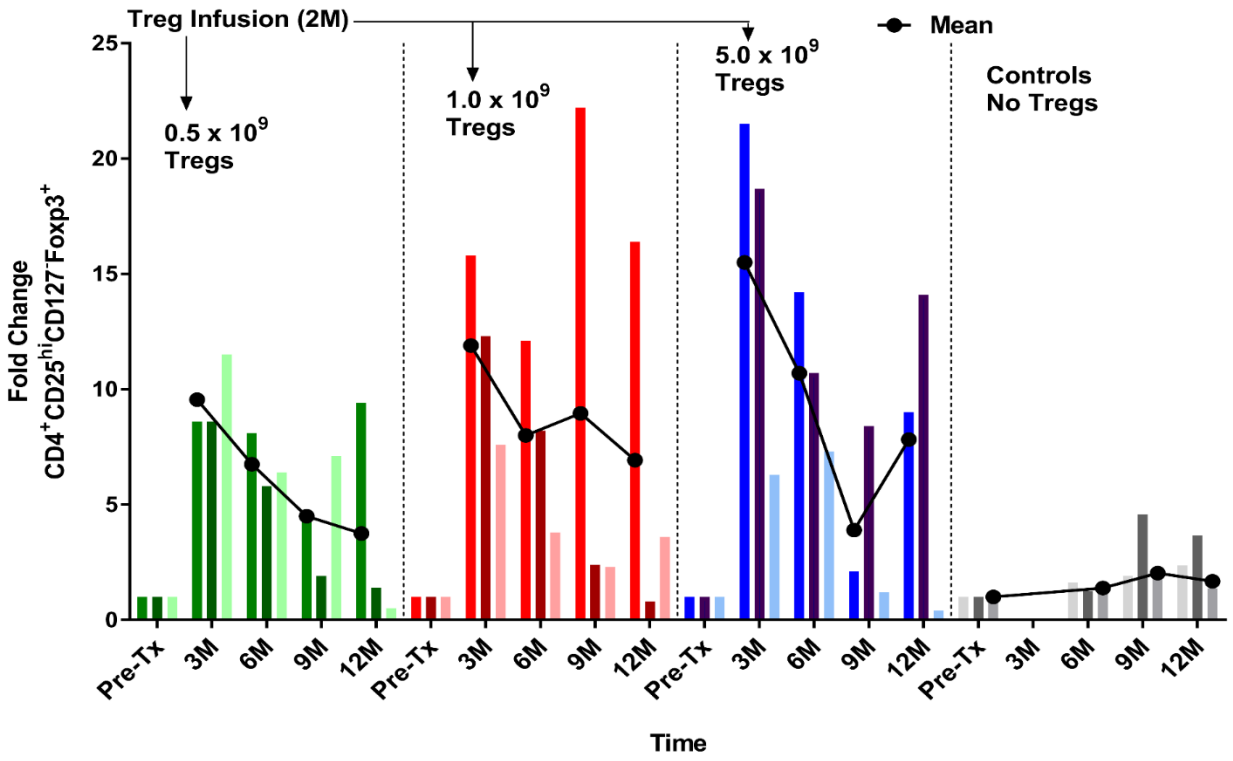
Patient Recruitment Schema



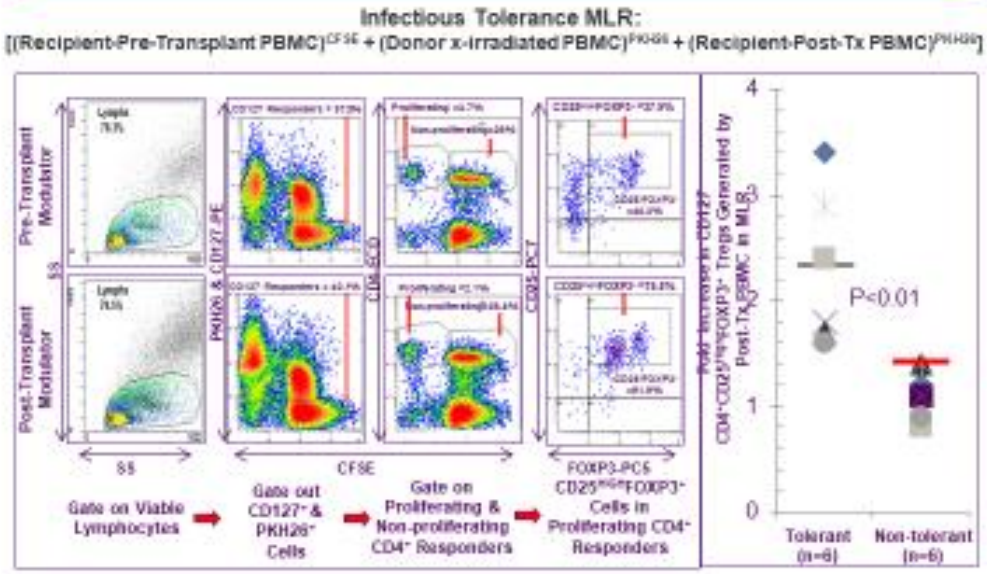
Increased and Sustained Treg frequencies in Tolerant recipients



Treg Percentage Change in Peripheral Blood of Phase 1 Expanded Treg Trial Patients



Possible Mechanism of Tolerance Induction: Infectious Tolerance



Clinical tolerance trials
Northwestern Transplant Center

- Simultaneous kidney/HSC in HLA mismatched related and unrelated recipients (FCRx)
- Sequential kidney/HSC in HLA-matched related recipients
- Adoptive therapy with Treg adoptive cell transfer (TRACT) in living donor kidney transplant recipients (Phase 1)

Hypothesis:

Use of a bioengineered donor derived HSCT (FCRx) with low intensity conditioning will allow for the establishment of durable donor macrochimerism and donor specific tolerance, with a minimal risk of GVHD

Science Translational Medicine
AAAS

Chimerism and Tolerance Without GVHD or Engraftment Syndrome in HLA-Mismatched Combined Kidney and Hematopoietic Stem Cell Transplantation
Joseph Leventhal *et al.*
Sci Transl Med **4**, 124ra28 (2012);
DOI: 10.1126/scitranslmed.3003509

www.ScienceTranslationalMedicine.org 7 March 2012 Vol 4 Issue 124 124ra28

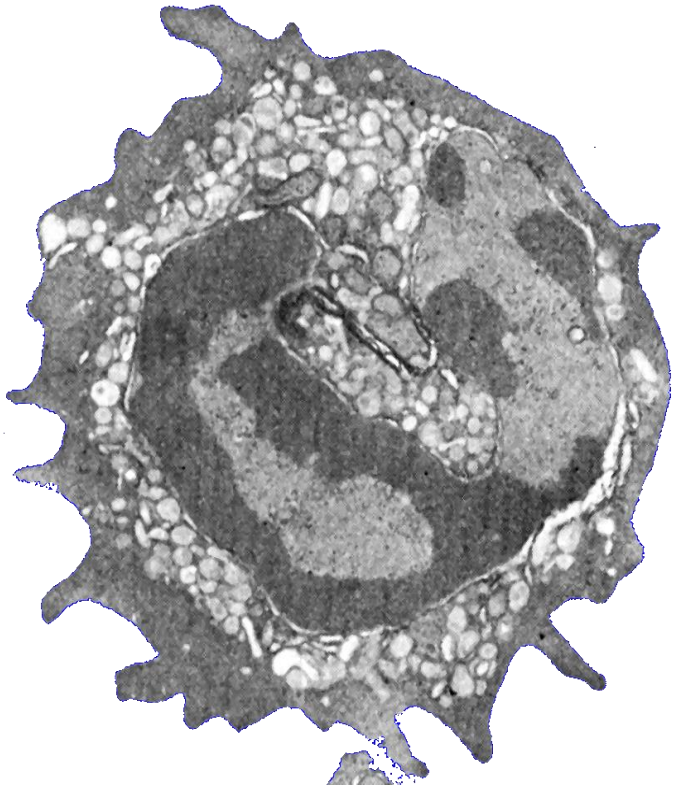
KIDNEY TRANSPLANT

Chimerism and Tolerance Without GVHD or Engraftment Syndrome in HLA-Mismatched Combined Kidney and Hematopoietic Stem Cell Transplantation

Joseph Leventhal,[1] Michael Abecassis,[1] Joshua Miller,[1] Lorenzo Gallon,[1] Kadiyala Ravindra,[2] David J. Tollerud,[2,3] Bradley King,[2,3] Mary Jane Elliott,[2] Geoffrey Herzig,[4] Roger Herzig,[4] Suzanne T. Ildstad[2,3]*

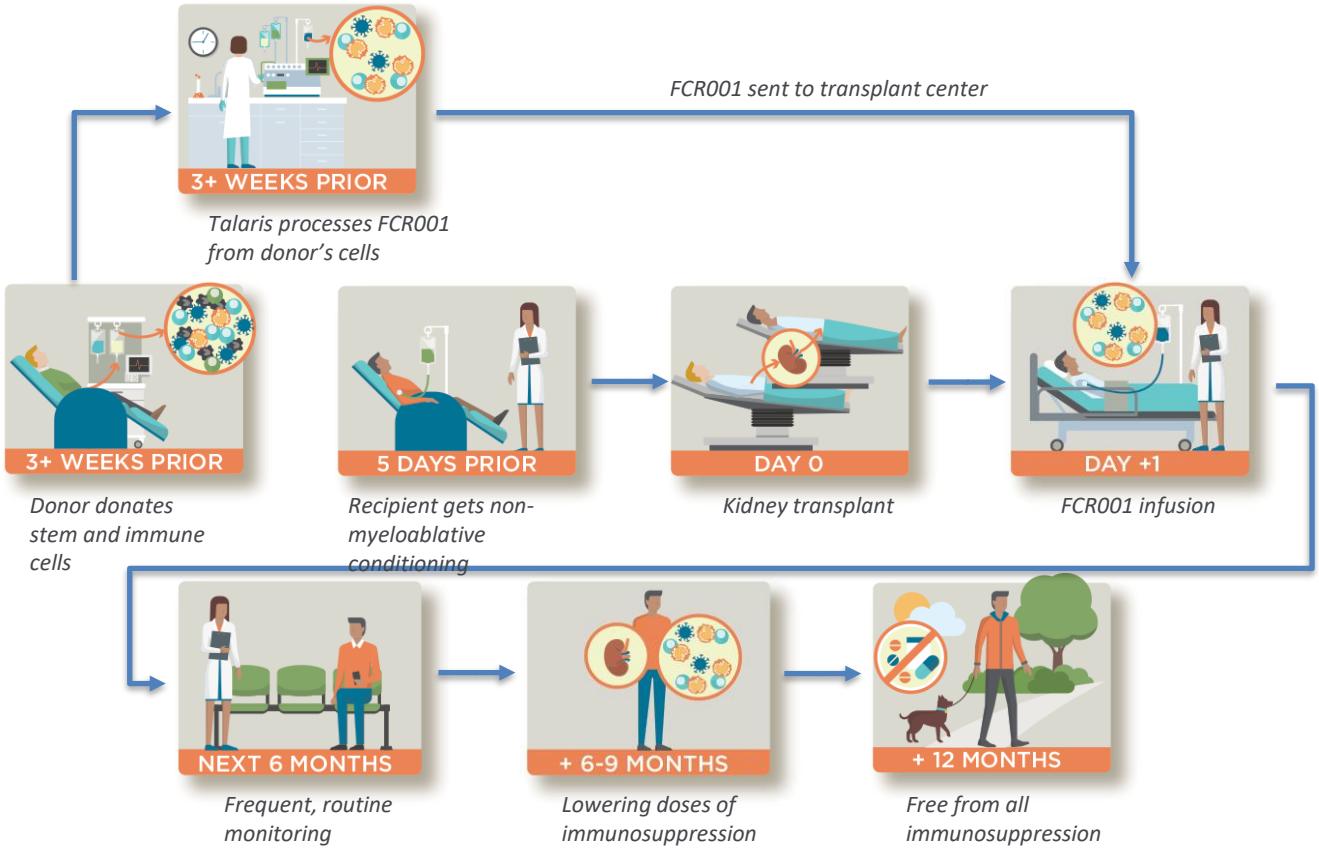
The toxicity of chronic immunosuppressive agents required for organ transplant maintenance has prompted investigators to pursue approaches to induce immune tolerance. We developed an approach using a bioengineered mobilized cellular product enriched for hematopoietic stem cells (HSCs) and tolerogenic graft facilitating cells (FCs) combined with nonmyeloablative conditioning; this approach resulted in engraftment, durable chimerism, and tolerance induction in recipients with highly mismatched related and unrelated donors. Eight recipients of human leukocyte antigen (HLA)–mismatched kidney and FC/HSC transplants underwent conditioning with fludarabine, 200-centigray total body irradiation, and cyclophosphamide followed by posttransplant immunosuppression with tacrolimus and mycophenolate mofetil. Subjects ranged in age from 29 to 56 years. HLA match ranged from five of six loci with related donors to one of six loci with unrelated donors. The absolute neutrophil counts reached a nadir about 1 week after transplant, with recovery by 2 weeks. Multilineage chimerism at 1 month ranged from 6 to 100%. The conditioning was well tolerated, with outpatient management after postoperative day 2. Two subjects exhibited transient

The Facilitating Cell



- $CD8^+$
- αβ/γδ TCR-
- **Distinct from Stem Cell (HSC)**
- **Promotes engraftment**
- **Prevent GVHD**
- Human FC Characterization: AJT 2016
- Immunomagnetic selection/enrichment for FC/HSC:FCRx
- IND#16834

FCR001: an allogeneic somatic cell therapy product derived from mobilized peripheral blood cells collected from the donor by apheresis. The product contains a minimum acceptable # of hematopoietic progenitor cells (CD34+), Facilitating Cells (CD8+/αβTCR-), and a specified number of αβ T cells.



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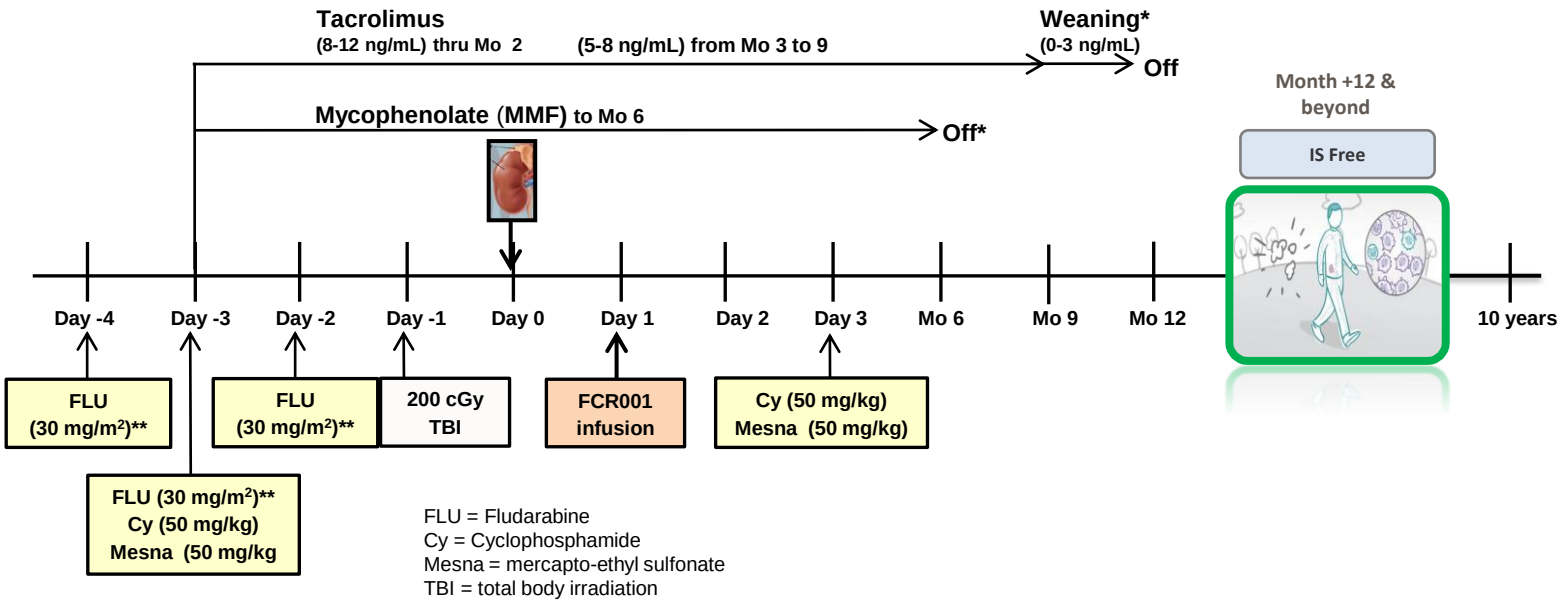
Phase 2 Trial Design



*patients demonstrating stable donor chimerism, no history of rejection, and adequate kidney function

** Hemodialysis 3 to 4 hours post administration

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Phase 2[1] Living Donor Kidney Status Summary

- 37 subjects transplanted between 2009-2016
- Safety profile of conditioning acceptable, outpatient management following discharge from CRU within week of transplant
- **26 of 37 subjects (70%) off all immunosuppression** (25 - 113 months)[2]
  - Following protocol adjustments (2011 & 2013), ***14 of 17 subjects (82%)*** off all immunosuppression
- Chimerism not dependent on HLA match (success in completely ***unmatched, unrelated*** pairs)
- Durably chimeric subjects show normal protocol biopsies at 24 months, whereas standard of care patients begin to show deterioration due to toxicity of immunosuppression and rejection
- No autoimmune disease recurrence in durably chimeric subjects
- Immunocompetent to respond to vaccination

[1] Ongoing Phase 2 study at Northwestern University and Duke University
[2] Updated January 2020

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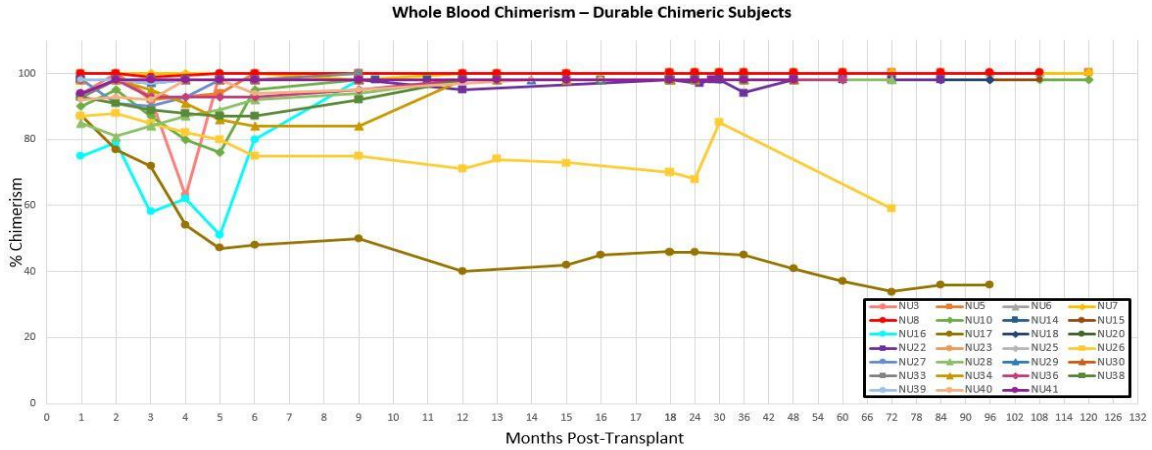
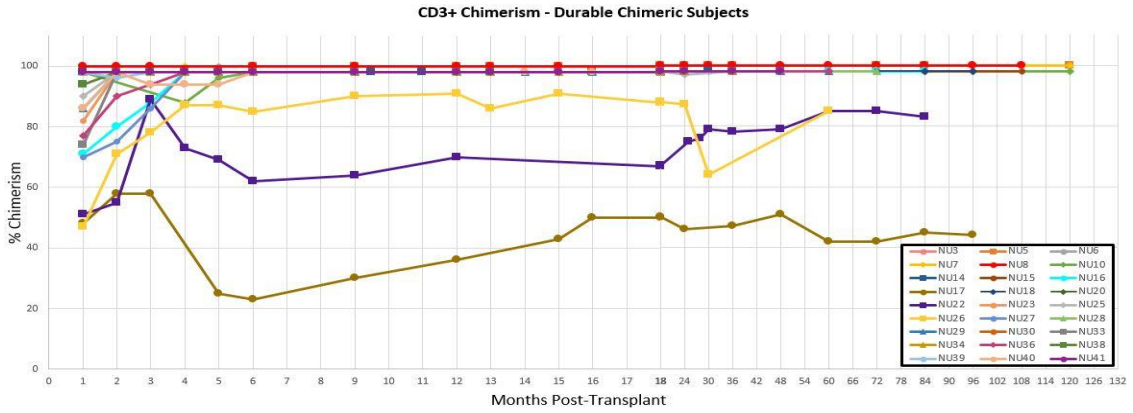
Patterns of Chimerism in Phase 2 Trial Subjects: Durable Chimerism





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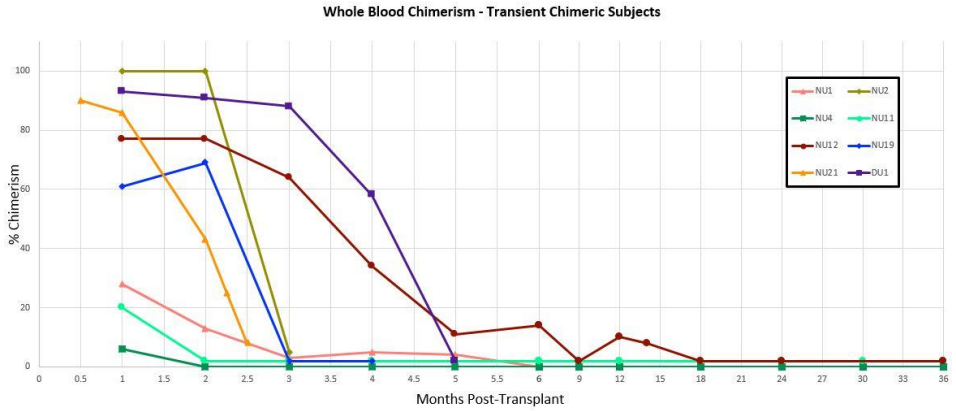
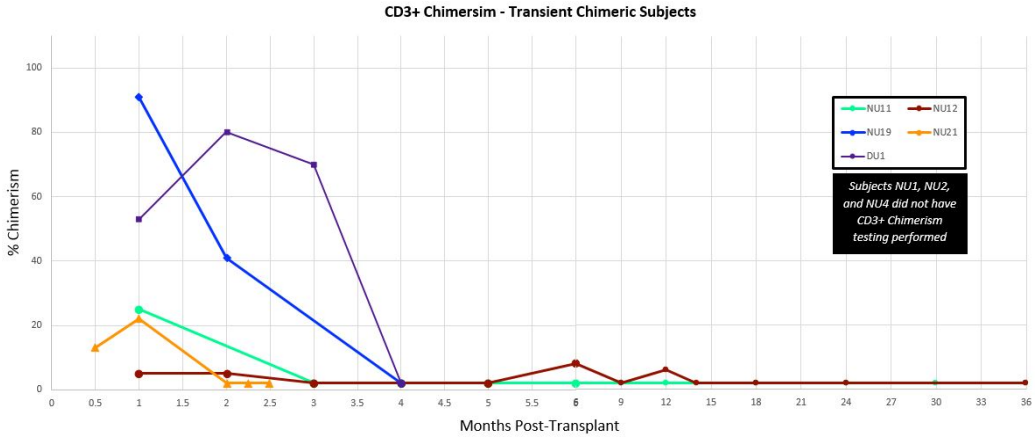
Patterns of Chimerism in Phase 2 Trial Subjects: Transient Chimerism





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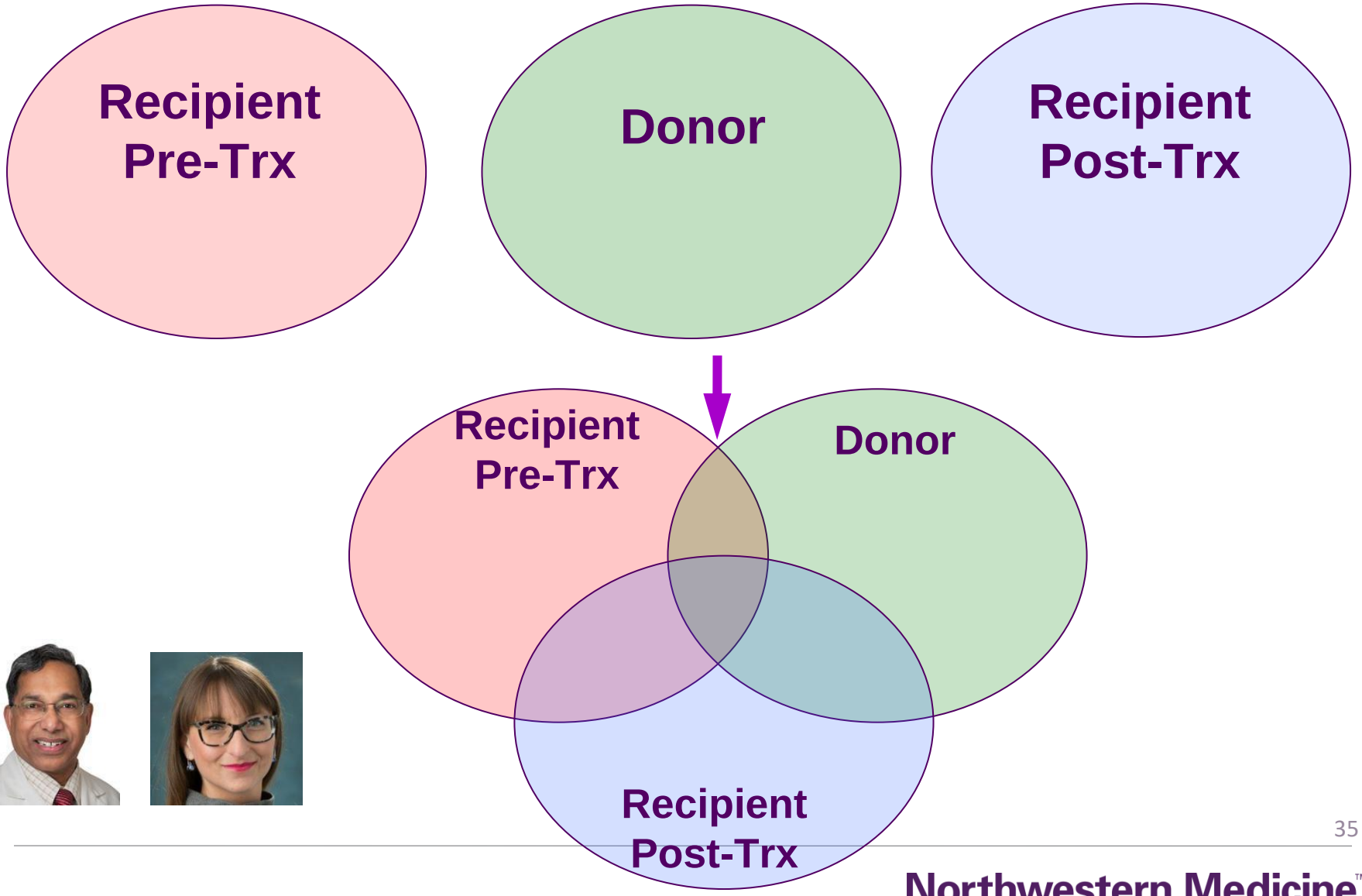
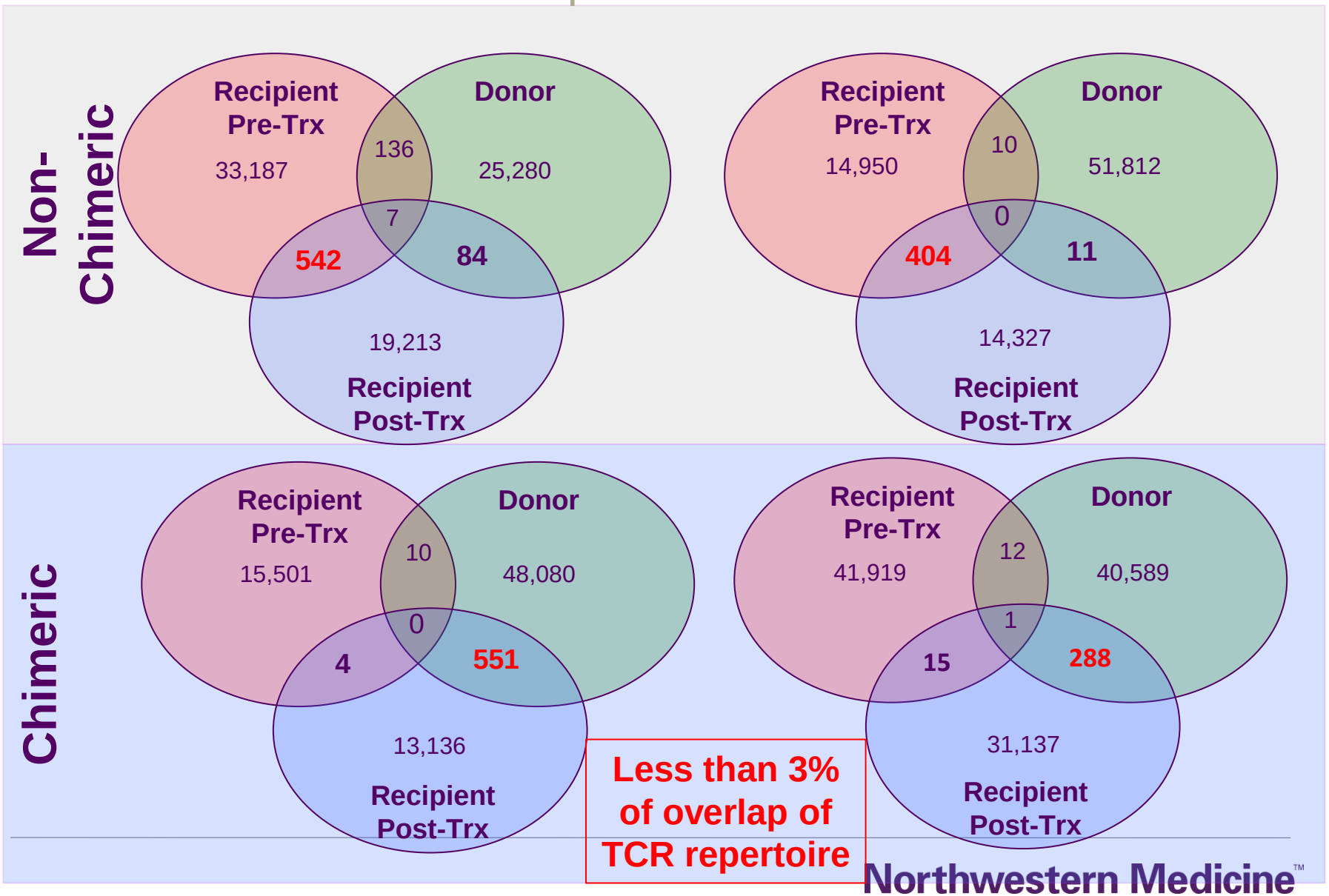
Comparison of unique TCRβ clones



Shifts in TCRβ repertoire in Chimeric and Non-Chimeric patients



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Intragraft Molecular Pathways Associated with Tolerance Induction via Facilitating Cells (FCRx)

Study Design: Cross-sectional

Study groups:

1) Paired Donor Kidney Biopsies (D; n=5), and paired donor for SIS samples SIS(d); n=2)
2) FCRx Biopsies (time to bx, mo after Tx: 17.6 mo-12-25)(FCRx; n=7)
3) Biopsies with histological diagnosis of ACR (R; n=10)
4) Biopsies without ACR, from kidneys with stable function and standard IS (SIS; n=10)

- Biopsies with ABMR (ABMR; n=10)
- Biopsies with CNIT (CNIT; n=12)
- Biopsies from normal kidneys (non-transplant) (NK; n=10)

Samples: Archival samples (FFPE blocks)

Evaluations: mRNA and miRNA expression analyses

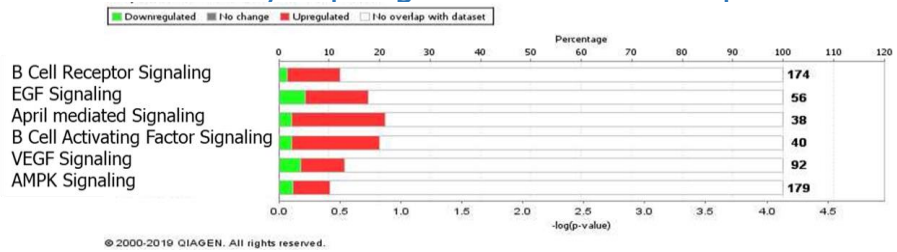
Data Analyses: Quality Control
Individual comparison analyses
Data integration

Formalin-fixed paraffin embedded samples (FFPE)
High Pure RNA Paraffin Kit (Roche, IN, USA)
SensationPlus™ FFPE Amplification/ WT Labeling Kit SensationPlus™
Affymetrix™ GeneChip® HG-U133A 2.0

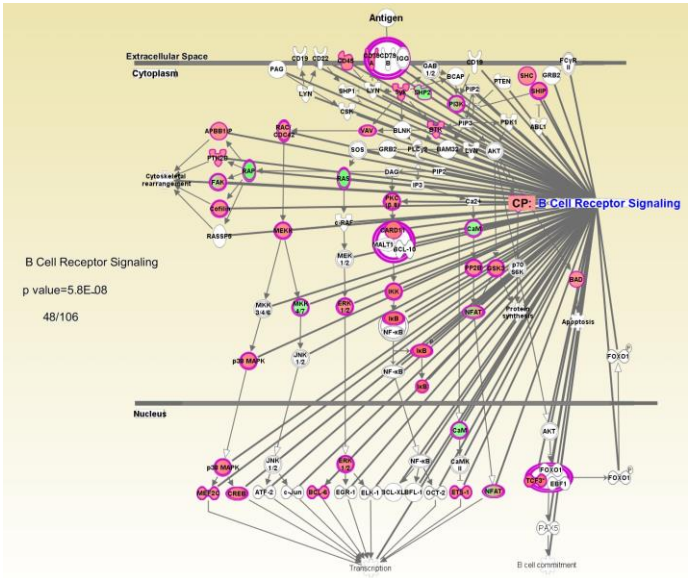
Pathway-focused miScript miRNA PCR Arrays (Qiagen)
84 mature miRNAs previously described as associated with immune response

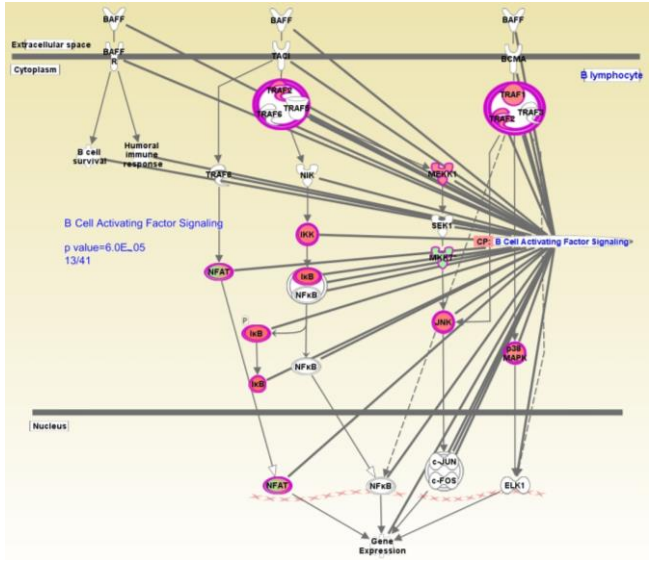
Intragraft Molecular Pathways Associated with Tolerance Induction via Facilitating Cells (FCRx)

Canonical Pathways up-regulated in FCRx compared to Standard Immunosuppression Samples with normal function



Pathway	p-value
B Cell Receptor Signaling	5.80E-08
EGF Signaling	9.90E-05
April mediated Signaling	1.90E-04
B Cell Activating Factor Signaling	6.00E-05
VEGF Signaling	4.6E-0.5
AMPK Signaling	5.50E-04





Transplant Research Institute
James D. Eason Transplant Institute

Conclusions from Pilot Study

- FCRx samples lack of activated pathways associated with alloresponse and graft *vs.* host
- Compared with SIS samples, differentially expressed genes associated with B cells were identified in FCRx samples
- FCRx samples presented similar profiles with paired donor samples differing mainly in active pathways associated with T cell exhaustion, DC maturation, and PD1-PDL1
- Enrichment of CD34+ cell specific genes is consistent with the notion that the CD34+ cells used for tolerance induction maybe homing to the allograft
- Most of these findings are linked with the predictive pathways described as likely associated with facilitating cell tolerance induction (Chhabra and lldstad, Current Opinion Trannspl 2018)

Transplant Research Institute
James D. Eason Transplant Institute

Weill Cornell Medicine

Identification Of A Molecular Signature Characterized By Dominance Of Negative Regulation Over Cytotoxic Effectors In Tolerant Kidney Allograft Recipients

John Lee[1], Joseph Leventhal[2], Carol Li[1], Andreas Katapodis[3], Suzanne Ildstad[4], and Manikkam Suthanthiran[1]

[1]Division of Nephrology and Hypertension, Department of Medicine, Weill Cornell Medicine, New York, NY, USA

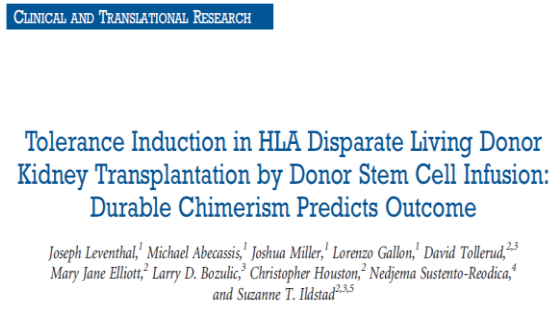
[2]Department of Surgery, Northwestern Medicine Feinberg School of Medicine, Chicago, IL, USA

[3]Novartis, Basel, Switzerland

[4]Regenerx, Louisville, KY, USA

Methods

- **To develop biomarkers of tolerance, we performed urinary cell mRNA profiling of kidney allograft recipients conditioned with facilitating cell enriched hematopoietic stem cells (FCRx Group) and kidney allograft recipients enrolled in CTOT-04 and treated with conventional immunosuppressive drugs.**


CLINICAL AND TRANSLATIONAL RESEARCH

Tolerance Induction in HLA Disparate Living Donor Kidney Transplantation by Donor Stem Cell Infusion: Durable Chimerism Predicts Outcome

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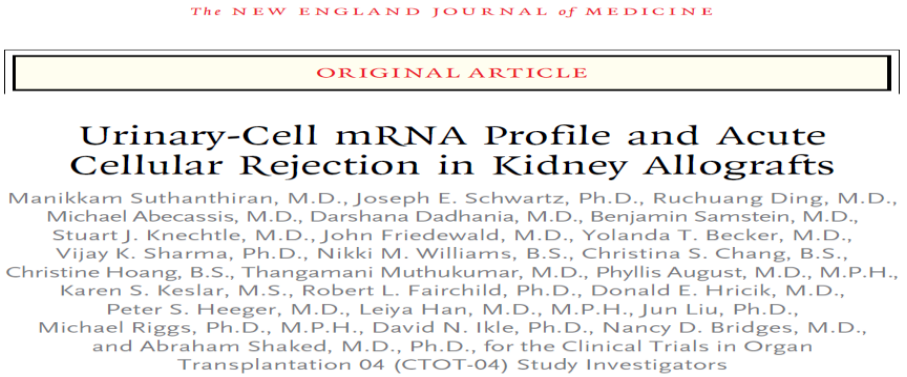
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ORIGINAL ARTICLE

Urinary-Cell mRNA Profile and Acute Cellular Rejection in Kidney Allografts

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FCRx Group

N=19 blinded urine samples,
N=10 kidney allograft recipients,
All 10 conditioned with a FCRx regimen

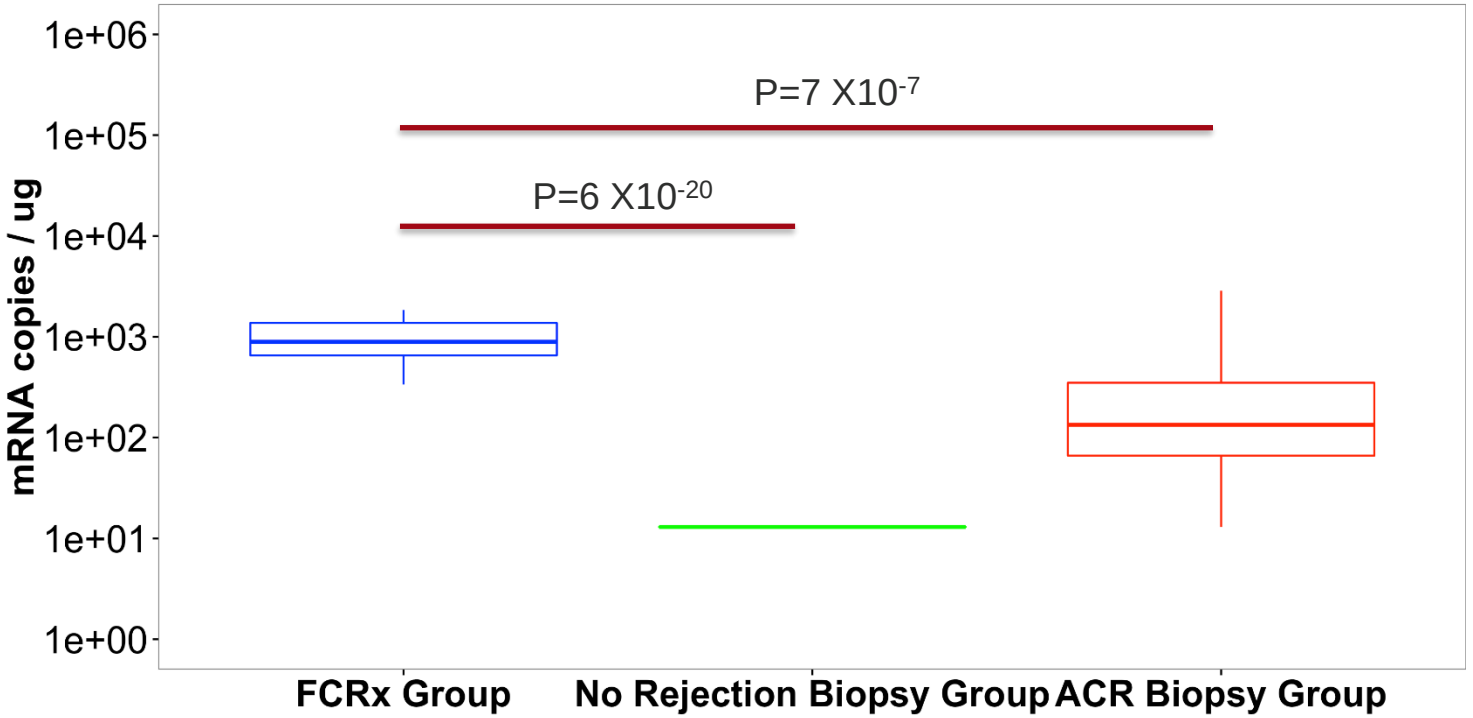
No Rejection Biopsy Group

N=163 blinded urine samples,
126 kidney allograft recipients

ACR Biopsy Group

N=43 blinded urine samples,
34 kidney allograft recipients

Urinary Cell Levels Of CTLA-4 mRNA In The FCRx Group, No Rejection Biopsy Group, And The ACR Biopsy Group



FCRx Group: 19 blinded samples, 10 patients

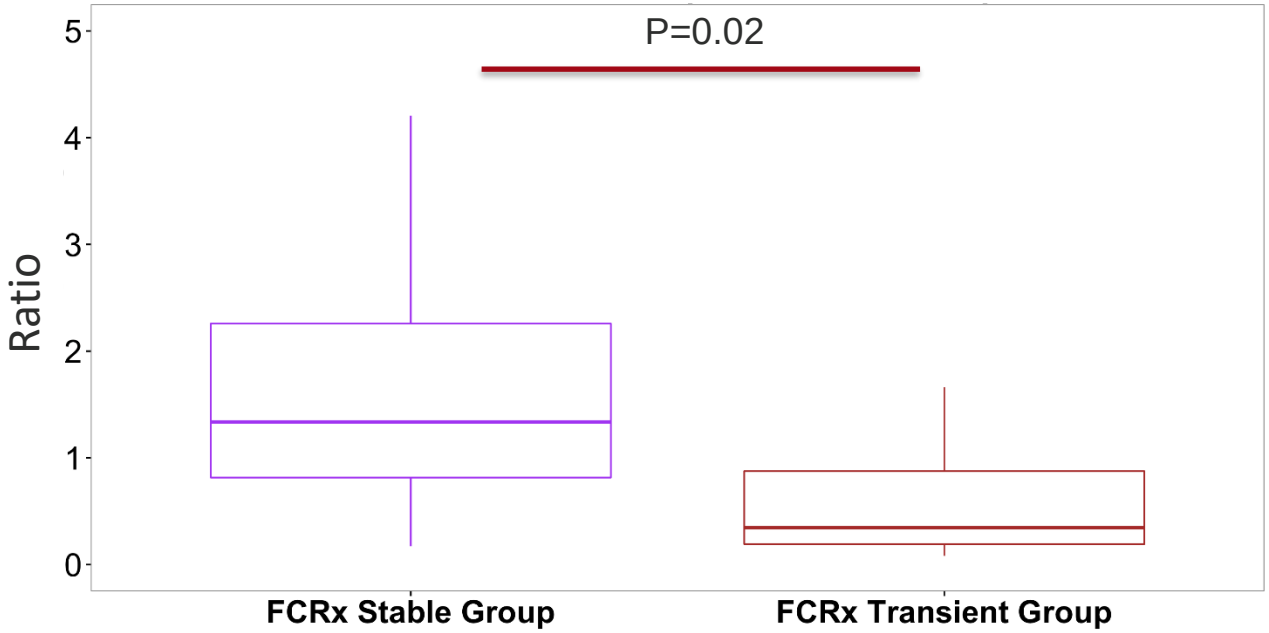
No Rejection Biopsy Group: 163 blinded samples, 126 patients

ACR Biopsy Group: 43 blinded samples, 34 patients

Urinary cell levels of CTLA-4 mRNA are significantly higher in the FCRx Group than in the No Rejection Biopsy Group ($P=6 \times 10^{-20}$)

Urinary cell levels of CTLA-4 mRNA are significantly higher in the FCRx Group than in the ACR Biopsy Group ($P=7 \times 10^{-7}$)

Ratios of CTLA-4 mRNA to Granzyme B mRNA + Perforin mRNA is Higher in the FCRx Stable Group than in the FCRx Transient Group



FCRx Stable Macrochimerism Group: 13 blinded samples, 7 patients

FCRx Transient Macrochimerism Group: 6 blinded samples, 3 patients

Ratio of CTLA-4 / GB + Perforin is significantly higher in the FCRx Stable Macrochimerism Group than in the FCRx Transient Macrochimerism Group

Conclusions

- **Urinary cell levels of CTLA-4 are uniquely higher in the FCRx Group than in the No Rejection Group and the ACR Group**
- **Urinary cell levels of several other mRNAs are not different between the FCRx group and No Rejection biopsy group.**
- **The ratio of CTLA-4 mRNA to GB mRNA + Perforin mRNA distinguishes FCRx Stable Group from the FCRx Transient Group**
- **Levels of CTLA-4 mRNA and the ratio of CTLA-4 to GB and Perforin are potential new biomarkers to identify tolerance and emphasize domination of negative regulation over cytotoxic effectors in tolerant kidney graft recipients.**

Why Don’t We Have a High Incidence of GVHD?

Control of Donor HSC cell composition

Robust deletional effect of nonmyeloablative conditioning

Peripheral Immune Regulation:

Tregs? MDSC? Bregs?

Immune Exhaustion?

What are the mechanisms underlying acquisition of immune competence in fully chimeric mismatched subjects?

Antigenic cross dressing

Persistence of recipient tissue resident APCs

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Questions?