“The greatest danger for most of us is not that our aim is too high and we miss it, but that it is too low and we reach it.”

Michelangelo
First Identical-Twin Kidney Transplant 1953

Performed on December 23, 1954 (PBB Hospital)

Joe Murray Receiving the Nobel Prize, 1990
Sayegh and Murray, Harvard Professorship and Chair Celebration, 2005
Transplantation 60 Years Later

- Transplantation is now the treatment of choice for end-stage organ failure
- Non life-saving organs: kidney (improved outcome versus dialysis) and pancreas
- Life-saving organs: liver, heart, lung
- Composite tissue: limbs, face, others
- Pancreatic islet cells: insulin replacement or cure for diabetes?
- Stem cells?
- Many challenges remain!
Transplantation 60 Years Later: Challenges

- Recipient: Donor supply pool
- Long-term immunosuppression
- Chronic attrition
- Changing face of rejection
- Tolerance
- Economy
- Commercialism
# Global Activity in Organ Transplantation

## 2012 Estimates

<table>
<thead>
<tr>
<th>Organ</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>76000</td>
</tr>
<tr>
<td>Liver</td>
<td>24000</td>
</tr>
<tr>
<td>Heart</td>
<td>5000</td>
</tr>
<tr>
<td>Lung</td>
<td>3000</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2000</td>
</tr>
</tbody>
</table>

≈ 110,000 organs transplanted per year
≤ 10% of global needs

104 countries reported to the Global Observatory on Organ Donation and Transplantation
South Africa, India and China - are 2008 estimates
Organ donation in the Middle East region

- **Gap is growing between supply and demand of organs in MESOT countries**
  - No. patients on waiting lists is constantly growing
  - Patients seek commercial transplantation

- **Living organ donation is the most widely practiced type of donation in the Middle East, including kidney and partial liver**
  - Donors are predominantly genetically related to recipients

- **Cadaveric organ donation has “great potential” in the region (primarily due to rate of accidents)**
  - Limited by continued debate within the medical community about the concept of brain death and inadequate public awareness of the importance of organ donation

- **MESOT**
  - Established in Turkey in 1987 as a non-profit
  - >29 country members, including all Arab countries, Iran, Turkey, Pakistan, and countries of central Asia
Most patients in MESOT countries die whilst waiting for organ transplantation.

- Organs from donations after cardiac death (DCD) can increase the donor pool by 15%–25%
  - Several ethical, legal, and social concerns need to be addressed to make DCD more widely accepted by the general population in the Middle East.
Total Transplantation Performed in MESOT per Organ (Jan-Dec 2012)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Live</th>
<th>Deceased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>6611</td>
<td>1379</td>
</tr>
<tr>
<td>Liver</td>
<td>1062</td>
<td>621</td>
</tr>
<tr>
<td>Heart</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>

The challenges of organ donation in the MEC region are complex

- Inadequate preventative medicine
- Uneven health infrastructure
- Poor awareness in the medical community
- Poor government support
- Understanding by the public of the importance of organ donation
- Lack of effective health insurance
- Lack of planning for organ procurement and transplant centers
MENOS:
Middle East Network for Organ Sharing
To address the growing problems of organ sales, transplant tourism and trafficking in organ donors in the context of the global shortage of organs, a Summit Meeting was held in Istanbul of more than 150 representatives of scientific and medical bodies from 78 countries around the world, and including government officials, social scientists, and ethicists.
The Declaration of Istanbul
Organ Trafficking and Transplant Tourism and Commercialism

152 participants from 78 countries

To halt unethical activities and to foster safe and accountable practices that meet the needs of transplant recipients while protecting donor

*Lancet. Vol 372 July 5, 2008*
Principles:

1. National governments, working in collaboration with international and non-governmental organizations, should develop and implement comprehensive programs for the screening, prevention and treatment of organ failure, which include:

- The advancement of clinical and basic science research;

- Effective programs, based on international guidelines, to treat and maintain patients with end-stage diseases, such as dialysis programs for renal patients, to minimize morbidity and mortality, alongside transplant programs for such diseases;

- Organ transplantation as the preferred treatment for organ failure for medically suitable recipients.
The Declaration of Istanbul on Organ Trafficking and Transplant Tourism

2. Legislation should be developed and implemented by each country or jurisdiction to govern the recovery of organs from deceased and living donors and the practice of transplantation, consistent with international standards:

- Policies and procedures should be developed and implemented to maximize the number of organs available for transplantation, consistent with these principles;
- The practice of donation and transplantation requires oversight and accountability by health authorities in each country to ensure transparency and safety;
- Oversight requires a national or regional registry to record deceased and living donor transplants;
- Key components of effective programs include public education and awareness, health professional education and training, and defined responsibilities and accountabilities.
The Declaration of Istanbul on Organ Trafficking and Transplant Tourism

3. Organs for transplantation should be equitably allocated within countries or jurisdictions to suitable recipients without regard to gender, ethnicity, religion, or social or financial status.
   
   – Financial considerations or material gain of any party must not influence the application of relevant allocation rules.

4. The primary objective of transplant policies and programs should be optimal short- and long-term medical care to promote the health of both donors and recipients.
   
   – Financial considerations or material gain of any party must not override primary consideration for the health and well-being of donors and recipients.
5. Jurisdictions, countries and regions should strive to achieve self-sufficiency in organ donation by providing a sufficient number of organs for residents in need from within the country or through regional cooperation.

– Collaboration between countries is not inconsistent with national self-sufficiency as long as the collaboration protects the vulnerable, promotes equality between donor and recipient populations, and does not violate these principles;

– Treatment of patients from outside the country or jurisdiction is only acceptable if it does not undermine a country’s ability to provide transplant services for its own population.
The Declaration of Istanbul on Organ Trafficking and Transplant Tourism

Proposals:

Consistent with these principles, participants in the Istanbul Summit suggest the following strategies to increase the donor pool and to prevent organ trafficking, transplant commercialism and transplant tourism and to encourage legitimate, life-saving transplantation programs:

• To respond to the need to increase deceased donation.

• To ensure the protection and safety of living donors and appropriate recognition for their heroic act while combating transplant tourism, organ trafficking and transplant commercialism.
Changing Face of Rejection: Why?

• Better immunosuppression against cell mediated alloimmune responses
• Redundancy of the immune system: emergence of other mechanisms of rejection
• Use of induction therapy, especially T cell depleting antibodies and T cell costimulatory pathways blockade (Tregs)
• Proliferation of minimization strategies
• Better detection assays and awareness by the Tx professionals (e.g., the C4d revolution)!
T Cells Play A Key Role in Rejection
De novo purine synthesis

IL-2 receptor

Activated Calcineurin

NFAT

Steroid

NFAT

Cell Cycle

Aza

G1

S

MMF

M

G2

De novo purine synthesis

Costimulatory Signal

Antigenic Signal

Polyclonals

OKT3 mAb

TCR

IL-2-R mAb

Rapamycin

Polyclonals

T cell

Ca^{2+}

CsA

FK506

IL-2

Aza

IL-2 GENE PROMOTOR
EVOLUTION OF TRANSPLANT IMMUNOSUPPRESSION

Corticosteroids
Azathioprine
1960
Cyclosporine
1970
OKT3
1980
Polyclonal antibodies
1990
Transfusions
2000
Splenectomy
Thoracic duct drainage
Graft irradiation
2020
Tacrolimus
Cyclosporine Neoral/Generics
MMF
Neoral/Generics
IL2R mAbs
Rapamycin
Thymo/Campath-1H
Anto-CD20
CTLA4Ig
Individualization
Minimization
Tolerance

How Do We Reject a Graft?
1-Recognition of alloantigen:
   T cell-APC interaction (importance of peripheral lymphoid organs for naïve T cell recognition)
   T cell-endothelial cell interaction

2-T cell activation and differentiation:
   T cell costimulation
   Cytokine and chemokine production
   Proliferation and clonal expansion
   Differentiation: Th-c1/Th-c2/Th-c17
STEPS IN ALLOGRAFT REJECTION

3-T helper cell function (CD4+ T cells):
- CD8+ T cells, B cells and monocytes/macrophages
- Endothelial cell activation

4-Effecter mechanisms leading to graft destruction:
- Lymphocyte mediated cytotoxicity (CD8+ T cells)
- Alloantibodies: complement and cell mediated cytotoxicity
- Delayed type hypersensitivity (macrophages)
Emergence of new mechanisms of rejection

1. B cell and antibody mediated rejection
2. Monocyte-mediated after T cell depletion
3. T-17 cells: new kids on the block
4. Imbalance of T-effector/Regulatory mechanisms
Figure 1

a) Non-T cell mediated rejection

b) T cells

c) Monocytes

Salama, Womer and Sayegh JI 2007
TH1/TH2 PARADIGM: The Happy Days!

Th0 Cell

- IL-12
- IL-4

Th1 Cell

- IFN-\(\gamma\)
- IL-2

Th2 Cell

- IL-4
- IL-5
- IL-10
- IL-13

Cell Mediated Immunity

Humoral Immunity
Allergy
Parasitic Infections
Heart transplantation across single MHC class II-mismatched strains

BM12 (H-2^{bm12})  B6 (H-2^{b})

- Spontaneous graft acceptance without immunosuppression
- Established model of chronic allograft rejection and CAV

Yang et al. Circulation 2008
bm12 into C57BL/6

*MHC class II mismatch model of CAV*

WT (develop CAV)

INF-g-/- (protected)

T-bet-/-(?)

heart tx
Dramatic acceleration of allograft rejection in Tbet−/− recipients
WT (DAY 14)  T-BET⁻/⁻ (DAY 14)  IFN-γ⁻/⁻ (DAY 14)

WT (DAY 56)  IFN-γ⁻/⁻ (DAY 56)

WT DAY 14  T-BET⁻/⁻ DAY 14  INF-γ⁺/⁺ DAY 14

WT DAY 56  INF-γ⁺/⁺ DAY 56
Current model of T helper cell differentiation

From Afzali B. et al.  
*Clinical and Experimental Immunology*, 148: 32-46.
Naive CD4+ T cells, after activation by signalling through the T-cell receptor and co-stimulatory molecules such as CD28 and inducible T-cell co-stimulator (ICOS), can differentiate into one of three lineages of effector T helper (TH) cells — TH1, TH2 or TH17 cells. These cells produce different cytokines and have distinct immunoregulatory functions. Interferon-γ (IFNγ) produced by TH1 cells is important in the regulation of antigen presentation and cellular immunity. The TH2-cell cytokines interleukin-4 (IL-4), IL-5 and IL-13 regulate B-cell responses and anti-parasite immunity and are crucial mediators of allergic diseases. TH17 cells have been shown to express IL-17, IL-17F, IL-21 and IL-22 (and IL-26 in humans) and to regulate inflammatory responses. TGFβ, transforming growth factor-β.
Th2 skewing in INFg$^{-/-}$ and Tbet$^{-/-}$ recipients

Increased Th17 cytokines in Tbet$^{-/-}$
Both CD4 and CD8 graft infiltrating cells produce **IL-17**
Absence of CD4$^+$ cells prolongs graft survival in Tbet$^{-/-}$
No evidence of chronic allograft rejection in absence of CD4$^+$ cells
IL-17 and IL-6 cytokine production is dramatically reduced in Tbet CD4 DKO compared to Tbet CD8 DKO and Tbet$^{-/-}$.
Anti-IL17 significantly prolongs allograft survival (bm12 heart) in Tbet−/−

![Graph showing the survival rate over time with control IgG and Anti-IL-17 treatments.](image)

- **X-axis**: Days (0 1 2 3 4 7 9 11 13)
- **Y-axis**: Percent survival
- **Legend**:
  - Control IgG
  - Anti-IL-17
- **Statistical Significance**: P<0.01

Injections of antibody (100μg/injection)
# Phenotype of graft infiltrating leukocytes in T-bet⁻/⁻ recipients with and without IL-17 neutralization

<table>
<thead>
<tr>
<th>Infiltrating cells</th>
<th>T-bet⁻/⁻ Control Ig (day 14)</th>
<th>T-bet⁻/⁻ IL-17 neutralization (day 14)</th>
<th>T-bet⁻/⁻ IL-17 neutralization (day 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>15.4 ± 2.1</td>
<td>3.2 ± 1.3</td>
<td>10.3 ± 3.0</td>
</tr>
<tr>
<td>Macrophages</td>
<td>21.6 ± 4.3</td>
<td>4.7 ± 1.8</td>
<td>13.6 ± 3.0</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>18.4 ± 5.6</td>
<td>1.2 ± 0.3</td>
<td>4.6 ± 1.9</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>96.1 ± 13.5</td>
<td>26.4 ± 6.5</td>
<td>71.7 ± 7.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>151.1 ± 18.3</td>
<td>34.1 ± 6.9</td>
<td>100.3 ± 13.7</td>
</tr>
</tbody>
</table>
Conclusions

- T-bet deficiency results in up-regulation of Th2/Th17 cytokines
- Accelerated allograft rejection in T-bet⁻/⁻ is independent of Th-1 cytokines
- Neutralizing IL-17 results in a prolonged graft survival
- CD4⁺ but not CD8⁺ T cell deficiency or depletion in T-bet⁻/⁻ mice affords dramatic protection from vasculopathy and facilitates long-term acceptance of the grafts

*IL-17 producing CD4 Th17 cells are important in allograft rejection and may serve as a novel target of strategies for prevention of transplant rejection and graft vasculopathy*
Acknowledgements

A novel role of CD4 Th17 cells in mediating cardiac allograft rejection and vasculopathy

Xueli Yuan,1 Jesus Paez-Cortez,1 Isabela Schmitt-Knosalla,1 Francesca D’Addio,1 Bechara Mfarrej,1 Michela Donnarumma,1 Antje Habicht,1 Michael R. Clarkson,1 John Iacomini,1 Laurie H. Glimcher,2 Mohamed H. Sayegh,1 and M. Javeed Ansari1,3

1Transplantation Research Center, Renal Division, Brigham and Women’s Hospital and Children’s Hospital Boston, Harvard Medical School and 2Harvard School of Public Health and Harvard Medical School, Boston, MA 02115
3Division of Nephrology and Division of Organ Transplantation, Northwestern University Feinberg School of Medicine, Chicago, IL 60611
IL-17-dependent cellular immunity to collagen type V predisposes to obliterative bronchiolitis in human lung transplants

CD4+ T cell–dependent, col(V)-specific cell-mediated immunity in lung transplant (Tx) recipients. (A) TV-DTH responses by PBMCs obtained from normal healthy controls (n = 6), renal transplant recipients with GPS at 2–10 yr after transplant (n = 5), or lung transplant recipients at 0.5–3.5 yr after transplant (n = 15). Lung primary disease types represented are chronic obstructive pulmonary disease (n = 7), cystic fibrosis (n = 3), idiopathic pulmonary fibrosis (n = 2), α-1 antitrypsin deficiency (n = 2), and other (n = 1). All had stable graft function on standard immunosuppression at time of testing. TV-DTH responses to EBV and TT/DT were determined separately and averaged to yield a positive control swelling response (Recall) for each subject. Responses to 5 μg col(II), col(IV), or col(V) were averaged from duplicate tests and are shown as individual data points. Horizontal bars denote group means. **P < 0.001 among treatment groups in response to a specific collagen, Wilcoxon rank-sum test. (B) TV-DTH responses (mean ± SEM) to TT/DT and col(V) in col(V)-reactive patients L52 and L84 in the presence of isotype control, anti–IFN-γ, anti–TNF-α, anti–IL-1β, or anti–IL-17 Abs. *P < 0.05 versus IgG, Student’s t test. (C) TV-DTH responses (mean ± SEM) to TT/DT and col(V) by sham-depleted whole PBMCs (10 × 106) or subset-depleted PBMCs (8 × 106) of patients L84 (α-1 antitrypsin deficiency) and L52 and L16 (chronic obstructive pulmonary disease). NT, not tested. *P < 0.05 versus whole PBMC, Student’s t test.

T helper cell differentiation

From Afzali B. et Al. 
Clinical and Experimental Immunology, 148: 32-46.
The Balance of Teff/Tregs Determines Outcome

**Graph Description:**
- **Y-axis:** Alloreactive T cell pool size.
- **X-axis:** Time post-transplant.
- **Areas:**
  - **Expansion:** Effector phase / acute rejection.
  - **Apoptotic deletion:** Induction.
  - **Stable tolerance:** Meta-stable tolerance.
  - **Memory / chronic rejection:** Stable tolerance.

**Key Points:**
- **No Rx:** Many reactive T cells.
- **Rx:** Few reactive T cells.
- **Regulatory T cells:**
  - Many reactive T cells → No Rx.
  - Few reactive T cells → Rx.

**Legend:**
- Expansion.
- Apoptotic deletion.
- Rejection.
- Tolerance.

**References:**
- Turka/Strom/Sayegh 2007
Regulatory T cell homeostasis

Thymus

CD4⁺CD25⁺ FoxP3⁺

autoantigen

CD4⁺CD25⁻ FOXP3⁻

Self or foreign antigen

Periphery

CD4⁺CD25⁺ FoxP3⁺

Natural T reg

CD4⁺CD25⁺ FoxP3⁺

Induced T reg

CD4⁺ Tr1 IL-10

CD4⁺ Th3 TGFβ

CD4⁺ effector

Naïve CD4⁺CD25⁻ FOXP3⁻

Natural T reg

Adaptive T reg

Courtesy, Jeff Bluestone
FOXP3 mRNA Levels are Higher in Patients with Successfully Reversed Acute Rejection Episode

Urine specimens were collected from 36 patients with acute rejection. Twenty six of the 36 acute rejections qualified as successful reversal and the remaining 10 did not. Urinary cell Foxp3 mRNA was significantly higher in the group with successful reversal than in the group with out reversal (mean±SE: 4.7±0.5 vs. 1.5±0.7, P=0.0009). The levels of CD25 (7.3±0.4 vs. 6.0±0.9, P=0.23), CD3ε (8.5±0.5 vs. 7.4±0.8, P=0.35), and perforin (7.8±0.5 vs. 7.3±0.7, P=0.43) were not informative of acute rejection outcome.
IMMUNE REACTIVITY IN STABLE AND HIGH RISK PATIENTS

Frequency of 60/million IFN-γ producing T cells

Stable

- < 60 spots/million: 77%
- > 60 spots/million: 23%

>1 rejection

- < 60 spots/million: 27%
- > 60 spots/million: 73%

p=0.02 by two tailed Chi squared test

Najafian et al JASN 2002
CD4^+CD25^- Mediated Regulation of the Indirect Alloresponse

*Stable Patients*
- < 60 spots per million cells
  - Regulation: 53%
  - No Regulation: 47%

*High Risk Patients*
- 60 spots per million cells
  - No Regulation

*P* = 0.018

Salama et al, JASN, 2003
B7/CD28 co-stimulation and T cell activation
hCTLA4Ig prolongs graft survival in fully MHC mismatch model
Costimulation Blockade with Belatacept in Renal Transplantation

Flavio Vincenti, M.D., Christian Larsen, M.D., Ph.D., Antoine Durrbach, M.D., Ph.D., Thomas Wekerle, M.D., Björn Nashan, M.D., Ph.D., Gilles Blancho, M.D., Ph.D., Philippe Lang, M.D., Josep Grinyo, M.D., Philip F. Halloran, M.D., Ph.D., Kim Solez, M.D., David Hagerty, M.D., Elliott Levy, M.D., Wenjiong Zhou, Ph.D., Kannan Natarajan, Ph.D., and Bernard Charpentier, M.D., for the Belatacept Study Group*
All patients received basiliximab, MMF, and corticosteroid-tapering regimen.
LEA29Y Phase II Trial: Results

- Equivalent acute rejection rates to CNI
- Improved GFR
- Less CAN by biopsy
- Relatively safe (need to watch for PTLD)
- What are the mechanisms of rejection while receiving LEA29Y?

Vincenti et al. NEJM 2005
B7/CD28 co-stimulation and T cell activation

Effector T cell

Regulatory T cell
B7/CD28 deficiency prolonged graft survival in fully allogeneic mismatched cardiac transplant model
LEA29Y (Belatacept): concerns

Cutting Edge: CD28 Controls Peripheral Homeostasis of CD4^+CD25^+ Regulatory T Cells

B7/CD28 deficiency shortened graft survival in single MHC class II-mismatched cardiac transplant model.

Graph showing graft survival over days after transplantation with different experimental groups:
- bm12 into B6
- bm12 into CD28 KO
- bm12 into B7.1/2 DKO
B7/CD28 deficient recipients showed a decreased number of CD4^+CD25^+Foxp3^+ T cells

\[ p=0.0047 \]

\[ p=0.0026 \]
B7/CD28 deficiency caused a significant increase in the ratio of CD4$^+$ effector memory/Treg

![Graph showing the ratio of CD4$^+$ effector memory/Treg in B6 WT, CD28 KO, and B7.1/2 DKO recipients of bm12 cardiac allografts. The graph indicates a significant increase in the ratio for B7.1/2 DKO compared to B6 WT and CD28 KO.]

- p=0.0102
- p=0.0015
Anti-B7.1/2 mAb also shortened survival of bm12 hearts in B6 recipients

Anti-B7.1/2 antibody
500 μg at day -10 and 250 μg
days -8, -6, -4, -2, 0
CD25\(^+\) T cell depletion shortened survival of bm12 hearts in B6 recipients

Anti-CD25 antibody
days -6 and -1
250ug
## Allograft Survival and % Tregs

<table>
<thead>
<tr>
<th>Bm12 into B6</th>
<th>% CD4⁺Foxp3⁺ (Mean ± SEM)</th>
<th>Median Survival Time (MST, Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7.1/2 DKO</td>
<td>2.67 ± 0.57</td>
<td>7.5</td>
</tr>
<tr>
<td>CD28 KO</td>
<td>3.63 ± 0.37</td>
<td>13</td>
</tr>
<tr>
<td>Anti-CD25 (pre-Tx)</td>
<td>4.99 ± 0.29</td>
<td>16</td>
</tr>
<tr>
<td>Anti-B7.1+ Anti-B7.2 (pre-Tx)</td>
<td>6.42 ± 1.13</td>
<td>19</td>
</tr>
<tr>
<td>B6 WT</td>
<td>13.18 ± 0.48</td>
<td>&gt;56</td>
</tr>
</tbody>
</table>
Conclusions

- This study demonstrates for the first time the paradoxical role of B7/CD28 co-stimulation in fully allogeneic and single MHC class II-mismatched grafts;

- B7/CD28 deficiency affects Treg generation and maintenance leading to an increase ratio of effector to regulatory T cells (Teff/Treg) and precipitating rejection in the MHC class II-mismatched model, a Treg-dependent model;

- These data suggests that B7 blockade may have paradoxical effects in recipients of MHC class II-mismatched grafts or in grafts dependent on Tregs for long-term survival, an issue that may have consequences in ongoing clinical trials with Belatacept (LEA29Y).
hCTLA4 decreases natural Tregs on naive mice
hCTLA4Ig has paradoxical effect on single MHC class-II mismatch model
Summary: BENEFIT (AJT, 2010)

- Belatacept regimens compared to CsA demonstrated superior renal function, less CAN, similar patient/graft survival, and a favorable cardiovascular/metabolic profile.
- Higher rates and grades of AR in belatacept groups had limited impact on renal function and graft survival.
- Safety profile of belatacept was generally similar to CsA.
- Higher number of PTLD cases in belatacept groups; most occurred in recipients with known risk factors (eg, EBV negative serology, T-cell depleting therapy).
- The LI regimen appeared to be preferable to the MI regimen.
Incidence of Acute Rejection Episodes

<table>
<thead>
<tr>
<th>Banff grade, n (%)</th>
<th>Belatacept MI</th>
<th>Belatacept LI</th>
<th>CsA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild acute (IA)</td>
<td>7 (3)</td>
<td>4 (2)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Mild acute (IB)</td>
<td>3 (1)</td>
<td>8 (4)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Moderate acute (IIA)</td>
<td>17 (8)</td>
<td>16 (7)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Moderate acute (IIB)</td>
<td>20 (9)</td>
<td>10 (4)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Severe acute (III)</td>
<td>2 (1)</td>
<td>1 (&lt;1)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Did not meet 20% NI margin
† Met 20% NI margin
Belatacept compared to CsA demonstrated

- **Efficacy**
  - Similar patient/graft survival
  - Better renal function
  - Better cardiovascular/metabolic profile
  - Similar rates of acute rejection

- **Safety and tolerability**
  - Similar rates of infection and malignancy
    - Higher number of PTLD; most occurred in recipients with known risk factors
  - Well tolerated as a monthly infusion
Incidence of Acute Rejection Episodes

<table>
<thead>
<tr>
<th>Banff grade, n (%)</th>
<th>Belatacept MI</th>
<th>Belatacept LI</th>
<th>CsA</th>
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<tr>
<td>Mild acute (IA)</td>
<td>0</td>
<td>4 (2)</td>
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<td>Mild acute (IB)</td>
<td>7 (4)</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Moderate acute (IIA)</td>
<td>10 (5)</td>
<td>17 (10)</td>
<td>17 (9)</td>
</tr>
<tr>
<td>Moderate acute (IIB)</td>
<td>16 (9)</td>
<td>8 (5)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Severe acute (III)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Effector T cells

Small pool:
Living donor tx, 1st transplant

High dose CTLA4Ig

Tregs

REJECTION

TOLERANCE
Effector T cells

Large pool:
Cadaver highly mismatched
ECD kidney transplant

Tregs

High dose
CTLA4Ig

REJECTION

TOLERANCE
Clinical Development of Cell Therapy with Tregs in Tx

1. Ex vivo expansion and infusion
2. In vivo expansion by agents
3. Super Treg?
4. Inhibiting counter-Treg signals?

Are we there yet?
..we thought we understood it all, but then something new comes up..?
True Tolerance!

BILLINGHAM, BRENT, AND MEDAWAR
The Promise:
Indefinite graft survival
No maintenance immunosuppression
No drug-related side effects
No chronic rejection
Improved long-term outcome

Neonatal Tolerance
CLINICAL TOLERANCE
WE NEED

• Strategies that reproducibly create tolerance in animals

• Refined immunological assays to:
  – Reproducibly detect stages of tolerance
  – Reproducibly detect incipient rejection
CLINICAL TOLERANCE

- Cadaver transplant recipients who received total lymphoid irradiation: Strober et al., NEJM 1987
- Previous recipients of bone marrow transplantation for therapy of hematological malignancies from the same donor: Sayegh et al., Ann. Int. Med. 1991
- Bone marrow and kidney transplantation for therapy of hematological malignancies (myeloma) and renal failure: Spitzer et al., Transplantation 1999 and AJT 2006
- Immunosuppression withdrawal planned or supervised (success in liver approximately 20% when attempted); Caveats: ? true tolerance versus rejection free state, ? Longevity, ? predictability of success
THE ROAD TO TRANSPLANTATION
TOLERANCE IN HUMANS

- De-novo tolerance inducing protocols followed by immunosuppression withdrawal

- Immunosuppression minimization: does it lead to tolerance?
Active clinical trials in solid organ transplantation from the immune tolerance network

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>Study objectives</th>
<th>Study phase</th>
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<tbody>
<tr>
<td>An Observational Study to Assess the Prevalence of a Tolerance Signature in Renal Transplant Recipients (ARTIST)</td>
<td>▪ To assess the frequency of a previously defined tolerance signature* in a broad population of renal transplant recipients</td>
<td>Phase I (ongoing)</td>
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</table>
| Gradual Withdrawal of Immunosuppression in Patients Receiving a Liver Transplant (AWISH) | ▪ To evaluate the safety of slow withdrawal of IS drugs, and whether slow withdrawal will help reduce the long-term side effects of these drugs in liver transplant recipients  
▪ To evaluate the effect of slow withdrawal of IS drugs on HCV re-infection rates | Phase II (ongoing)        |
| Immunosuppression Withdrawal for Pediatric Living-Donor Liver Transplant Recipients (WISP-R) | ▪ To determine whether IS drugs can be safely withdrawn over a minimum of 9 months from children who received liver transplants at least 4 years ago                                                  | Phase I (ongoing)        |
| ITN Registry of Tolerant Kidney Transplant Recipients                          | ▪ Registry to identify kidney transplant patients who have maintained a functioning allograft, despite the cessation of all immunosuppression                                                                    | Recruiting               |
| Renal Allograft Tolerance through Mixed Chimerism                               | ▪ To evaluate the safety and efficacy of complete withdrawal of IS drugs following combined kidney and bone marrow transplants from the same donor                                                              | Phase II (suspended)     |

* Registry trial (ITN507ST) identified a signature from kidney recipients with functional tolerance as defined by stable renal function while remaining off IS drugs
IS, immunosuppressant; HCV, hepatitis C virus; ALT, alemtuzumab; TAC, tacrolimus; pts, patients, ESLD, end-stage liver disease
There are critical hurdles in achieving transplantation tolerance

<table>
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<tr>
<th>Scientific</th>
<th>Nonscientific</th>
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<td>2. Strategy: central or peripheral depletion in association with drug minimization or novel adjunctive therapies.</td>
<td>2. Ethical issues with complete immunosuppression withdrawal and risk of graft loss.</td>
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<tr>
<td>3. Confirmation in large animal models and the reproducibility in patients.</td>
<td>3. Optimizing the tolerogenic regimens in association with industry.</td>
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<td>4. Defining the mechanisms through which innate immunity, immune memory, and infections prevent or break tolerance induction.</td>
<td>4. Financing the trials.</td>
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<td>5. Tolerance trial end points.</td>
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</tbody>
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IMMUNE MONITORING

what, when, and where to look?
Surrogate markers in organ transplantation: bench to bedside

- Define the immunological basis of alloimmune responses / therapeutic targets
- Deliver blueprints for clinical management
- Provide safety for implementation of immunosuppression minimization, withdrawal and tolerance strategies
Why develop surrogate markers?

- Organ dysfunction is a late event
- Biopsy findings may not be predictive of clinical event and/or immune response
- Immunosuppressive drug levels do not reflect status of immune response
- Current measures do not allow “tailoring” of immunosuppression
- Immunosuppression minimization or withdrawal is monitored by clinical events or biopsies (both are late events), and not by status of immune response
- Clinical tolerance strategies require assays to measure the immune response
How to develop surrogate markers?

- Understand the pathophysiology of the process
- Develop an assay/assays that detects the particular relevant step in the pathophysiology
- Timing is critical, dynamic
- Sensitivity
- Specificity
- Predictive
- Amenable to change after an intervention
What are the characteristics of an ideal biomarker assay test/s?

- High positive and negative predictive values
- Diagnose rejection before/early after occurrence
- Guides drug optimization, minimization, withdrawal
- Rapid-easy to perform in most centers
- Non-invasive
- Inexpensive

Battery rather than a single test!
Biomarkers in Tx: Challenges!

- Where to look: periphery versus graft tissue?
- Adequacy of sample collections
- Quality assurance and control, reproducibility
- Handling massive amount of data
- Bioinformatics
- Statistical analysis
- Clinical utility: change in behavior
- Incorporation into endpoints
Learn From Experience!

- ITN and European Tolerance Consortium
- CTOT
- CCTPT (now CTOT-C)
- CIT
- NIH Genomics Tx Consortium
- Other non-Tx consortia (ACE, TrialNet, etc.)
Most patients will be on single immunosuppressive agent

Proliferation of biologicals

Increasing number of tolerant recipients but not wide spread applicability of tolerance protocols

Stem cell transplantation will work in some diseases but not others

A breakthrough in biomarkers may occur but clinical applicability will be debated

Malignancy will become the most important single factor determining long term outcome

The dream of unlimited number of organs and tolerance will continue
Unlimited Organs  Tolerance

Mohamed H. Sayegh, M.D. 2001 Presidential Address  American Society of Transplantation
STEM CELLS: FUTURE ORGAN/TISSUE REGENERATION?
Wahal yastawee 2allatheen ya3lamoun wallatheen la ya3lamoun?