Fellows Symposium on Transplantation Medicine

Friday, September 23
1:10 pm - 1:30 pm

Trends and Challenges in Transplantation

John S. Gill, MD

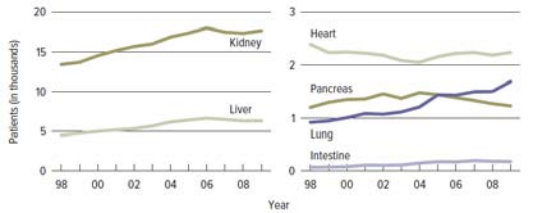
September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

Overview of Solid Organ Transplantation in the US: *SRTR Annual Data Report 2010*

John S. Gill MD, MS
UBC, Vancouver

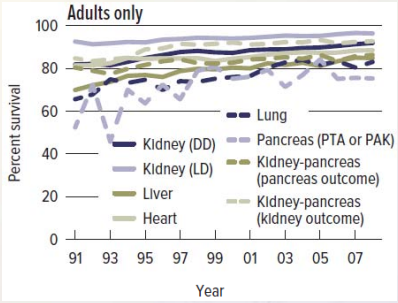
Solid Organ Transplant Comparisons

Transplants performed during the year (adult & pediatric combined)



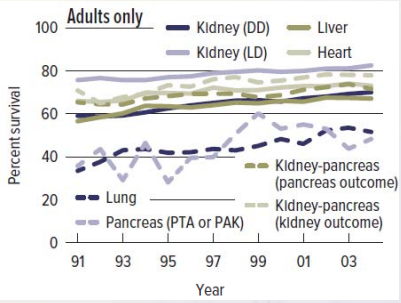
3

One-year all-cause graft survival



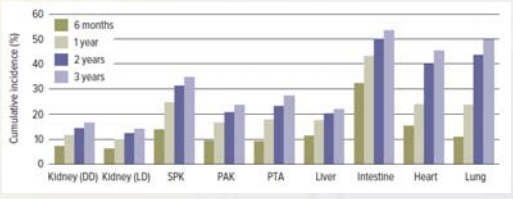
4

Five-year all-cause graft survival



5

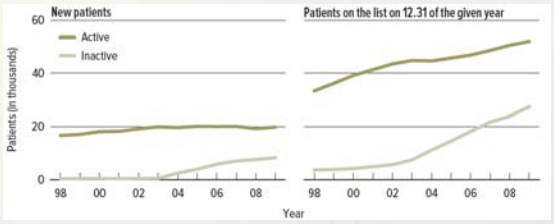
Cumulative incidence of first acute rejection



6

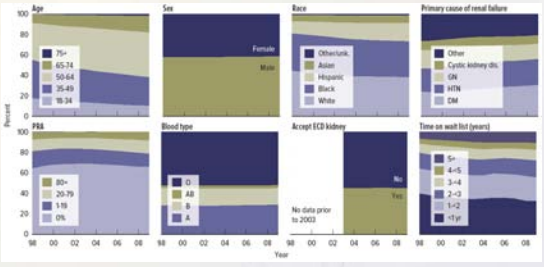
Kidney

Adult patients waiting for a kidney transplant



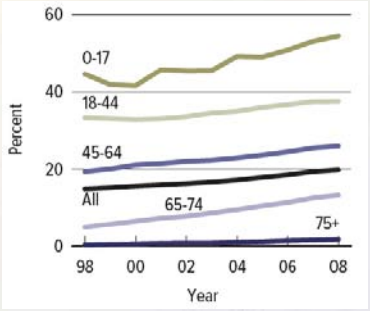
8

Characteristics of adult patients waiting for a kidney transplant



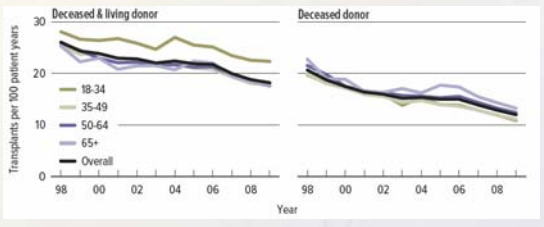
9

Prevalent dialysis patients listed for a kidney transplant, by age



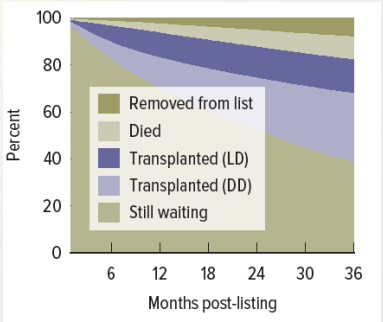
10

Transplant rates among adult patients listed for a kidney transplant, by age



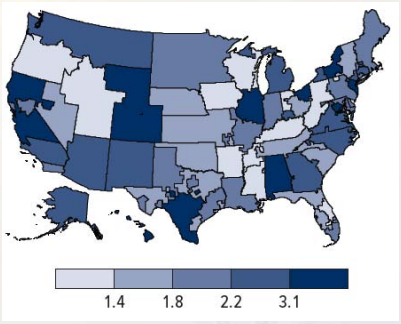
11

Kidney transplant waiting list status by month after listing, among new adult listings in 2006



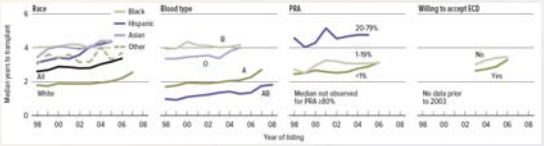
12

Median years to deceased donor adult kidney transplant in 2009, by DSA



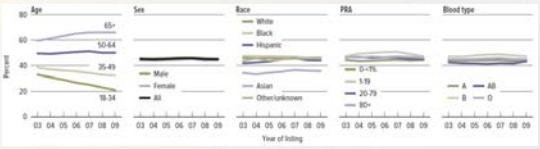
13

Median years to kidney transplant for listed adult patients



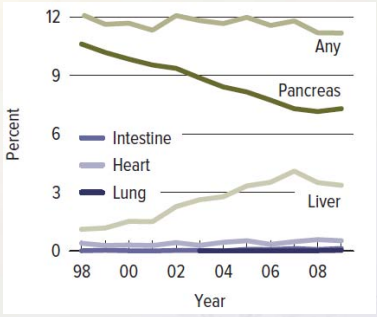
14

Adult listings willing to accept an ECD kidney



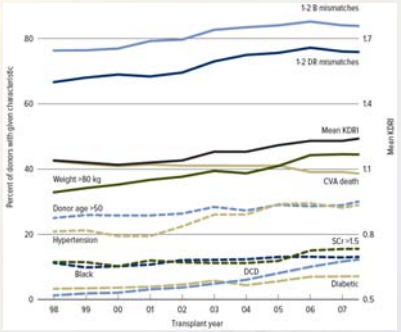
15

Deceased donor kidney recipients transplanted with another organ



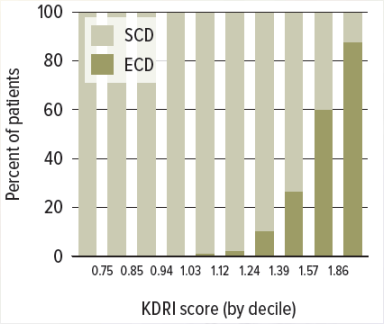
16

Major components of the kidney donor risk index (KDRI) over time



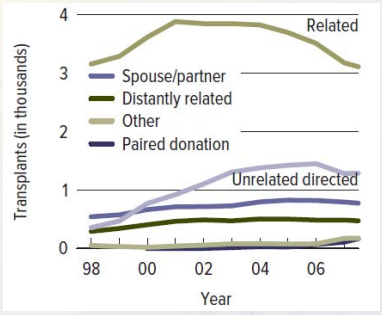
17

Kidney donor risk index (KDRI) scores for ECD & SCD kidneys, 2009



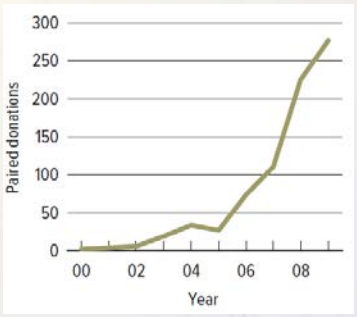
18

Kidney transplants from living donors, by donor relation



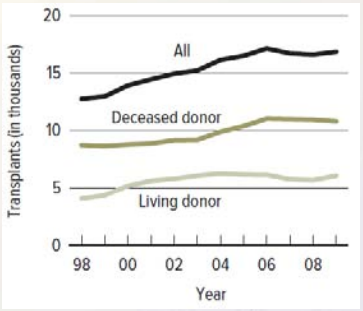
19

Paired kidney donations



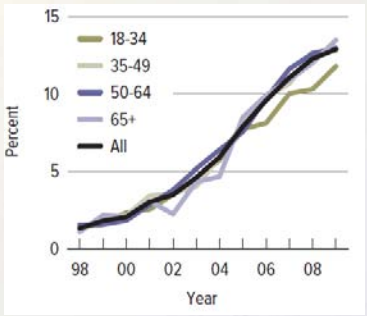
20

Total adult kidney transplants (includes SPK)



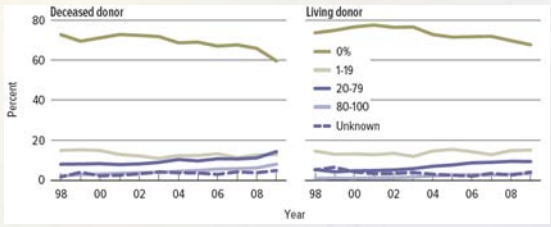
21

Use of DCD kidneys among adult, kidney alone transplant recipients, by recipient age



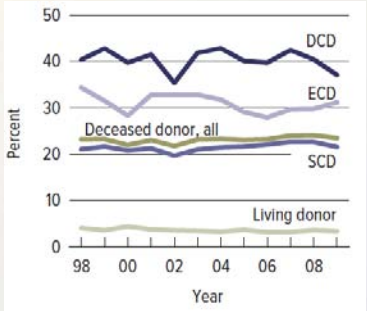
22

PRA at time of kidney transplant in adult recipients



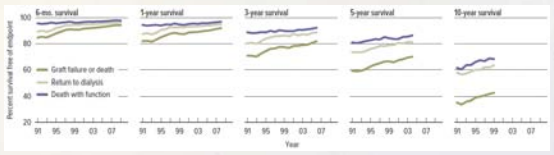
23

Delayed graft function among adult kidney transplant recipients



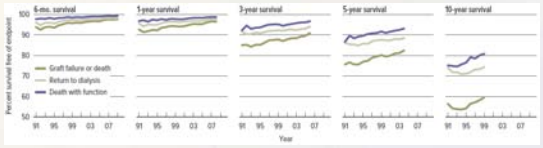
24

Outcomes among adult kidney transplant recipients: deceased donor



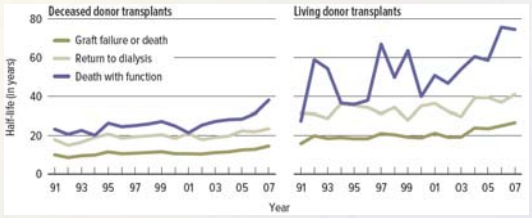
25

Outcomes among adult kidney transplant recipients: living donor



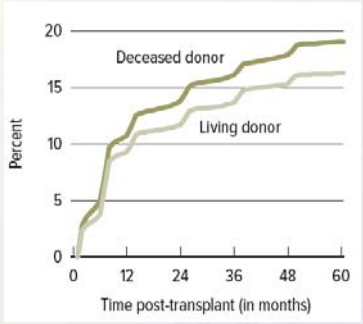
26

Half-lives for adult kidney transplants with a functioning graft ≥1 year



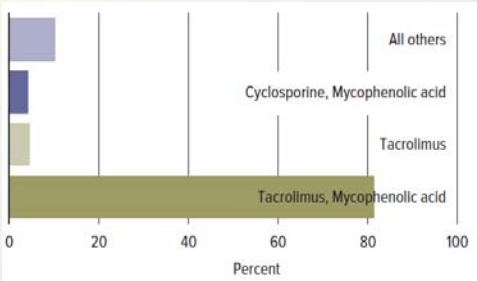
27

Incidence of first acute rejection for adult kidney transplants 2005–09



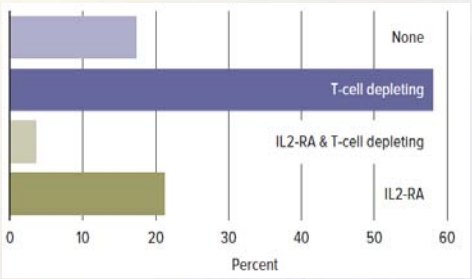
28

Initial immunosuppression in adult kidney transplant recipients in 2009 (+/- steroids)



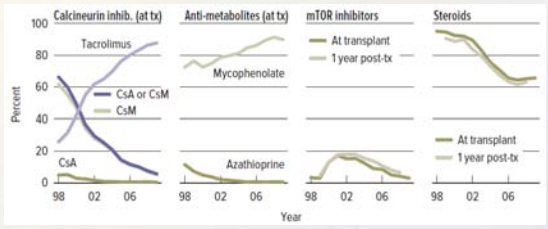
29

Induction agents used adult kidney transplant recipients, 2009



30

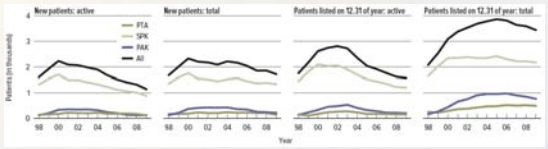
Immunosuppression use in adult kidney transplant recipients



31

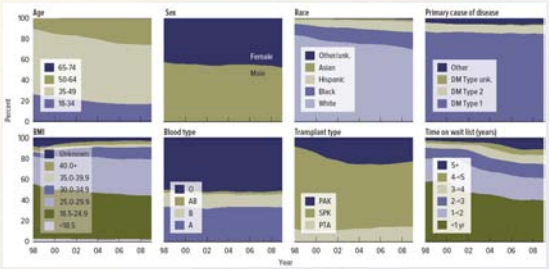
Pancreas

Adult patients waiting for a pancreas transplant



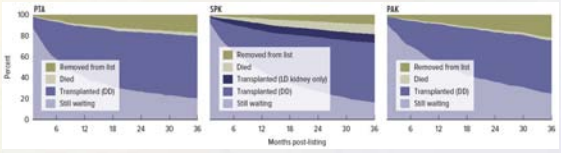
33

Characteristics of adult patients waiting for a pancreas transplant



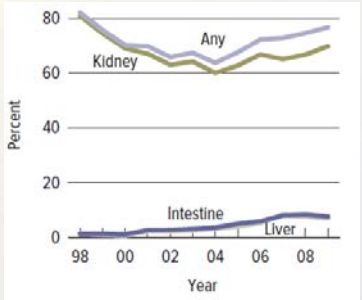
34

Pancreas waiting list status by month after listing among new adult listings in 2006



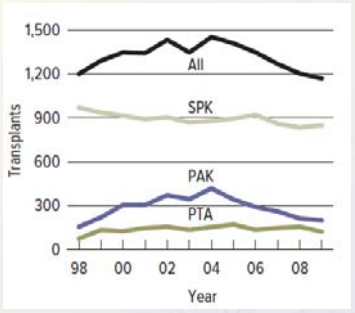
35

Deceased donor pancreata transplanted with another organ



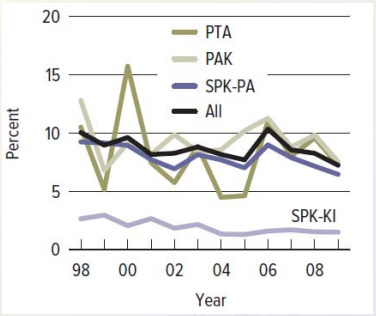
36

Total number of adult pancreas transplants



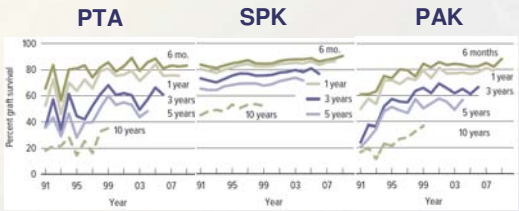
37

Early (<6 weeks) graft failure among adult pancreas transplant recipients



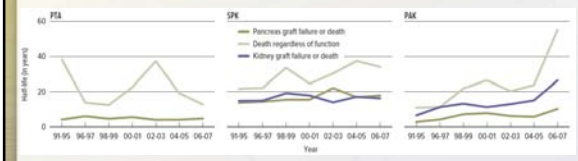
38

Pancreas failure among adult recipients



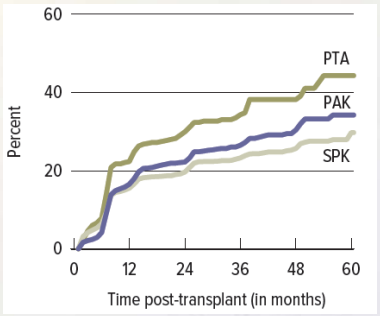
39

Half-lives for adult pancreas transplants functioning for at least 1 year



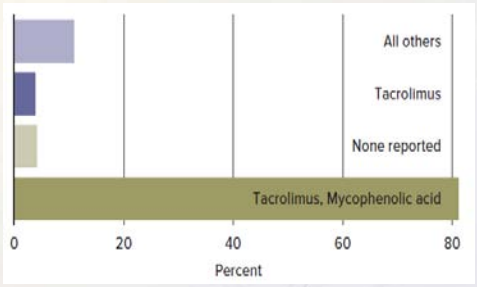
40

Incidence of first acute rejection among adults receiving a pancreas in 05–09

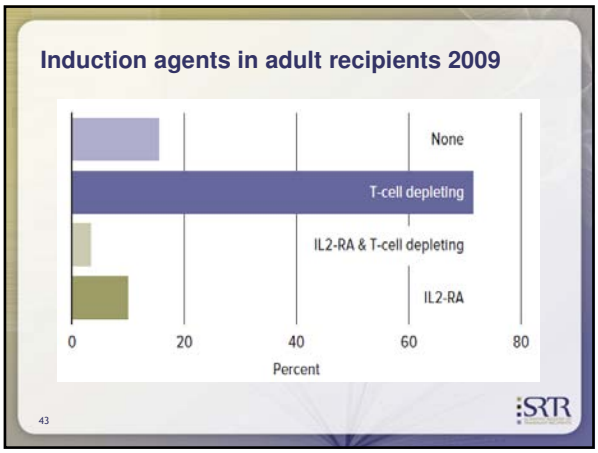


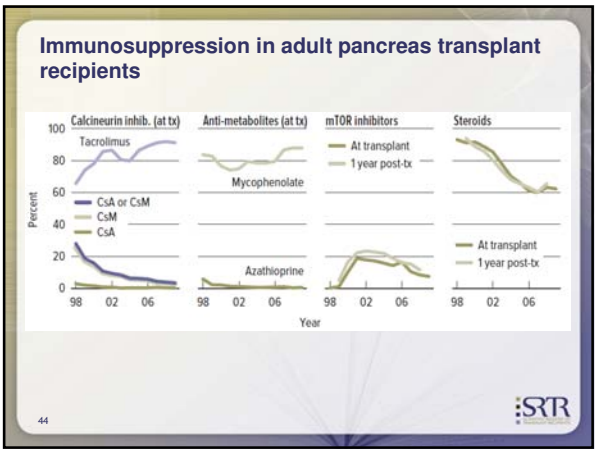
41

Initial immunosuppression in adult pancreas recipients 2009 (+/- steroids)

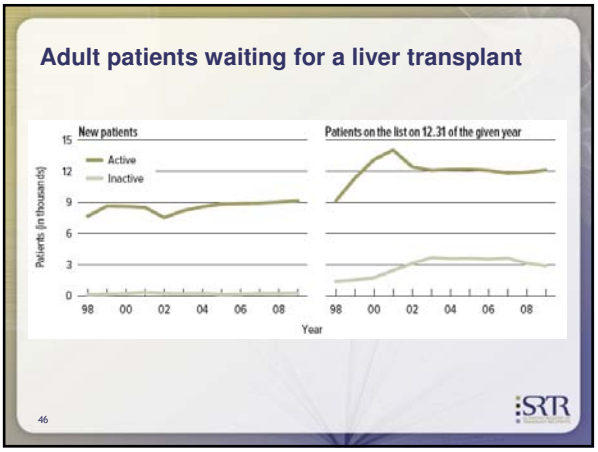


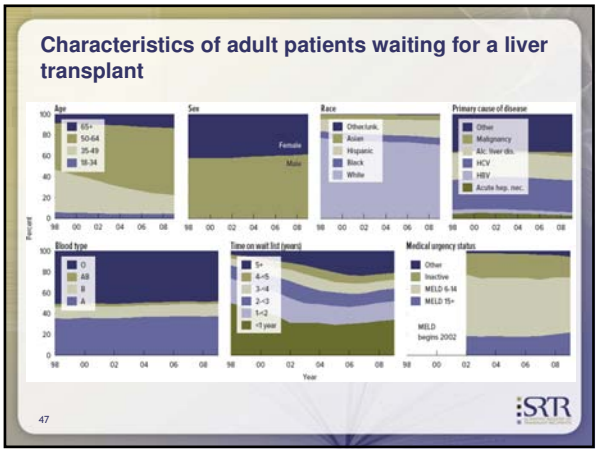
42

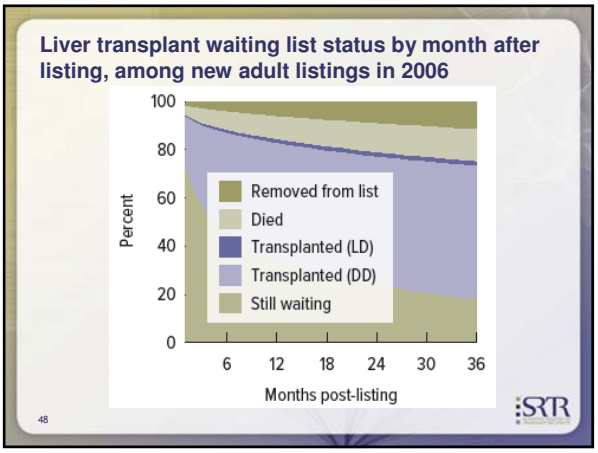

Induction agents in adult recipients 2009
None
T-cell depleting
IL2-RA & T-cell depleting
IL2-RA
0
20
40
60
80
Percent
43


Immunosuppression in adult pancreas transplant recipients
Calcineurin inhib. (at tx)
Anti-metabolites (at tx)
mTOR inhibitors
Steroids
Tacrolimus
CsA or CsM
CsM
CsA
Mycophenolate
Azathioprine
At transplant
1 year post-tx
Percent
Year
44

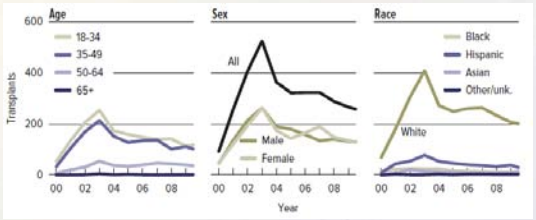

Liver
SRTR
SCIENTIFIC REGISTRY OF TRANSPLANT RECIPIENTS


Adult patients waiting for a liver transplant
New patients
Patients on the list on 12.31 of the given year
Active
Inactive
Patients (in thousands)
Year
46


Characteristics of adult patients waiting for a liver transplant
Age
Sex
Race
Primary cause of disease
Blood type
Time on wait list (years)
Medical urgency status
Year
47

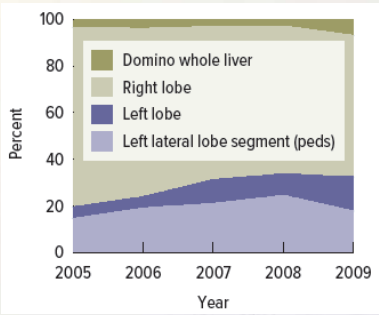

Liver transplant waiting list status by month after listing, among new adult listings in 2006
Removed from list
Died
Transplanted (LD)
Transplanted (DD)
Still waiting
Percent
Months post-listing
48

Liver donations from living donors



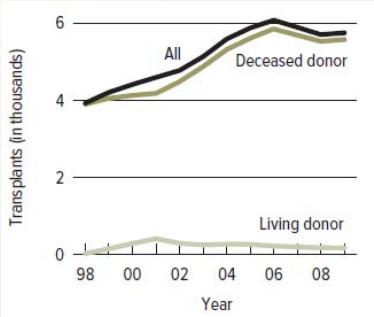
49

Living donor liver transplant graft type



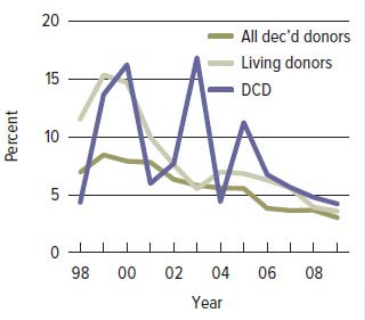
50

Total adult liver transplants



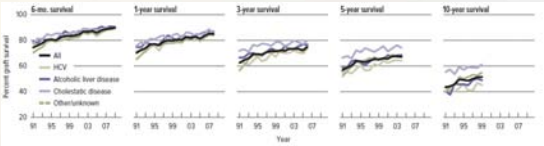
51

Early (<6 weeks) liver graft failure among adult recipients



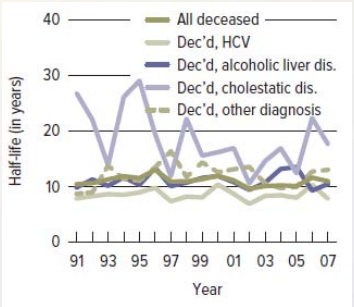
52

Liver graft failure among adult recipients: deceased donor



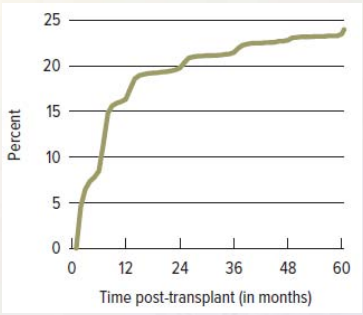
53

Half-lives of adult deceased donor livers surviving at least one year



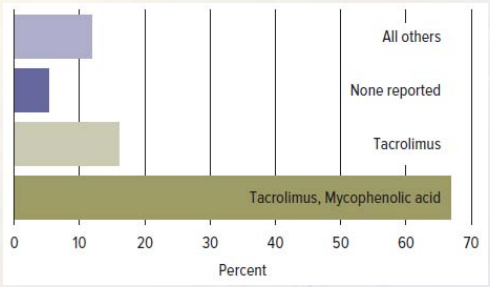
54

Incidence of first acute rejection among adult liver transplants in 2005–2009



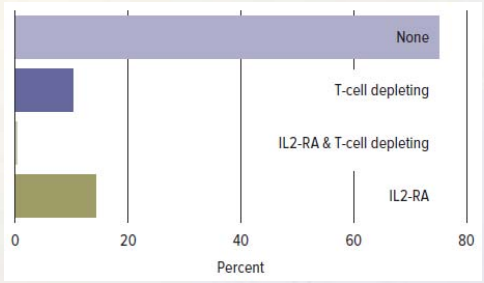
55

Initial immunosuppression in adult liver transplants in 2009 (+/- steroids)



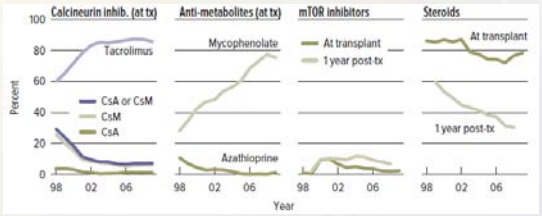
56

Induction agents in adult liver transplant recipients, 2009



57

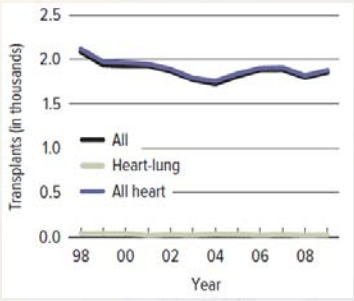
Immunosuppression in adult liver transplant recipients



58

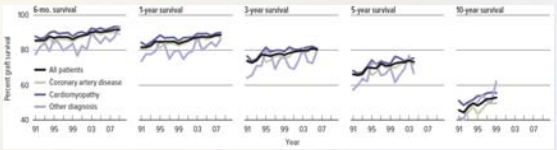
Heart

Total adult heart & heart/lung transplants



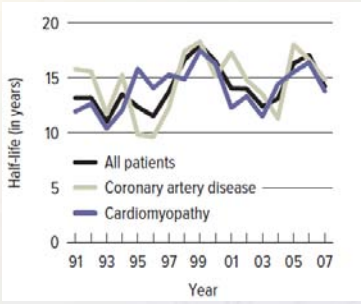
60

Graft failure among adult heart transplant recipients



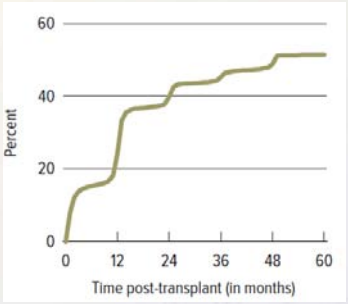
61

Half-lives for adult heart transplants surviving for at least one year



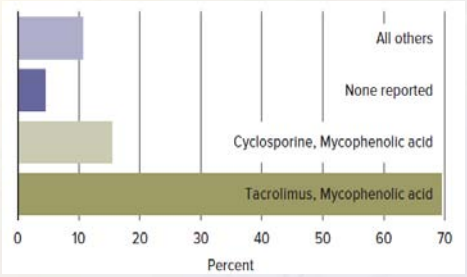
62

Incidence of first acute rejection among adult heart transplant recipients in 2005–2009



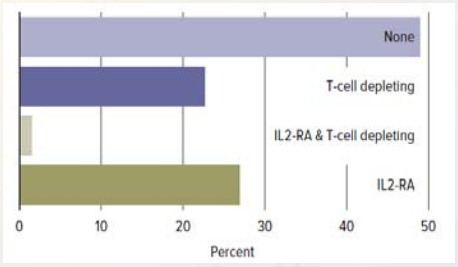
63

Initial immunosuppression in adult heart recipients, 2009 (+/- steroids)



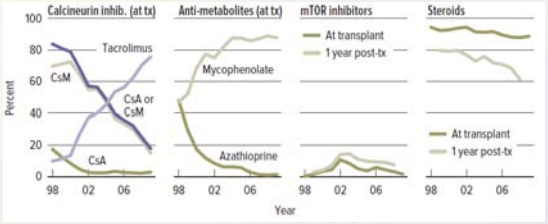
64

Induction agents used in adult heart transplant recipients, 2009



65

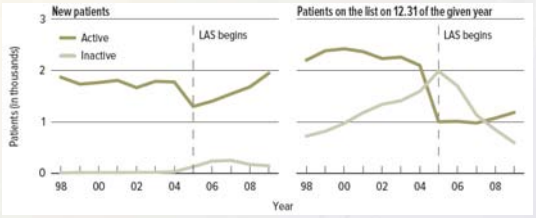
Immunosuppression use in adult heart transplant recipients



66

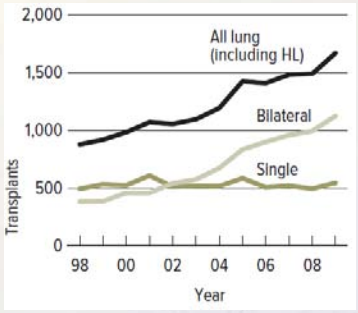
Lung

Adult patients waiting for a lung transplant



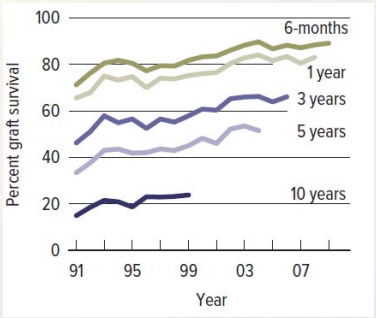
68

Total adult lung transplants



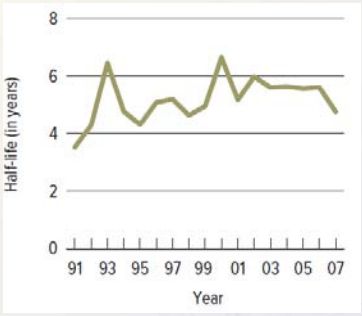
69

Graft failure among adult lung recipients



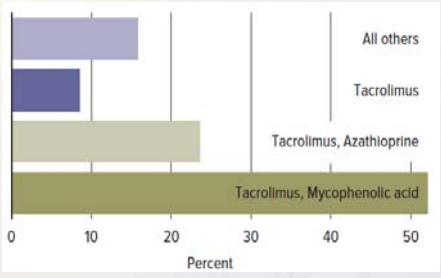
70

Half-lives for adult lung transplants surviving at least one year

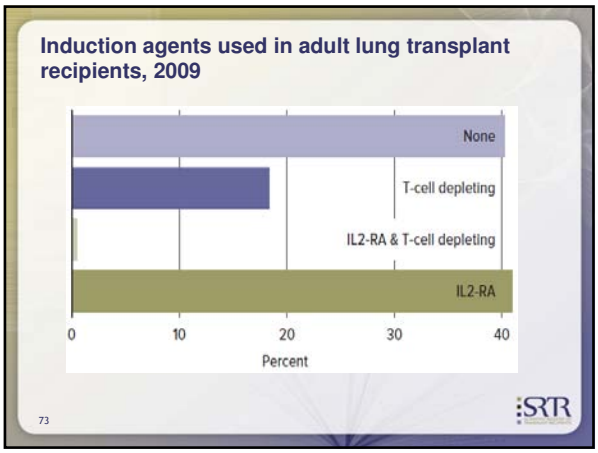


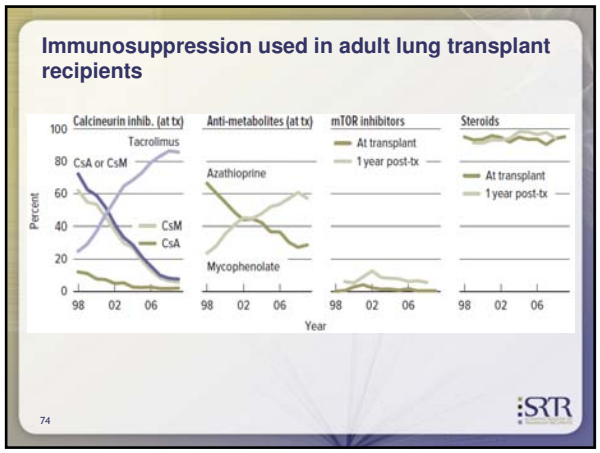
71

Initial immunosuppression in adult lung transplant recipients, 2009 (+/- steroids)



72


Induction agents used in adult lung transplant recipients, 2009
None
T-cell depleting
IL2-RA & T-cell depleting
IL2-RA
0
10
20
30
40
Percent
73
SRTR


Immunosuppression used in adult lung transplant recipients
Calcineurin inhib. (at tx)
Anti-metabolites (at tx)
mTOR inhibitors
Steroids
Tacrolimus
CsA or CsM
CsM
CsA
Azathioprine
Mycophenolate
At transplant
1 year post-tx
Percent
Year
98
02
06
74
SRTR

Fellows Symposium on Transplantation Medicine

Friday, September 23
1:30 pm - 2:00 pm

Understanding Immunosuppression: From Bench to Bedside

Kenneth A. Newell, MD, PhD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

Understanding Immunosuppression: From Bench to Bedside

AST Fellows Symposium 2011

Kenneth A. Newell
Emory University

Grapevine, TX
September 23, 2011

Outline

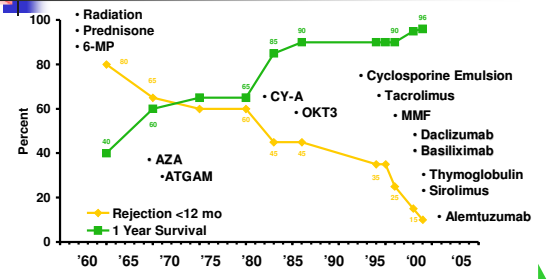
- Evolution of Immunosuppression
- The drugs – mechanisms and toxicities
- Immunosuppressive regimens – how we use them
- Infections and malignancies covered in later presentations
- Pivotal trials and new studies
- New agents – in the pipeline and failed

Evolution of immunosuppression

- 1954 – successful renal transplant
  - Identical twin transplant – no immunosuppression
- 1959 – first successful allograft
  - non-identical dizygotic twin transplant with sublethal total body irradiation
- 1962 – first successful unrelated allograft
  - Azathioprine – 6 MP derivative (Sir Roy Calne)
  - Patient survived over 1 year
- 1963 – successful reversal of rejection by temporary treatment with high-does prednisone (200 mg/day)
  - Starzl et al. *Surg. Gynecol. Obstet.* 117:385, 1963

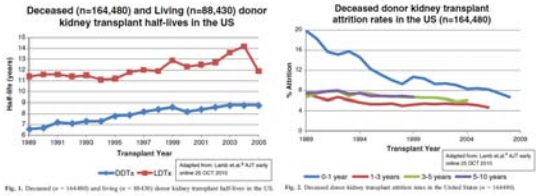
Cadaveric Renal Allograft Survival

"Better living through pharmacology?"



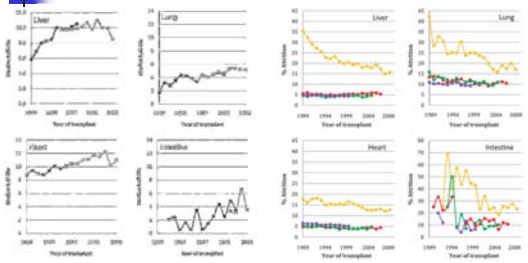
Deficiency Sufficiency Efficiency

Why develop new drugs?



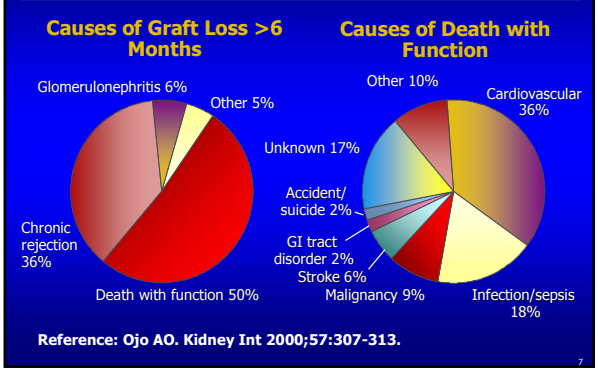
Sundus A. Lodhi and Herwig-Ulf Meier-Kriesche
Nephrol Dial Transplant (2011) 26: 15–17

Not just a problem for kidneys



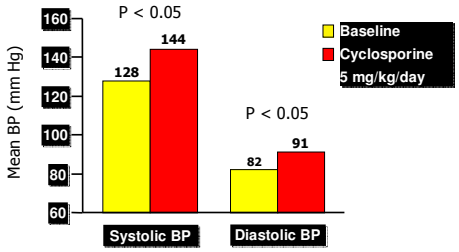
Lodhi SA et al, AJT 2011;11:1226

Causes of Graft Loss



Reference: Ojo AO. Kidney Int 2000;57:307-313.

BP in patients with psoriasis receiving CSA



Stiller MJ et al. *J Am Acad Dermatol*, 27:434, 1992

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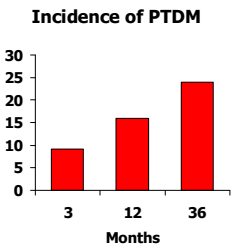
Post-transplant diabetes mellitus



- Risk factors
  - AA race
  - Hispanic ethnicity
  - Male donor
  - HLA mismatches
  - Hepatitis C
  - BMI > 30
  - Tacrolimus
- PTDM associated with
  - Graft failure
  - Death

Kasiske BL et al. Am J Transplant 3:178, 2003

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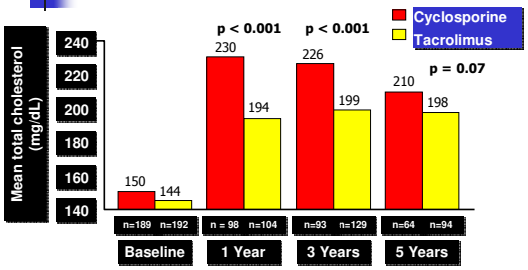
Post-transplant hyperlipidemia



Pirsch JD et al. Presented at Transplant 2000; Chicago, IL May 13 – 17, 2000

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The drugs (we have)

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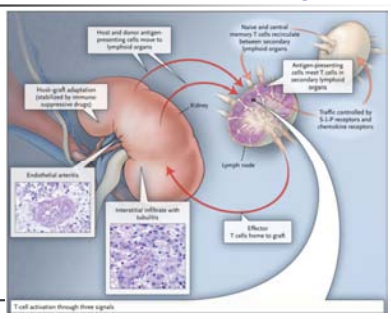
Process of acute rejection



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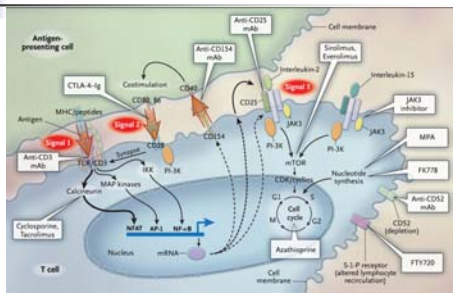
Halloran NEJM 2004; 351(26):2715

Types of rejection

- Hyperacute – affects primarily kidney, pancreas, and heart allografts
  - Occurs within minutes to hours
  - Mediated by anti-donor antibodies present at the time of transplantation
    - Anti-HLA or blood group antibodies
    - Arise through pregnancy, transfusion, transplantation
- Acute – primary target of immunosuppressive drugs
  - Occurs within days to years
  - Mediated by T cells
  - Incidence varies from 10 - >70% by organ
- Chronic – actually refers to a number of conditions that result in fibrosis (usually at later time points) and progressive allograft dysfunction
  - Immunologic
  - Drug-induced
  - Recurrent disease
  - Ischemia/reperfusion injury

3 signal model of T cell activation



 Halloran NEJM 2004; 351(26):2715

Therapeutic Categories

- Biologics – proteins with immunosuppressive properties
  - Polyclonal Antibodies
  - Monoclonal Antibodies – i.e., anti-CD3, anti-CD52, anti-CD20, anti-IL2R
  - Fusion Proteins – i.e., LEA29Y (CTLA4-Ig)
- Small Molecules
  - Pathway inhibitors – i.e., CNI, mTOR inhibitors, anti-proliferatives, etc

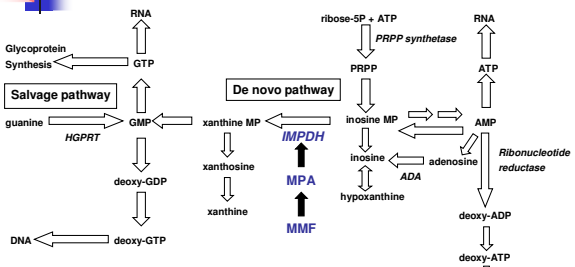
Corticosteroids

- MOA incompletely understood
  - Effects both dependent and independent of binding glucocorticoid receptors
    - Steroid/GR complex binds DNA or DNA binding proteins to alter gene transcription
  - Impaired production of many cytokines and inflammatory mediators
  - Impaired APC and inflamatory cell function and trafficking
- Toxicities: HTN, diabetes, weight gain, bone disease, psychosis, peptic ulcer, acne, etc.

Anti-proliferatives
Inhibit T and B cell proliferation

- Imuran (azathioprine) – metabolized to 6-MP
  - purine analogue
  - Toxicities: leukopenia, thrombocytopenia, hepatotoxicity
- Mycophenolate mofetil (MMF; also enteric coated)
  - De novo purine synthesis inhibitor
    - Inhibits the enzyme IMPDH – critical in synthesis of quanosine nucleotides
  - Toxicities: leukopenia, thrombocytopenia, GI toxicity
- Leflunomide; FK778
  - De novo pyrimidine synthesis inhibitor
    - Inhibits the enzyme DHODH
  - Toxicities: rare leukopenia, rare thrombocytopenia

Purine biosynthesis: inhibition of the de novo pathway by MMF

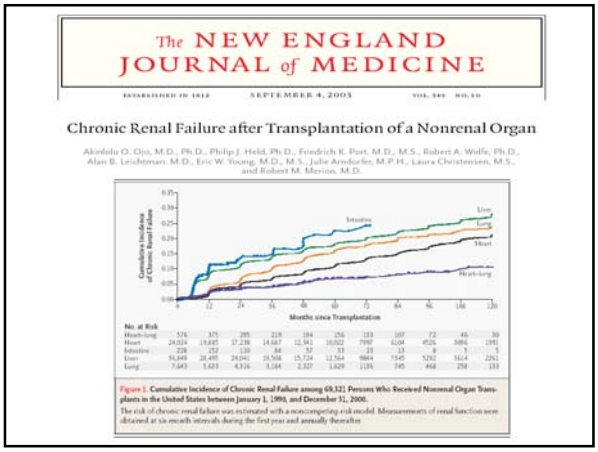


Calcineurin inhibitors (CNI)

- Cornerstone of immunosuppressive regimens
  - Inhibit production of numerous cytokines including IL-2, -3, -4, -5, IFNγ, TNFα, CM-CSF, etc.
- Cyclosporine - introduced 1983
  - Binds cyclophilin – complex then inhibits calcineurin-mediated dephosphorylation of NFKB and its consequent nuclear translocation thereby inhibiting cytokine production
- Tacrolimus – macrolide antibiotic
  - Binds the immunophilin FK binding protein 12 – complex then binds calcineurin

Toxicities of CNI

- Major shared toxicities:
  - Nephrotoxicity
  - Neurotoxicity
- CSA > tacrolimus:
  - gingival hypertrophy
  - hair growth
  - Hypertension
  - hyperlipidemia
- Tacrolimus > CSA:
  - post-transplant diabetes mellitus



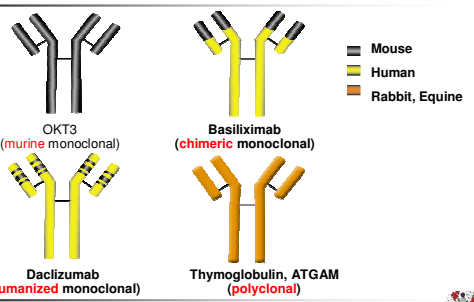
mTOR inhibitors

- During T cell activation cytokine receptors activate TOR
  - Sirolimus, a macrolide antibiotic, binds FKBP12 at the same site as tacrolimus but due to structural differences does not inhibit calcineurin. Everolimus is a derivative of sirolimus
  - TOR inhibition blocks proliferation by impairing signaling events mediated by IL-2/IL-2R binding as well as signaling by other growth factors (IL-15 and others)
  - Anti-proliferative effect may inhibit chronic rejection
- Toxicities:
  - Delayed wound healing (anti-proliferative effect)
  - Anemia and thrombocytopenia
  - Hypercholesterolemia/lipidemia
  - May exacerbate nephrotoxicity of calcineurin inhibitors

Induction

- Definition – a short course of treatment initiated at the time of transplantation
- Aim – reduce the large number of T cells capable of responding to alloantigens (i.e., 5 % of the T cell repertoire thereby blunting the vigorous early response

Structures of Immunosuppressive Antibodies



Induction Agents – all antibodies

- Lymphocyte depletion
  - Polyclonal – Thymoglobulin (MALG and ATGAM no longer available)
    - Immunization of horses, goats or rabbits with human lymphocytes or thymocytes
  - Monoclonal
    - OKT3 (anti-CD3) – T cell specific, depletion persists 1 – 3 weeks (no longer available)
    - Alemtuzumab (Campath 1H; humanized anti-CD52) – binds CD52 on all T and B cells as well as many monocytes, macrophages, and NK cells; depletes T cells and B cells to a lesser degree, depletion lasts for months
- IL-2R antagonists – binds IL-2R α chain expressed by activated cells
  - Little obvious toxicity

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Thymoglobulin: Target Antigens

*TCR $\alpha\beta$	*CD5	CD 58	HLA-Class I
*CD3	*CD6	*CD50	β2-microglobulin
CD 4	CD7	*CD54	CD80
*CD8	*CD11a	CD102	CD86
*CD2	*CD49d,e,f	*CCR7	
CD 28	*β7 Integrin	*CCR5	
CD 45	CD 18	*CXCR4	

*High functional activity (modulation at 1 µg/mL)

Bonnefoy-Bérard et al. *Transplantation.* 1991;51:669. Bonnefoy-Bérard et al. *Immunology.* 1992;77:61-67. Bonnefoy-Bérard et al. *Blood.* 1992;79:2164. Bonnefoy-Bérard et al. *J Heart Lung Trans.* 1996;15:435. Bourdage et al. *Transplantation.* 1995;59:1194.
Michallet et al. *Transplantation.* 2002; In press.

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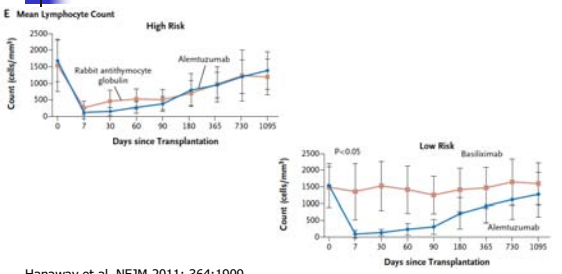
Lymphocyte recovery after induction



Hanaway et al. NEJM 2011; 364:1909

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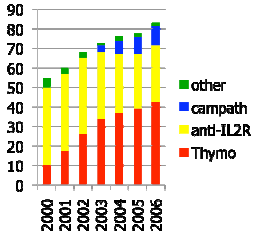
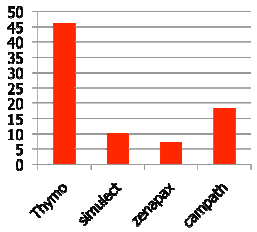
Induction and steroid use in renal transplantation

Induction use by year



CS avoidance by agent



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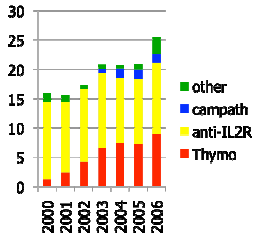
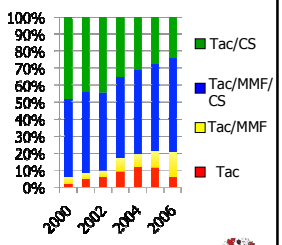
Induction and steroid use in liver transplantation

Induction use by year



Common regimens



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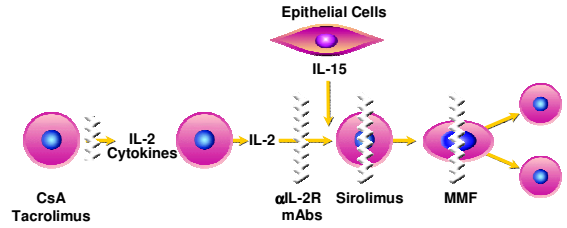
The regimens

- Only 1 agent representing a new class of immunosuppressant has been introduced in the last 11 years
- Thus transplantation increasingly relies on new combinations of existing agents in an attempt to improve efficacy and safety

Goals of combination therapy



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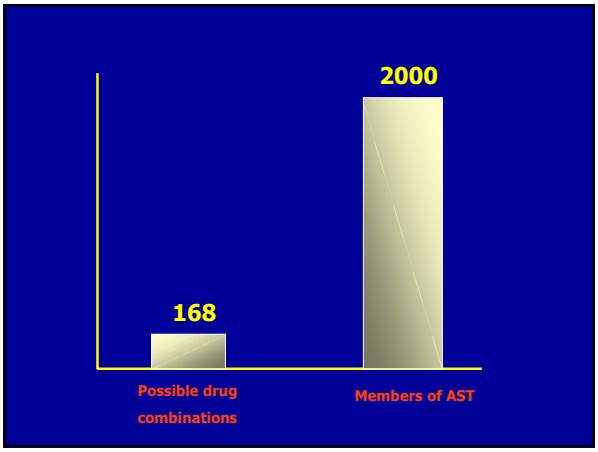
Immunosuppressive Chaos

Can decrease dose of MMF/Sirolimus
Can replace CNI with Rapamycin
CsA AZA Pred
Can give fixed dose
CsA MMF Pred
Can dose on C-2 level
Tacro MMF Pred
Can stop Calcineurin Inh
Can dose on levels
Tacro Sirolimus Pred
Can never use steroids
CsA Sirolimus Pred
Can decrease dose of CNI
Can never use MMF/AZA or Sirolimus



Commonly used regimens

- Tac/MMF or MPA/CS
  - Kidney 49%, liver 48.1%, heart 48.7% and lung 45.7%
- Tac/MMF or MPA
  - Kidney 24.3%, liver 12.8%, heart 3.8% and lung 1.5%
- CSA/MMF or MPA/CS
  - Kidney 6.7%, liver 3.0%, heart 28.5% and lung 6.3%
- Tac/CS
  - Kidney 1.7%, liver 21.5%, heart 1.9% and lung 3.9%
- Tac alone
  - Kidney 2.0%, liver 5.5%, heart 0.6% and lung 0.1%
- MMF or MPA/CS
  - Kidney 2.7%, liver 1.0%, heart 0.9% and lung 0.2%

2007 OPTN/SRTR Annual Report
Immunosuppression Use for Maintenance prior to discharge

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Pivotal trials

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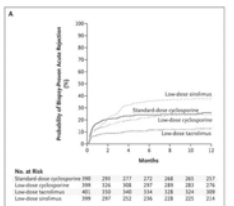
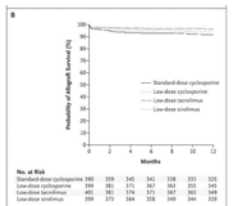
ORIGINAL ARTICLE

Reduced Exposure to Calcineurin Inhibitors in Renal Transplantation

NEJM 2007;357:2562





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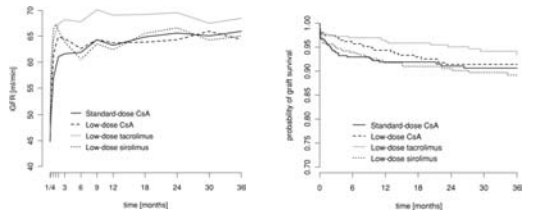
Symphony - 3 year follow-up



Ekberg et al. AJT 2009; 9:1876

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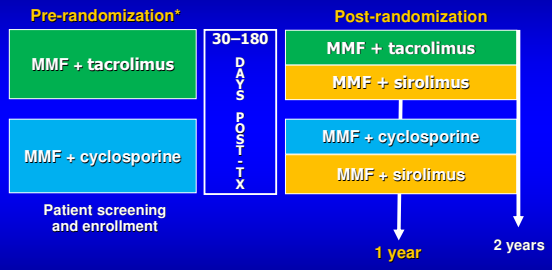
Spare the Nephron - Trial Design



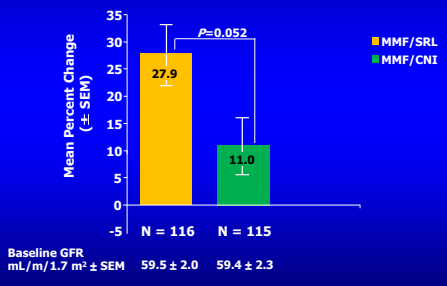
*Randomization pre-stratified by CNI type at screening

Target population = 305 single-organ renal allograft recipients

38

Mean % Change in Measured GFR

Baseline to Month 12



Baseline GFR mL/m/1.7 m² ± SEM	59.5 ± 2.0	59.4 ± 2.3

39

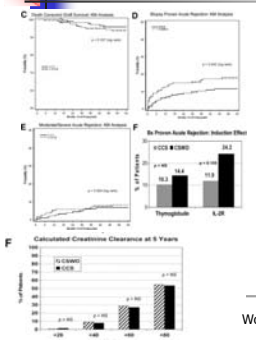
Efficacy Outcomes, n (%)

	MMF/SRL* N=148	MMF/CNI* Total N=150	MMF/CNI* MMF/TAC N=119
Biopsy-proven acute rejection	10 (7%)	10 (7%)	8 (7%)
Days from randomization, Mean (SEM)	322.2 (5.8)	378.7 (5.3)	381.4 (4.9)
Death	0 (0%)	3 (2%)	2 (2%)
Graft loss	3 (2%)	4 (3%)	3 (3%)

*P = NS for MMF/SRL vs MMF/CNI.

40

Early steroid withdrawal in renal transplantation



- Study design: Tac/MMF/Ab induction with steroid cessation at 7 days vs 5 mg/day
- Results
  - CSWD increased early ACR (mostly mild)
  - Similar long-term allograft survival and function
  - Improved cardiovascular risk (PTDM, triglycerides, weight gain)

Woodle et al. Ann Surg 2008;248:564

Bench to bedside

- Studies guiding the clinical development of belatacept
  - Bluestone, Turka, Larsen mouse
  - Larsen, Kirk, Kenyon – NHP
  - BMS phase II and III studies leading to approval of NULOJIX® summer 2011

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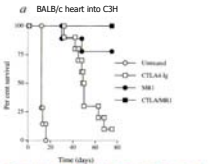
CTLA4-Ig – murine models

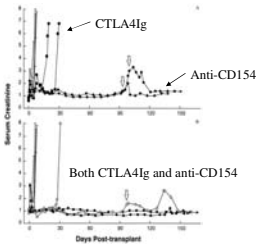
- CTLA4-Ig blocks rejection of human islets by mice (Lenschow et al. Science 1992;257:751)
- CTLA4-Ig blocks rejection of heart allografts in rats (Turka et al. PNAS;89:11102)
- CTLA4-Ig in combination with anti-CD40L blocks rejection of heart allografts in mice (Larsen et al. Nature 1996;381:434)



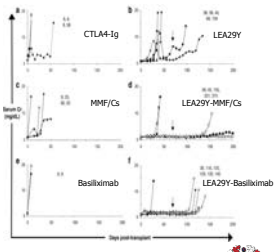
Larsen et al. Nature 1996;381:434

Blockade of CD28/B7 in NHPs

Kirk et al. PNAS 1997;94:8789



Larsen et al. AJT 2005;5:443



LEA29Y/belatacept/NULOJIX®

- Belatacept phase II
  - IM103-100 - bela (2 dosing regimens) vs. CSA with anti-IL2R mAb, MMF and steroids; bela arms better renal function, equal rejection, favorable metabolic profile
  - IM103-034 – steroid avoidance – Thymoglobulin induction, maintenance with bela MMF or bela + sirolimus
  - Belatacept in liver transplantation – study stopped – tac + MMF vs. bela (2 dosing regimens) + MMF or bela + MMF + anti-IL2R mAB
- Belatacept phase III
  - BENEFIT – same design as IM103-100
  - BENEFIT EXT – similar to IM103-100 for ECD transplants

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Costimulation Blockade with Belatacept in Renal Transplantation

Flavio Vincenti, M.D., Christian Larsen, M.D., Ph.D., Antoine Durbach, 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*

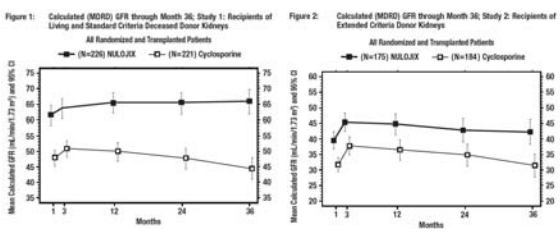
Table 2. Incidence of Primary and Secondary Efficacy End Points.

End Point	Intensive Belatacept (N=74)	Less-Intensive Belatacept (N=71)	Cyclosporine (N=73)
Primary efficacy end point			
Clinically suspected and biopsy-proven acute rejection at 6 mo — no. (%)	5 (7)	4 (6)	6 (8)
Absolute difference in rate from cyclosporine group — percentage points (exact 95% CI)*	−1.5 (−11.3 to 8.3)	−2.6 (−12.5 to 6.7)	—
Secondary efficacy end points			
Mild acute rejection (grade IA) — no. (%)	2 (3)	0	1 (1)
Mild acute rejection (grade IB) — no. (%)	0	0	1 (1)
Moderate acute rejection (grade IIA) — no. (%)	2 (3)	3 (4)	2 (3)
Moderate acute rejection (grade IIB) — no. (%)	1 (1)	1 (1)	2 (3)
Subclinical rejection — no. (%)	7 (9)	14 (20)	8 (11)
Treated episode of subclinical rejection — no. (%)	6 (8)	11 (15)	5 (7)

* CI denotes confidence interval.

NEJM 2005;353:770

Belatacept preserves renal function



From belatacept package insert

Belatacept and rejection

Table 6: Efficacy Outcomes by Years 1 and 3 for Study 1: Recipients of Living and Standard Criteria Deceased Donor Kidneys

Parameter	NULOJIX Recommended Regimen N=226 n (%)	Cyclosporine (CSA) N=221 n (%)	NULOJIX-CSA (97.3% CI)
Efficacy Failure by Year 1	49 (21.7)	37 (16.7)	4.9 (−3.3, 13.2)
Components of Efficacy Failure*			
Biopsy Proven Acute Rejection	45 (19.9)	23 (10.4)	
Graft Loss	5 (2.2)	8 (3.6)	
Death	4 (1.8)	7 (3.2)	
Lost to follow-up	0	1 (0.5)	
Efficacy Failure by Year 3	58 (25.7)	57 (25.8)	−0.1 (−9.3, 9)
Components of Efficacy Failure*			
Biopsy Proven Acute Rejection	50 (22.1)	31 (14)	
Graft Loss	9 (4)	10 (4.5)	
Death	10 (4.4)	15 (6.8)	
Lost to follow-up	2 (0.9)	5 (2.3)	
Patient and graft survival†			
Year 1	218 (96.5)	206 (93.2)	3.2 (−1.5, 8.4)
Year 3	206 (91.2)	192 (86.9)	4.3 (−2.2, 10.8)

From belatacept package insert

Belatacept and PTLD

Table 2: Summary of PTLD Reported in Studies 1, 2, and 3 Through Three Years of Treatment

Trial	NULOJIX Non-Recommended Regimen (N=477) EBV Positive (n=406)	EBV Negative (n=43)	EBV Unknown (n=28)	NULOJIX Recommended Regimen[1] (N=472) EBV Positive (n=404)	EBV Negative (n=48)	EBV Unknown (n=20)	Cyclosporine (N=476) EBV Positive (n=398)	EBV Negative (n=57)	EBV Unknown (n=20)
Study 1									
CNS PTLD	1	1							
Non-CNS PTLD		1		2				1	
Study 2									
CNS PTLD	1	1		1	1				
Non-CNS PTLD					1				
Study 3									
CNS PTLD		2							
Non-CNS PTLD			1						
Total (%)	2 (0.5)	5 (11.6)	1 (3.6)	3 (0.7)	2 (4.1)	0	0	1 (1.8)	0

Black box warning

WARNING: POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER, OTHER MALIGNANCIES, AND SERIOUS INFECTIONS

Increased risk for developing post-transplant lymphoproliferative disorder (PTLD), predominantly involving the central nervous system (CNS). Recipients without immunity to Epstein-Barr virus (EBV) are at a particularly increased risk; therefore, use in EBV seropositive patients only. Do not use NULOJIX (belatacept) in transplant recipients who are EBV seronegative or with unknown EBV serostatus [see *Contraindications (4)* and *Warnings and Precautions (5.1)*].

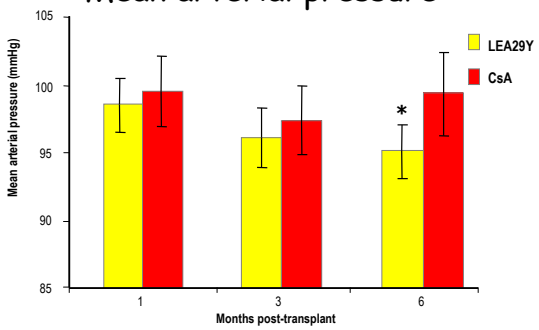
Only physicians experienced in immunosuppressive therapy and management of kidney transplant patients should prescribe NULOJIX. Patients receiving the drug should be managed in facilities equipped and staffed with adequate laboratory and supportive medical resources. The physician responsible for maintenance therapy should have complete information requisite for the follow-up of the patient [see *Warnings and Precautions (5.2)*].

Increased susceptibility to infection and the possible development of malignancies may result from immunosuppression [see *Warnings and Precautions (5.1, 5.3, 5.4, 5.5)*].

Use in liver transplant patients is not recommended due to an increased risk of graft loss and death [see *Warnings and Precautions (5.6)*].

From belatacept package insert

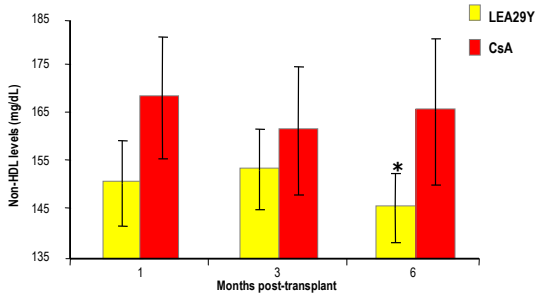
Mean arterial pressure



* p<0.05 compared with CsA

Non-HDL lipid levels

29% of LEA29Y-treated patients were using lipid-lowering medications compared with 41% of CsA-treated patients at 6 months



* p<0.05 compared with CsA

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Alemtuzumab Induction in Renal Transplantation

Michael J. Hanaway, M.D., E. Steve Woodle, M.D., Shamkant Mulgaonkar, M.D., V. Ram Peddi, M.D., Dixon B. Kaufman, M.D., Ph.D., M. Roy First, M.D., Richard Croy, M.A., and John Holman, M.D., for the INTAC Study Group*

NEJM 2011;364:1909

Study design

- 139 high-risk patients randomized
  - alemtuzumab – one 30 mg dose
  - Thymoglobulin – 6 mg/kg total dose
- 335 low-risk patients randomized
  - alemtuzumab – one 30 mg dose
  - basiliximab – 40 mg over 4 days
- All patients received tacrolimus, MMF, and 5 days of glucocorticoids
- Primary end point – BCAR at 6 and 12 months
- Patients followed for 3 yrs. for safety and efficacy end points

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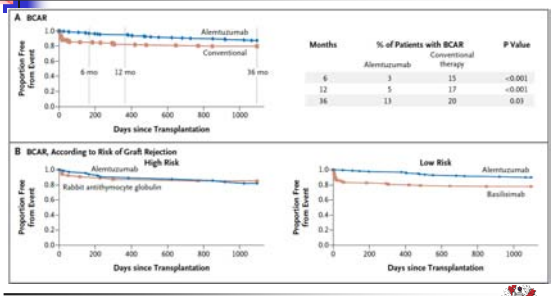
Biopsy confirmed acute rejection



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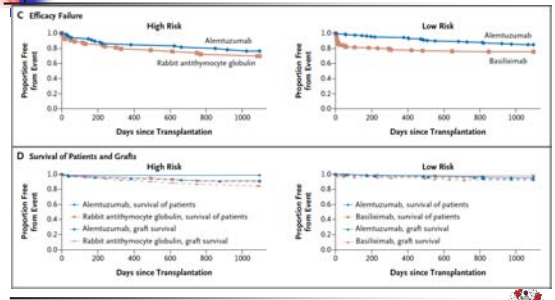
NEJM 2011;364:1909

Long-term outcomes



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NEJM 2011;364:1909

Examples of ongoing studies

- Allan D. Kirk – ongoing tolerance study in renal transplant recipients sponsored by the FDA
- Cooperative Trials in Organ Transplantation
  - CTOT-09
    - "Immune Monitoring and CNI Withdrawal in Low Risk Recipients of Kidney Transplantation"
    - PI Peter Heeger (Mt. Sinai School of Medicine)
  - CTOT-10
    - "Optimization of Belatacept Usage As a Means of Avoiding CNI and Steroids in Renal Transplantation"
    - PI Christian P Larsen (Emory University)

EMORY HEALTHCARE

Tolerance to renal allografts - A rational approach

- PI Allan Kirk (clinicaltrials.gov identifier NCT00565773, ATC 2011 Abstract 56)
- Targeted enrollment 20 patients
  - T cell depletion – alemtuzumab
  - Costimulation blockade – belatacept
  - Transient mTOR inhibition – sirolimus
  - 10 patients receive donor BM infusion
- Spaced weaning of sirolimus at 1 yr and belatacept at 2 yrs

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Results – ATC 2011 Abstract 56

Sunday May 1Room 204C 3:03 PM

- Rejection*: 1 of 19 with early rejection day 10 (responded to 3 day pulse of CS)
- 11 protocol biopsies at 1 yr: 2 Banff grade 1 subclinical rejections
- No alloantibodies detected*
- No malignancies, significant infections or CMV
- BK (n=7) & EBV (n=4) viremia (↓ IS)
- Repertoire repopulation: increased FoxP3+CD4+CD25+ & transitional type B cells

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CTOT-09 Immune monitoring and CNI withdrawal

- Primary objective – develop a strategy of immune monitoring to facilitate safe withdrawal of CNI in low-risk kidney transplant recipients
- Primary endpoint – percentage of patients with incremental IF/TA scores >2 at 18 months
  - Secondary endpoints
    - Estimated GFR
    - Incidence of ACR
    - Allograft and patient survival
    - Development of new DSA
    - Donor-specific T cell memory determined by Elispot
    - Frequency of successful withdrawal of tacrolimus
    - Change in IF/TA scores

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CTOT-09 Study design

- Recipients of living donor kidneys will receive induction with Thymoglobulin and maintenance therapy consisting of tacrolimus, MMF, and prednisone
- Patients without ACR, with no rejection on a 6 month protocol biopsy, and no DSA will be randomized to tac withdrawal vs. tac maintenance (2:1)
- The two groups will be compared with respect to histologic evidence of chronic injury and renal function at 18 months

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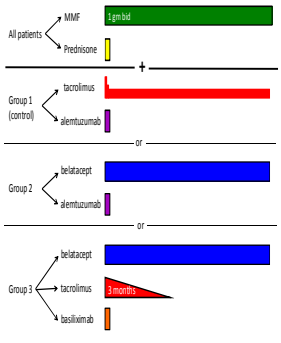
CTOT-10

Group 1:
- Investigational agents: Campath® (alemtuzumab);
- Long-term Prograf® (tacrolimus) or generic;
- CellCept® (mycophenolate mofetil- MMF) or generic, and
- 4 day course of MEDROL® (methylprednisolone)

Group 2:
- Investigational agents: NULOJIX® (belatacept);
- Investigational agents: Campath® (alemtuzumab);
- CellCept® (mycophenolate mofetil- MMF) or equivalent,
- 4 day course of MEDROL® (methylprednisolone)

Group 3:
- Induction: Simulect® (basiliximab);
- Investigational agents: NULOJIX® (belatacept);
- 85 day course of Prograf® (tacrolimus) or equivalent;
- CellCept® (mycophenolate mofetil- MMF) or equivalent,
- 4 day course of MEDROL® (methylprednisolone)



National Institutes of Allergy and Infectious Diseases

Study Endpoints

- The primary objective is to evaluate NULOJIX® (belatacept) based regimens as a means of improving long term graft function without increasing the risks of immunologic graft injury by avoiding both CNI and corticosteroids.

Primary Endpoint

- Major study endpoints will be determined for each participant 52 weeks after enrollment. However, patients will be followed until the end of the study (52 to 156 weeks) with additional endpoint assessments.
- The primary endpoint is mean glomerular filtration rate (GFR) calculated for each treatment group using the CKD-EPI equation at 52 weeks.

National Institutes of Allergy and Infectious Diseases

Secondary Endpoints

Histological Evidence of Rejection and Graft Dysfunction

- The incidence of clinically suspected and biopsy proven acute rejection ***(CSBPAR- refer to the study definitions page)*** within the first 24 weeks as defined by histologic evidence of rejection and graft dysfunction.

Measures of Renal Function and Injury

The following secondary endpoints will measure renal function and injury at weeks 52, 104 and 156:

1. Proportion of subjects with eGFR < 60 mL/min/1.73 m2 measured by CKD-EPI.
2. Change in CKD stages from baseline.
3. Proportion of subjects with defined CKD stage 4 or 5.
4. Mean calculated eGFR using MDRD 4 variable model.
5. The slope of eGFR by CKD-EPI over time based on serum creatinine collected at all visits indicated on the Schedule of Events.
6. The incidence of delayed graft function ***(DGF- refer to study definitions page).***
7. An increase of one or more grades of CAN/IFTA when comparing the implantation and subsequent protocol biopsies.
8. Incidence of CAN/IFTA grade I, II or III.

National Institutes of Allergy and Infectious Diseases

Secondary Endpoints

Incidence and Severity of Rejection and Anti-Donor Reactivity

1. The incidence of acute cellular rejection grade equal to or > than IA, by the Banff 2007 criteria, within the first 52 weeks.
2. The severity of first and highest grade of acute cellular rejection within the first 52 weeks.
3. The incidence of antibody mediated rejection ***(AMR- refer to the study definitions page)***.
4. The type of treatment of rejection.
5. The prevalence of *de novo* anti-donor HLA antibodies at 52 weeks.

Measures of Cardiovascular and Metabolic Parameters

1. The incidence of new onset diabetes after transplant or impaired fasting glucose
2. The incidence of treated diabetes between day 14 and week 52.
3. HbA1c measured
4. Standardized BP measurement and use of HTN medications
5. Fasting lipid profile (Total Cholesterol, non-HDL Cholesterol, LDL, HDL, and triglyceride) and use lipid lowering medications
6. Total daily prescribed pill number

National Institutes of Allergy and Infectious Diseases

Secondary Mechanistic Endpoints

Mechanistic assays will be performed at baseline, days 28 & 84, and weeks 24, 36, 52, 72, 104 and 156 or as specified.

Immune Reactivity and Function

1. Multiparameter flow cytometric enumeration and phenotyping of peripheral blood leukocyte subsets including T cell subsets, B cells, DC, NK cells. (Emory Cellular Core Laboratory).
2. Protective immunity (Emory Viral Surveillance Core Laboratory).

a. Viral load monitoring – EBV, CMV, Polyoma BK & JC.
b. Assessment of the quantity and quality (poly-functional cytokine production) of CMV- and EBV- specific T cells (Tetramer, intracellular cytokine production after peptide or viral lysate challenge) and viral-specific antibody.

3. Anti-donor responses

a. Donor-specific antibody (Emory HLA Clinical Laboratory).
b. Immunohistochemistry of for-cause and 52 week protocol renal allograft biopsies (Emory Pathology Core Laboratory).
c. Gene expression, mRNA profiling in blood, urine and tissue (University of Alabama Molecular Core Laboratory).

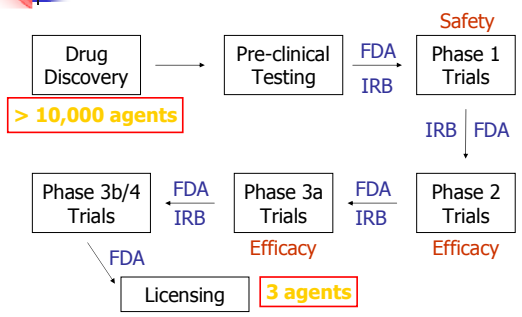
4. Serum and Urine proteins, selected validated biomarkers of Acute and Chronic kidney injury. (University of Alabama Protein Assay Core Laboratory).

National Institutes of Allergy and Infectious Diseases

New agents in the pipeline

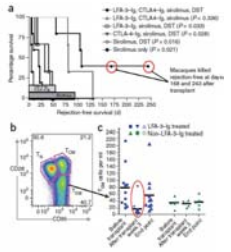
Emory Healthcare

Organization/Time Line for Clinical Trials



Alefacept–targeting memory T cells

- CD4 and CD8 memory T cells express CD2
- Alefacept (LFA3-Ig) binds CD2 and depleted memory T cells
- LFA3-Ig, CTLA4-Ig, sirolimus, and DST promotes long-term graft survival



Weaver Nat Med 2009;15:746

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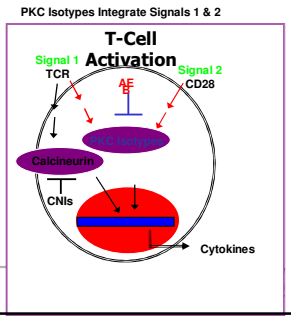
NVP-AEB071 (Novartis)

- Protein Kinase C (PKC) isotypes are centrally involved in signaling pathways downstream of the TCR (Signal1) and the CD28 (Signal2) co-receptor.
- AEB071 inhibits PKC isotypes with high potency and selectivity (IC_{50}=1-5 nM).
- AEB071 potently blocks T-cell activation (IC_{50}=6 nM) but not IL-2 driven T-cell proliferation (IC_{50} > 1 μ M).
- AEB071 is a novel IS acting via inhibition of PKC.

AEB071 mode of action



WTC Abstracts: 57, 2954, 2964, 2006

EMORY HEALTHCARE

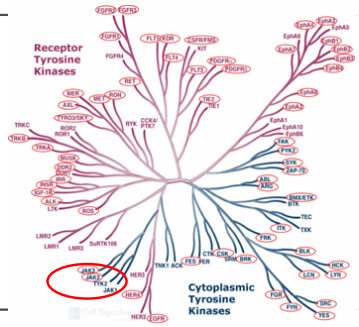
Oral NVP- AEB071 Prolongs Survival Times of Cynomolgus Monkey (NHP) Renal Tx Recipients & is Well Tolerated in Phase I Trials

Compound	Dose (mg/kg/d)	Median Survival Time (days)
No Treatment	**---**	**6**
AEB071	**20**	**7**
CsA	**20**	**7**
MPA	**30**	**15**
AEB071 + CsA	**20 + 20**	**> 100**
AEB071 + MPA	**20 + 30**	**62**

- **Phase I Results in Healthy Human Volunteers & Psoriasis Patients**
- **Phase 2 trials for renal Tx now underway. Goal: CNI replacement**

EMORY HEALTHCARE — WTC Abstracts: 57, 392, 546, 550, 741

Tyrosine Kinases



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CP-690,550 (Pfizer)

- Targets Janus kinase 3 (JAK3) a cytoplasmic tyrosine kinase associated with the common gamma chain of cytokine receptors of the interleukin (IL)-2 family, including IL-4, -7, -9, -15, and -21
- Potential to inhibit Th1 and Th2 cells as well as homeostatic activation and memory responses
- Clinical trials give 2 dosing regimens of CP-690,550 OR tacrolimus, in combination with MMF and prednisone to first time kidney transplant recipients for 6 months with an option for extension.
- Potential for CNI avoidance and Signal one avoidance

EMORY HEALTHCARE

Anti-CD40 Monoclonal Antibody 4D11(Kirin/Astellas)

- In-licensed from Kirin Brewery Company
- Fully human antagonistic anti-CD40 monoclonal antibody (IgG4)
- Blocks CD40/CD40L interaction, and inhibits both humoral (?) and cellular immunity
- Antibody causes lowering in the antibody-dependent cellular cytotoxicity (ADCC) and/or complement-development cytotoxicity (CDC)

EMORY HEALTHCARE

Agents targeting B cells

Human Leukocyte Antigen Antibodies and Chronic Rejection: From Association to Causation

Paul I. Terasaki and Junchao Cai

(*Transplantation* 2008;86: 377–383)

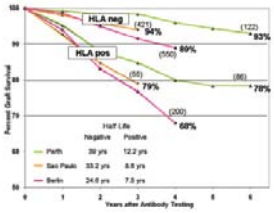


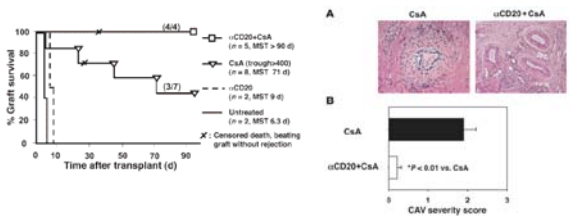
TABLE 1. HLA/MICA antibodies found in serial serum samples of patients before chronic failure

Center	Total fail	With antibody	%	Reference
Miami	25	23	92	Mizutani et al. (40)
Nagoya	30	26	87	Kinukawa et al. (41)
Maastricht	12	7	58	Van den Berg-Loonen et al. (43)
Greenville	26	24	92	Ozawa et al. (42)
Total	93	80	86	

Preemptive CD20+ B cell depletion attenuates cardiac allograft vasculopathy in cyclosporine-treated monkeys

Shahrooz S. Kelishadi,[1,2] Agnes M. Azimzadeh,[1] Tianshu Zhang,[1,2] Tiffany Stoddard,[1,2] Emily Welty,[1] Christopher Avon,[1] Mitch Higuchi,[1] Amal Laaris,[1,2] Xiang-Fei Cheng,[1] Christine McMahon,[3] and Richard N. Pierson III[1,2]

The Journal of Clinical Investigation http://www.jci.org Volume 120 Number 4 April 2010



B cell types and their role in txpl

Naive B cells	Memory B cells	Plasma cells	Plasmablasts
$CD20^{+/-}$, $CD27^-$, $CD138^-$, $CD38^+$	$CD20^+$, $CD27^+$, $CD138^-$, $CD38^+$	$CD20^-$, $CD27^+$, $CD138^+$, $CD38^{++}$	$CD20^-$, $CD27^+$, $CD138^+$, $CD38^{++}$
Begin in marrow, migrate to secondary lymph nodes	Have undergone significant maturation, but not terminally differentiated	Terminally differentiated B cells	Likely a short-lived antibody-secreting cell
Require T-cell help and significant maturation to develop into memory B cells and plasma cells	Reside in lymph nodes and spleen; common in germinal centers	Long-lived antibody-secreting cell	Produced early in immune responses or when memory B cells convert to antibody-secreting cells
Do not secrete antibody	Rapidly convert to plasmablasts (and maybe long-lived PCs) after restimulation with antigen	Primarily reside in bone marrow and are a major source of persistent donor-specific alloantibody production in sensitized patients	Reside in germinal centers
Diverse population that is continuously generated. Pool from which memory B cells and plasma cells develop in nonsensitized recipients. May need to be 'tolerized' using tolerance protocols	Dependence on T-cell help is unclear Likely play an important role in early antibody mediated rejection in sensitized recipients	Resistant to most common immunosuppressive agents	

Stegall et al. Curr Opin in Org Transplt 2010, 15:451-455

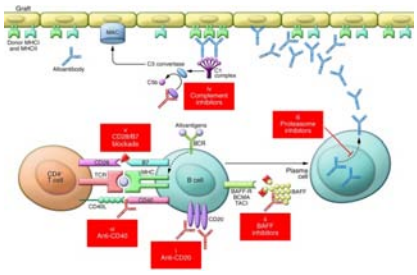
Impact of immunotherapies on B cells

Drug	Target	Preimmune/resting B-cells			Antigen-experienced B-cells		
		TR	FO	MZ	GC	MEM	LLPCs
Rituximab	CD20	D	D	PD	<	PD	ND
Atacicept	BLyS, APRIL	XX	D	D	<	PD	D
Belimumab	BLyS	XX	D	D	<	PD	X
Epratuzumab	CD22	D	D	PD	<	PD	ND
Bortezomib	Proteosome	ND	ND	ND	ND	ND	D
MPA	IMPDH	ND	ND	ND	<	ND	ND
CNI	Calcineurin	ND	ND	ND	<	ND	ND
Steroids	Multiple	ND	ND	ND	<	ND	ND

MPA indicates mycophenolic acid; CNI, calcineurin inhibitor; IMPDH, inosine monophosphate dehydrogenase; D, depletion; PD, partial depletion; ND, nondepleting, <, limits formation; X, evidence of depletion after 1 year; XX, sustained.

RF Parsons et al. Transplantation Reviews 2010;24:207

B cell-directed therapies



Knechtle SJ et al. J Clin Invest 2010; 120:1036-9

Regulatory B cells (Breg)

(Mauri C and Blair PA Nat Rev Rheum 2010; 6: 636-643)

Table 1 | Defining features of B_{reg} cells in animal models

Mouse models of disease	B_{reg} cell phenotype	Cytokines produced	Mechanisms of action	Reference(s)
$TCR\alpha^{-/-}$ colitis	$CD1d^{hi}$ ($CD21^{hi}CD62^{hi}$ $IgM^{hi}CD23^{hi}$)	IL-10, IL-12	IL-10-dependent $CD1d^{+/-}$, $CD40^{-/-}$ and $CD86^{-/-}$ B cells fail to suppress disease; induction of IL-12-producing B cells required for suppression of disease	Mizoguchi et al. (1997)[7], (2002)[8], (2000)[9], Sugimoto et al. (2007)[10]
$Gai2^{-/-}$ colitis	$CD19^{hi}IgM^{hi}$ (colitis is associated with a lack of T2-MZP and MZ B cells)	IL-10	Suggested recruitment and induction of $CD8^{+}$ T_{reg} cells and $CD1d^{+}NK1.1^{+}$ T cells; B cells require MHC class I and antigen peptide transporter 1 expression	Wei et al. (2008)[37], (2006)[38]
EAE	Unknown phenotype	IL-10	Recruitment and induction of $FOXP3^{+}$ T_{reg} cells; activation of B cells requires CD40 expression and, possibly, indirect inhibition of T_H1 and T_H17 cell responses via suppression of dendritic cells; possible role of stimulation of TLRs in B-cell activation	Fillatreau et al. (2002)[2], Lampropoulou et al. (2008)[27], Mann et al. (2007)[28]
EAE and contact hypersensitivity and experimental lupus	$CD19^{hi}CD1d^{hi}CD5^{+}$ B10 cells ($IgM^{hi}CD21^{+}CD23^{-}$ $CD24^{hi}CD93^{-}$)	IL-10	TLR2 and TLR4 stimulation required for expansion; CD40 ligation induces IL-10 competence; induce $FOXP3^{+}$ T_{reg} cell expansion in $CD19^{-/-}$ NZB/W lupus model	Yanaba et al. (2008)[29], (2008)[30], (2008)[31], Matsushita et al. (2008)[32], Watanabe et al. (2010)[33]
CIA	MZ B cells ($CD21^{hi}CD23^{-}$ $CD24^{hi}IgM^{hi}IgD^{lo}CD1d^{hi}$)	IL-10	Generated by apoptotic material in vivo; induce IL-10-producing T cells	Gray et al. (2007)[34]
CIA and MRL/lpr lupus	T2-MZP B_{reg} cells $CD19^{+}$ $CD21^{hi}CD23^{+}CD24^{hi}IgM^{hi}$ $CD93^{+}CD1d^{hi}$	IL-10	IL-10-dependent induction of adaptive T_{reg} cells; directly suppress $CD4^{+}$ T-cell cytokine production and proliferation in vitro; CD40 stimulation required for activation	Mauri et al. (2003)[6], Evans et al. (2007)[35], Blair et al. (2008)[36]
Nonobese diabetic mice	None	IL-10, TGF-β	TLR2 and TLR4 stimulation required for IL-10-dependent action; IgM stimulation required for IL-10-mediated suppression; possible role for B-cell Fas ligand expression in activation	Hussain et al. (2007)[39], Tian et al. (2001)[40]

Abbreviations: B_{reg} cell, regulatory B cell; CIA, collagen-induced arthritis; EAE, experimental autoimmune encephalomyelitis; FOXP3, forkhead box protein P3; IL, interleukin; MZ cell, marginal-zone cell; T2-MZP cell, transitional 2 marginal-zone precursor cell; TCR, T cell receptor; TGF-β, transforming growth factor β; T_H1 cell, type 1 T-helper cell; T_H17 cell, type 17 T-helper cell; TLR, Toll-like receptor; T_{reg}, regulatory T cell.

EMORY HEALTHCARE — EMORY TRANSPLANT CENTER

Arrested development

- Anti-B7 mAb (Wyeth/Genetics Institute) – blocks costimulatory signals
  - Efficacy in phase II trials
  - Development halted secondary to economics
- Anti-CD154 (CD40L) mAb (Biogen) - blocks costimulatory signals
  - Efficacious in NHP and clinical trials of renal transplantation in humans
  - Development halted as a result of vascular thrombosis
- FTY720 – sphingosine 1-phosphate agonist - traps lymphocytes in the LNs
  - Effective in experimental transplant models and phase II trials of renal transplantation
  - Development halted due to lack of efficacy in phase III trials and multiple toxicities (cardiac, pulmonary and occular)
- Efalizumab – anti-LFA1 mAb – inhibits cell migration and costimulation
  - Genentech – approved for treatment of psoriasis
  - Effective in phase II trials of renal transplant
  - Withdrawn from the market following several cases of PML

EMORY HEALTHCARE — EMORY TRANSPLANT CENTER

Therapeutic goals for emerging regimens

- Immunosuppression tailored to individual needs (recipient age, delayed function, immunologic risk)
- Reduced metabolic consequences of immunosuppression (eg, hypertension, hyperlipidemia, glucose intolerance, bone loss)
- Reduced morbidities (eg, infection, neoplasms)
- Improved compliance (simple dosing, fewer side effects)

Ultimate goal: improve allograft survival and function or decrease drug toxicities

EMORY HEALTHCARE — EMORY TRANSPLANT CENTER

Fellows Symposium on Transplantation Medicine

Friday, September 23
2:00 pm - 2:30 pm

Histocompatibility Techniques 101

Peter W. Nickerson, MD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

Histocompatibility Techniques 101

AST Fellows Course
Dallas 23 Sept 2011

Peter Nickerson
Flynn Family Chair in Renal Transplantation
Professor of Internal Medicine and Immunology

University of Manitoba
Health Sciences Centre Winnipeg
Manitoba Centre for Proteomics and Systems Biology

Objectives

1] To review HLA gene structure.

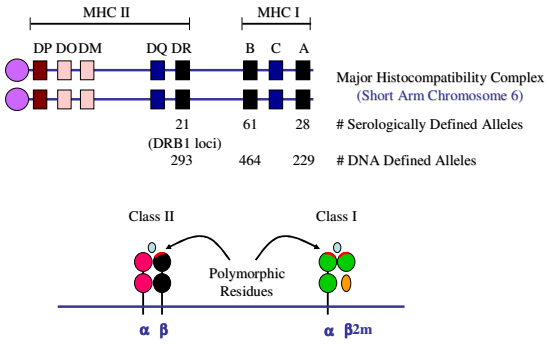
2] To review the impact of HLA matching on outcome.

3] To review evolution of laboratory techniques to detect HLA antibodies.

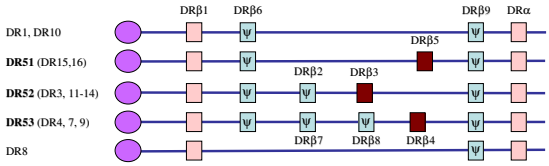
4] To understand the basis of the calculated PRA (cPRA)

5] To understand the predictive value of the "Virtual Crossmatch"

HLA Genetics:



MHC Class II DR Haplotypes (Linkage Disequilibrium)



HLA Disparity:
Effect of Matching on Graft Survival

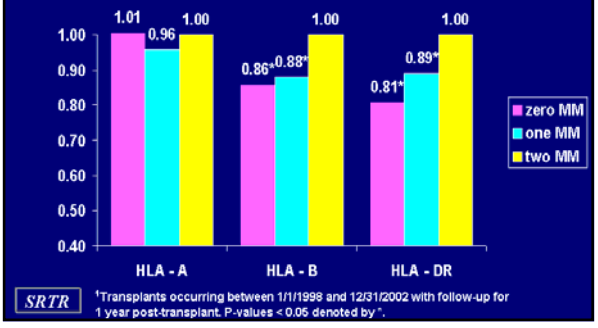
% Graft Survival

T1/2	T1/2*
13.4	13.0
11.3	8.9
9.3	8.3

Time Post-Transplant

(Opelz et al, Rev Immunogenetics (1999) 334)
* (Terasaki, Clinical Transplants (2000) 497)
(Takemoto et al, NEJM (2000) 1078)

RR of Allograft Failure During 1st Year, by HLA- A,B,DR MM[1]

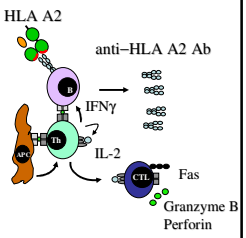


Pre-Transplant Assessment for Immune Memory

T and B - cell memory occur together

Pregnancy associated sensitization
Transplantation (1996) 62:672

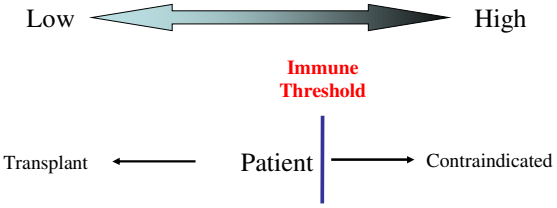
Transfusion associated sensitization
Transplantation (1990) 45:987



Antibody and T-cell mediated rejection occur together

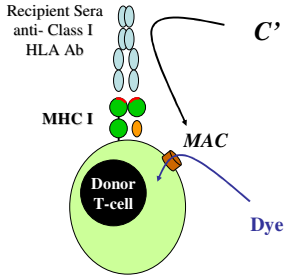
Ab mediated rejection: T2 + T3 Tubulitis 50% (12/24) of cases
Transplantation (1996) 61:1586

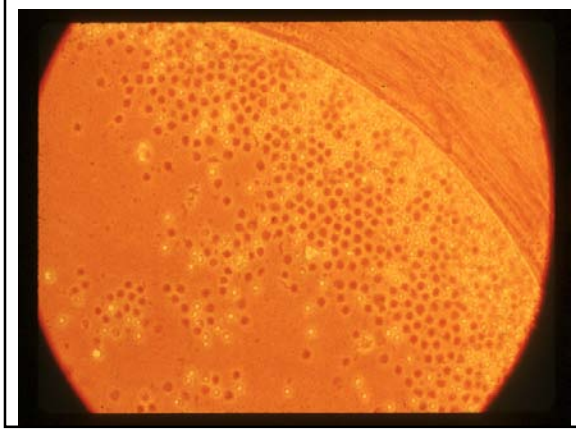
Detecting Immune Memory Pre-Transplant as a Measure of Risk for Early Graft Loss or Rejection



In Vitro Testing for Donor Reactive HLA antibodies

CDC T-cell crossmatch





Donor Reactive HLA Ab = Immune Threshold

T-cell	Accelerated Rj	Functioning
CDC +ve	**24**	6
CDC -ve	8	**187**

(Patel and Terasaki. NEJM (1969) 280:735)

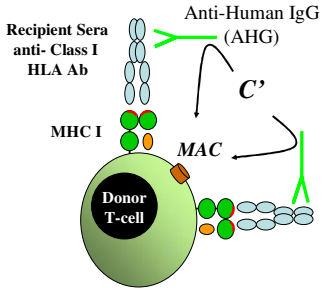
Clinical Paradigm (1969)

Low Risk ⟷ High Risk

Immune Threshold

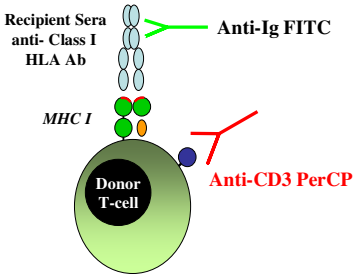
T-cell CDC CXM –ve	T-cell CDC CXM +ve
Transplant ←	Patient → Contraindicated

In Vitro Testing for anti-Donor HLA antibodies:
AHG-CDC T-cell crossmatch Enhances Sensitivity



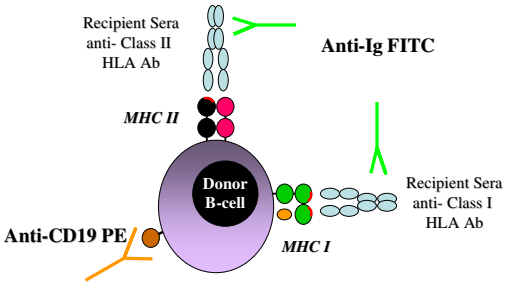
Dilution anti-HLA Ab	AHG CDC	NIH CDC
1:1	8	8
1:2	8	2/4
1:4	8	1
1:8	8	1
1:16	6	1
1:32	2	1

Flow Crossmatch (FCXM)

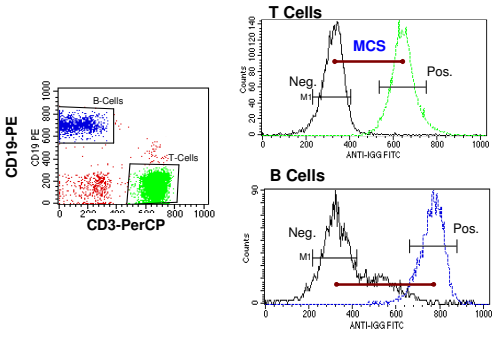


(Garovoy et al, Tran Proc (1983) 15:2939)

B-cell Crossmatch



3-Color Flow Cytometric Crossmatch



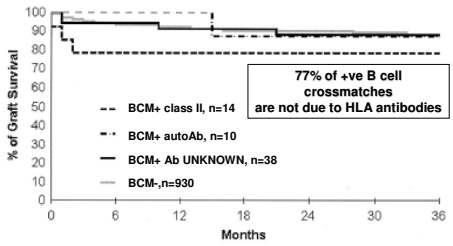
Flow Cross-match (FCXM)
is more sensitive than CDC methods

+ Sera Dilution	FCXM T-cell	AHG-CDC T-cell	NIH-CDC T-cell
1:1	Pos	8	8
1:2	Pos	8	2/4
1:4	Pos	8	1
1:8	Pos	4/6	1
1:16	Pos	2	1
1:32	Pos	1	1
1:64	Pos	1	1
1:128	Neg	1	1

Utility of FCXM in Primary Renal Transplantation Literature

	Population	CDC-XM	T FCXM +	% T FCXM+	Effect of T FCXM +
1987 Cook (n=196)	Primary	CDC	40 channels	18%	Early Loss: **22% vs. 7%**
1990 Mahoney (n= 67)	Primary	AHG	40 channels	18%	Early Loss: **33% vs. 7%** 1 year: 67% vs. 85%
1993 Ogura (n=841)	Primary	CDC	50 channels	18%	Early Loss: **20% vs. 7%** 1 year: 75% vs. 82%
1996 LeFor (n=214)	Primary	AHG	50 channels	7%	1 year: 75% vs. 86%
1997 Pelletier (n=102) **(No Difference)**	Primary	Amos	40 channels	18%	Rejection: 67% vs. 51% 1 year: **86% vs. 98%**
1998 Kimball (n=157)	Primary	Amos	40 channels	14%	Rejection: 51% vs. 25% 1 year: **44% vs. 97%**
1999 Kerman (n= 97) **(No Difference)**	Primary (Cadaveric)	AHG	80 channels		Rejection: 44% vs. 40% 1 year: **81% vs. 83%**
2001 Karpinski (n= 143)	Primary	AHG	40 channels	13%	Early Loss: **33% vs. 11%**

B-cell Crossmatches are Frequently False +ve



Le Bas-Bernardet,et al Transplantation 75:477,2003

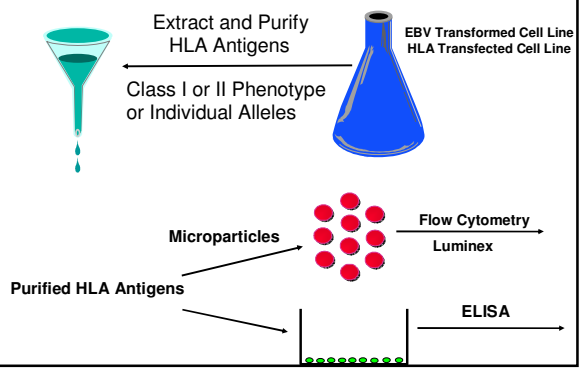
Can we validate that a + Flow CXM is due to a Donor Specific HLA Antibody (i.e. True Positive)?

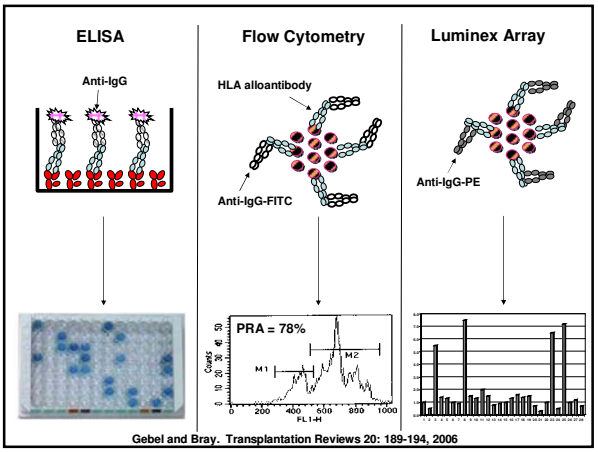
HLA Antibody Detection

Panel Reactive Antibody (PRA) → Screening for HLA Ab

- Cell Based → Complement Dependent Cytotoxicity
- Solid Phase Based
  - **ELISA**
  - **Luminex**
  - **Flow Cytometry**

Solid Phase, Antigen Specific Assays





Gebel and Bray. Transplantation Reviews 20: 189-194, 2006

Sensitivity of PRA Screening for HLA Ab by differing methodologies

	POSITIVE	NEGATIVE
CDC	102	162
AHG-CDC	116 (+13%)	148
ELISA	127 (+10%)	137
FLOW	139 (+10%)	125

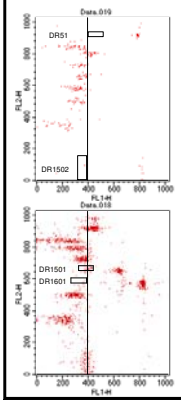
Gebel and Bray, Transplantation 69:1370-1374, 2000.

HLA Antibody Detection

Specificity Analysis

- Solid Phase Based Single Antigen Analysis
  - ELISA
  - Luminex
  - Flow Cytometry

Flow Single Antigen Beads (Class II)



Patient has HLA antibodies against

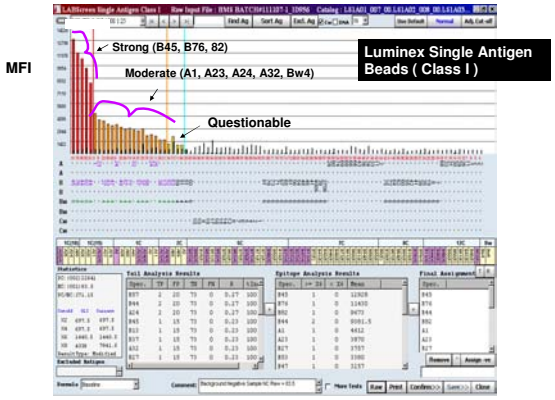
DR 1501 / DR1502
DR 1601
DR 51
} DR 2

(Sensitization Hx: Preg x3, Husband DR15)

How much Antibody?

Titer (Serial Dilution (1:256))
MCF
MESF

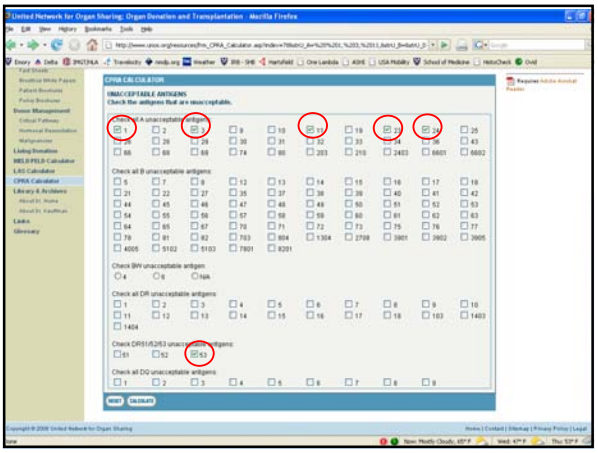
Caveat: not all beads have equal density of Ag

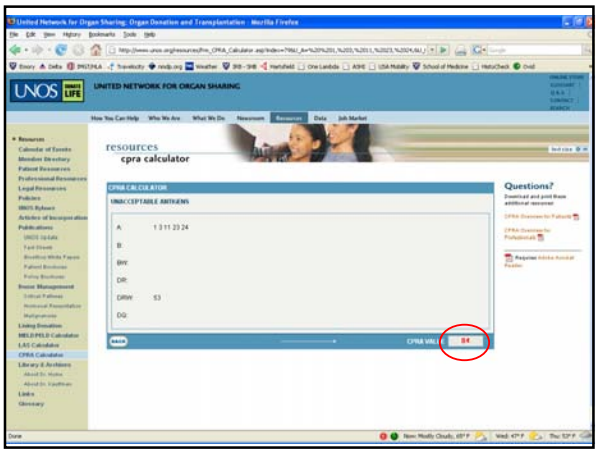


Calculated PRA (cPRA)

Calculated probability of reactive alloantibodies (cPRA) is used to predict crossmatch outcome.

cPRA is based on COMPLETE antibody specificity and the frequencies of HLA antigens present in a donor population; Local, Regional, National





Kidney Registrations on the Waiting List, 01/15/10

Registrants With No Previous Kidney Transplants

Cecka et al AJT 2011

HLA Antibody Detection

Issue to consider next:

How reliably can solid phase assays predict the crossmatch?

➔ "Virtual Crossmatch"

All Serum Donors

OVERALL COMPATIBLE/INCOMPATIBLE

Cell Based Flow Crossmatch

Solid Phase Donor Specific Antibody		Pos.	Neg.
	Pos.	395	30
	Neg.	44	111

Sensitivity= 93% Specificity = 72%

Paul Warner, ASHI Annual Meeting Oct 2008

Chicago Single Centre Study

OVERALL COMPATIBLE/INCOMPATIBLE

Cell Based Flow Crossmatch

Solid Phase Donor Specific Antibody		Pos.	Neg.
	Pos.	480	83
	Neg.	63	854

Sensitivity= 85% Specificity = 93%

Anat Tambur, Am J Transplant (2009)

Possible Interpretations of DSA- FCXM+

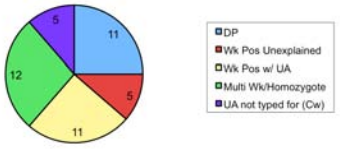
Low Risk Situations

- Auto-antibody
- Unknown serum factor confounding flow cross-match

High Risk Situations

- Blood transfusion since last solid phase assessment
- Mismatched HLA Antigen(s) not present on bead set used
- Mismatched HLA Antibody not routinely tested for (i.e. Cw, DP, DQα)
- Mismatched HLA Antigen(s) present on bead set but HLA conformation change on beads leads to false –ve bead test
- Allele specific HLA Antibody and self bead reactivity ignored

Causes of Unexpected Positive XMs n = 44



Paul Warner, ASHI Annual Meeting Oct 2008

All Serum Donors

OVERALL COMPATIBLE/INCOMPATIBLE

Cell Based Flow Crossmatch

Solid Phase Donor Specific Antibody		Pos.	Neg.
	Pos.	434	30
	Neg.	5	111

Sensitivity= 94% Specificity = **96%**

Paul Warner, ASHI Annual Meeting Oct 2008

Chicago Single Centre Study

OVERALL COMPATIBLE/INCOMPATIBLE

Cell Based Flow Crossmatch

Solid Phase Donor Specific Antibody		Pos.	Neg.
	Pos.	480	83
	Neg.	63	854

Sensitivity= 85% Specificity = 93%

61 of 63 were never tested for all mismatched donor HLA antigens

→35 of 63 had donor specific antibody on further testing (esp HLA-Cw)
→11 of 63 had 0% PRA (i.e. "False Positive" FCXM)

Anat Tambur, Am J Transplant (2009)

Explaining Flow Crossmatch Positive when Virtual ?-ve

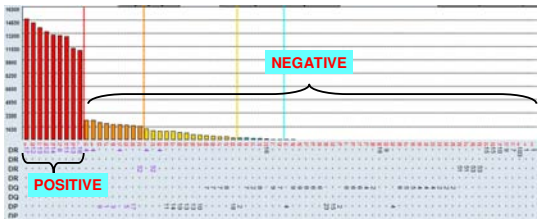
Need to Consider:

Antibodies to HLA Cw, DQβ, DQα, DP, and Allele Specific

Major Issue:

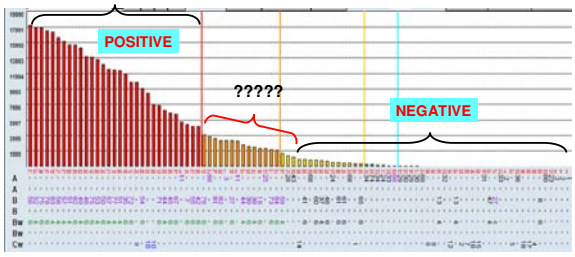
What threshold (MFI) on solid phase beads should be used to infer a true HLA Ab specificity is present?

LUMINEX HD antibody specificity data or "how the story began…"



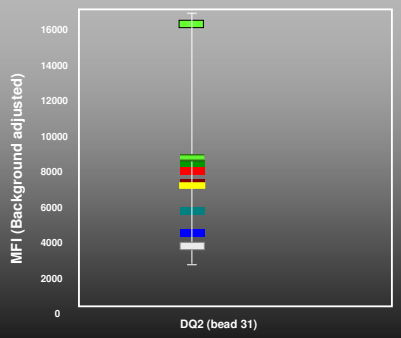
41

LUMINEX HD antibody specificity data or "how the story began…" - 2



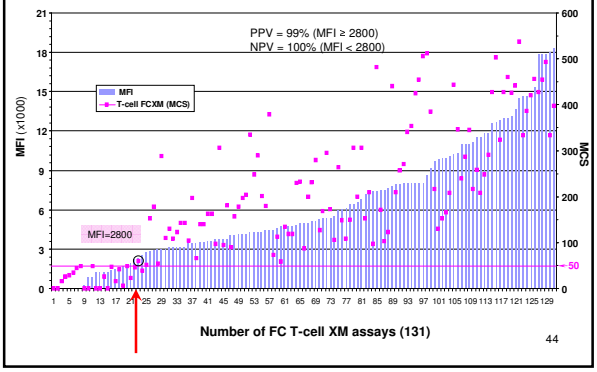
42

Canadian Evaluation of Inter-laboratory MFI Variation



43

CORRELATION BETWEEN SIGNAL INTENSITY (MFI) AND T- cell FCXM (MCS) RESULTS (mono-specific anti-HLA class I sera)



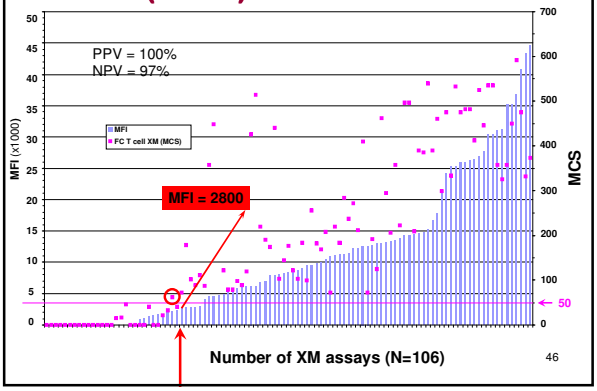
44

Cumulative effect of anti-HLA class I multi-specific sera

Serum #	Ab1	Ab2	Ab3	Ab4	MFI sum	T (MCS)
1	490	368	509	396	1763	22
2	52	174			226	0
3	174	86			260	0
4	412	405			817	0
5	400	720			1120	0
6	720	720	200		1640	0
7	1500	600			2100	32
8	630	1040	440	306	2416	40
9	443	374	604		1421	40
10	360	167	812	129	1500	0
11	1000	1800			2800	72
12	1000	1800			2800	178
13	1862	2256			4118	87
14	2000	2600			4600	357
15	2400	700	2600		5700	79
16	2400	2273	2131		6804	220
17	2550	3100	2400		8050	103

45

MULTI-SPECIFIC anti-CLASS I ANTIBODY MFI AND MCS (T-FCXM)



46

HLA Antibody Detection

➔"Virtual Crossmatch"

Very good correlation with the actual crossmatch if:

[A] attention to weak antibodies and
[B] those not routinely tested for Cw, DP, DQα

How does it perform in actual clinical practice?

Virtual Crossmatch: Biologic Significance

The Swiss are doing it prospectively with Kidneys

86% Virtual CXM & FCXM Concordant
7% Virtual CXM - & FCXM + ➔ Excellent Func^{c} & Normal Bx at 3 & 6 mo
7% Virtual CXM + & FCXM - ➔ 25% Subclinical Ab Mediated Rejection despite Thymo + IVIG

Bielmann et al, Am J Transplant (2007)7:626

United Network for Organ Sharing (UNOS)
Transplant Rates/1000 Active Patient Years

PRA/ CPRA	2001 Tx Rate	2002 Tx Rate	2009 Tx Rate	2010 Tx Rate
80-84	194	119	358	489
85-89	144	128	223	377
90-95	140	128	171	239
>95	98	76	97	69

Solid Phase Assays

Cecka, et al. Am J Transplantation 2011

Summary

Transplant Programs have seen a revolution in technology

- HLA Typing → Molecular (low to high (allele) resolution)
- HLA Ab Screen → Solid Phase (increase sensitivity)
- HLA Ab Specificity → Solid Phase (increase in resolution)
- Donor Specific HLA Ab → Flow Crossmatch (increase sensitivity)

Some issues to consider next:

- Needs to standardize HLA Ab quantitation (common language)
- Further studies needed to define relative risks of low levels of donor specific antibodies (e.g. detected by solid phase only)
- Further studies needed to validate significance of HLA Cw, DP, DQα antibodies, and non-HLA Ab (e.g. MICA, MICB)

Fellows Symposium on Transplantation Medicine

Friday, September 23
2:55 pm - 3:25 pm

Rejection from the T Cell's Perspective and Effector Mechanisms and the Basics of Graft Injury

Peter S. Heeger, MD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

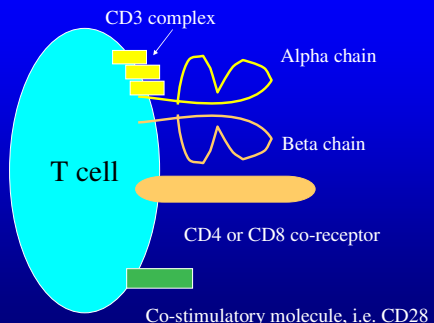
Transplant rejection from the T cell point of view

Peter S. Heeger, M.D.
Nephrology Division, Dept of Medicine
Recanati Miller Transplant Institute
The Mount Sinai School of Medicine
New York, NY

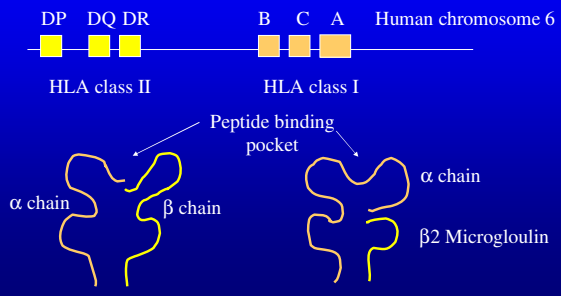
Definitions

- Isograft (syngeneic)-identical to self
  - Identical twins
  - Inbred mice
- Allograft-between individuals of the same species
  - rapidly rejected by naive mice and by "naïve" humans
  - Alloimmunity derives from alloreactive B and T cells
- Xenograft-between species
  - Example: Pig to human
  - Rapidly rejected by naive mice and by "naive" humans

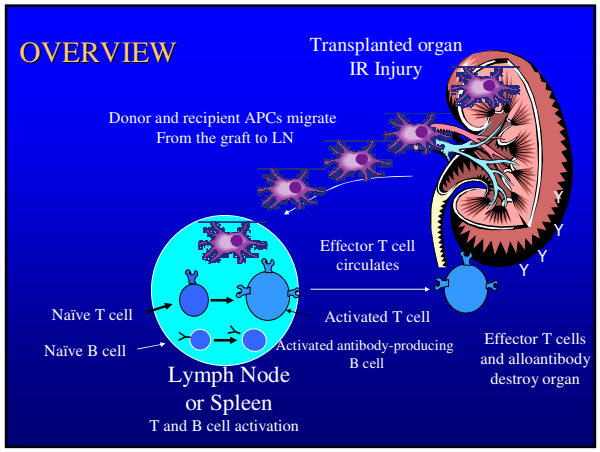
T cells and TCRs



Major Histocompatibility Molecules



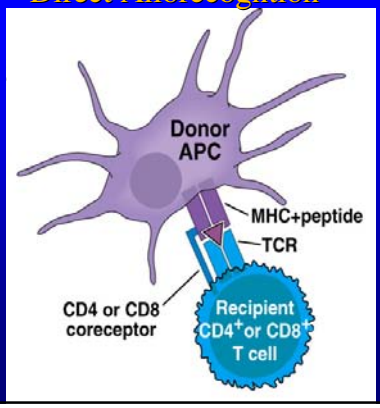
Schematic of MHC Class I Crystal Structure



Phases of the Alloimmune Response

- **Antigen recognition**
- T cell and B cell activation, differentiation and expansion
- Effector functions
- Resolution of the response with residual memory

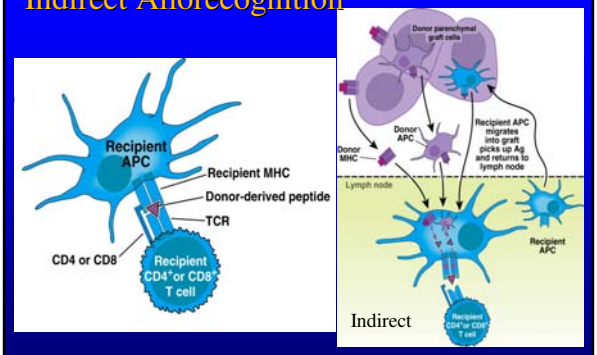
Direct Allorecogntion



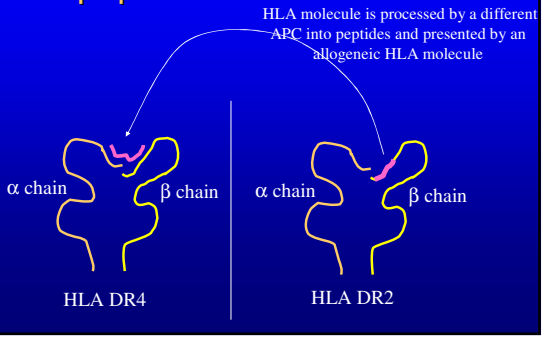
Direct alloreactivity

- CD4 and CD8 T cells are directly primed to donor MHC: peptide complexes at high frequency
- The ability to recognize donor MHC must be due to chance cross reactivity because the recipient T cells were never "trained" to recognize foreign MHC molecules
- T cells responding through the direct pathway are thought to account for episodes of acute cellular rejection

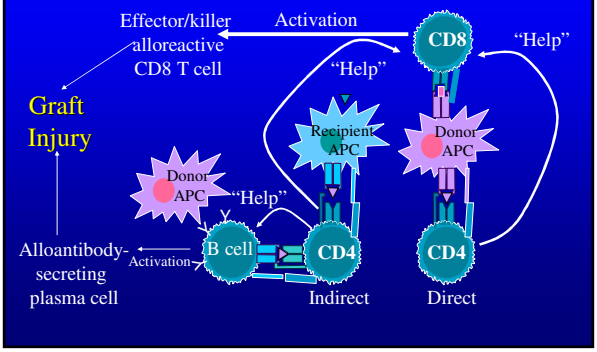
Indirect Allorecognition



Where to the indirectly presented peptides come from?



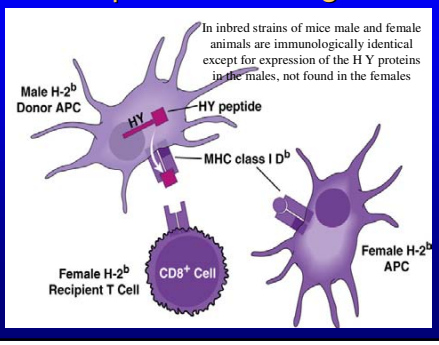
Interactions between direct and indirect



Minor Transplantation Antigens

- A recipient can reject a graft matched at all MHC loci (graft from one MHC-matched sibling to another, for example)
- Minor antigens are non-MHC, donor-derived peptide determinants expressed in the context of MHC molecules common to the recipient and the donor

Molecular Basis for Minor Transplantation Antigens

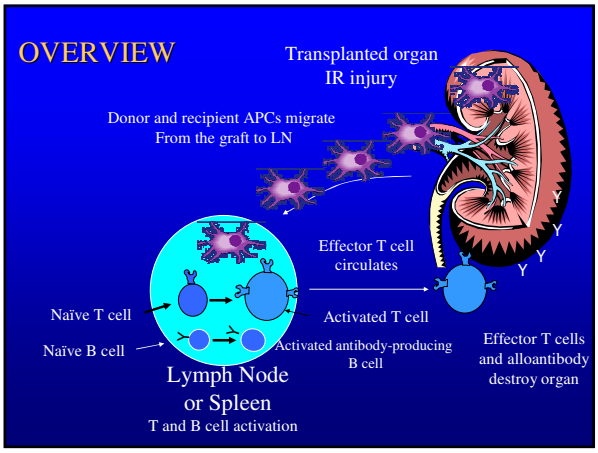


Known Minor Histocompatibility Antigens

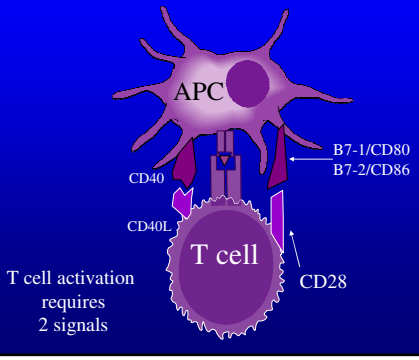
- H-Y (male antigens) *Smcy, Uty*
- Mitochondrial proteins MTFα, MTFβ
- myosin related protein HA-2
- Other
  - H13
  - Mx1
  - beta 2-microglobulin

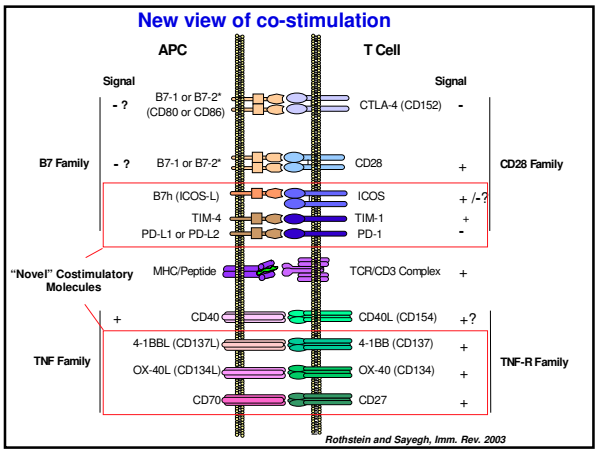
Phases of the alloimmune response

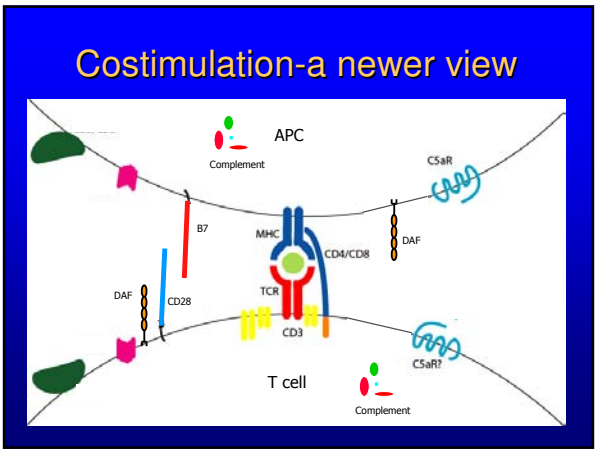
- Antigen recognition
- **T cell and B cell activation, differentiation and expansion**
- Effector functions
- Resolution of the response with residual memory

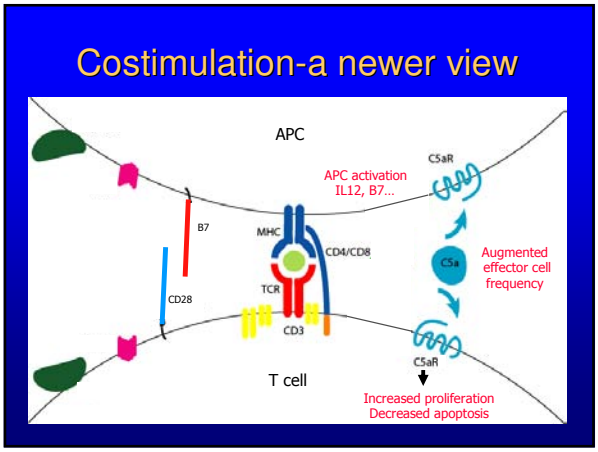


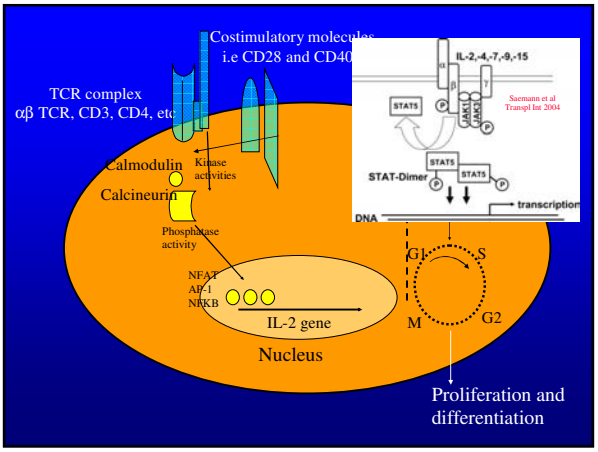
Costimulation-the old view

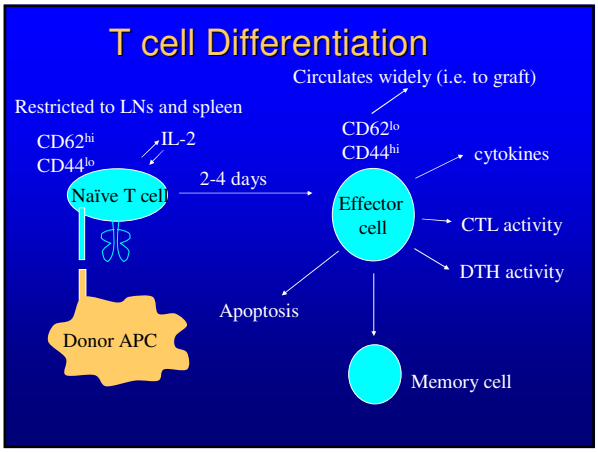


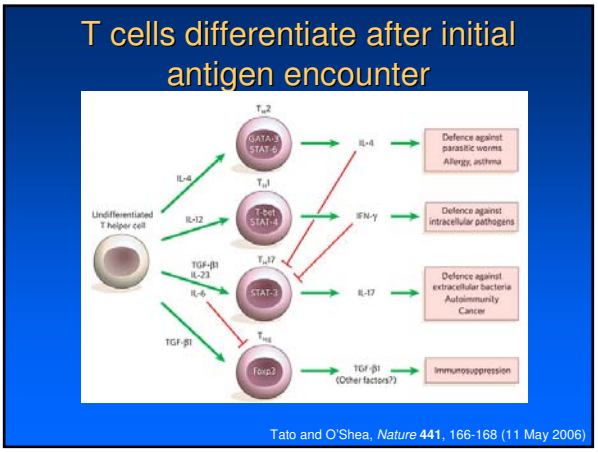

New view of co-stimulation
APC
T Cell
Signal
Signal
B7-1 or B7-2*
(CD80 or CD86)
CTLA-4 (CD152)
B7 Family
B7-1 or B7-2*
CD28
CD28 Family
B7h (ICOS-L)
ICOS
TIM-4
TIM-1
PD-L1 or PD-L2
PD-1
"Novel" Costimulatory Molecules
MHC/Peptide
TCR/CD3 Complex
CD40
CD40L (CD154)
TNF Family
4-1BBL (CD137L)
4-1BB (CD137)
OX-40L (CD134L)
OX-40 (CD134)
CD70
CD27
TNF-R Family
Rothstein and Sayegh, Imm. Rev. 2003


Costimulation-a newer view
APC
Complement
C5aR
B7
MHC
CD4/CD8
DAF
DAF
CD28
TCR
CD3
T cell
C5aR?
Complement

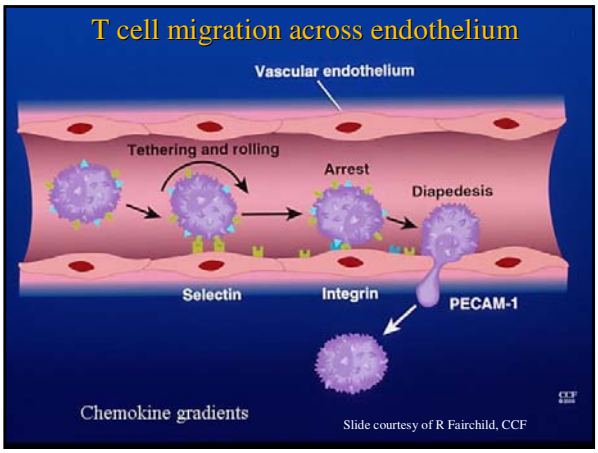

Costimulation-a newer view
APC
APC activation
IL12, B7...
C5aR
B7
MHC
CD4/CD8
C5a
Augmented
effector cell
frequency
CD28
TCR
CD3
C5aR
T cell
Increased proliferation
Decreased apoptosis


Costimulatory molecules
i.e CD28 and CD40
TCR complex
αβ TCR, CD3, CD4, etc
Calmodulin
Kinase
activities
Calcineurin
Phosphatase
activity
NFAT
AP-1
NFKB
IL-2 gene
Nucleus
IL-2,-4,-7,-9,-15
Saemann et al
Transpl Int 2004
STAT5
JAK1
JAK3
STAT-Dimer
transcription
DNA
G1
S
M
G2
Proliferation and
differentiation


T cell Differentiation
Circulates widely (i.e. to graft)
Restricted to LNs and spleen
CD62hi
CD44lo
IL-2
Naïve T cell
2-4 days
CD62lo
CD44hi
Effector
cell
cytokines
CTL activity
DTH activity
Apoptosis
Donor APC
Memory cell


T cells differentiate after initial
antigen encounter
Undifferentiated
T helper cell
IL-4
IL-12
TGF-β1
IL-23
IL-6
TGF-β1
GATA-3
STAT-6
T-bet
STAT-4
STAT-3
Foxp3
IL-4
IFN-γ
IL-17
TGF-β1
(Other factors?)
Defence against
parasitic worms
Allergy, asthma
Defence against
intracellular pathogens
Defence against
extracellular bacteria
Autoimmunity
Cancer
Immunosuppression
Tato and O'Shea, Nature 441, 166-168 (11 May 2006)

T cell migration across endothelium



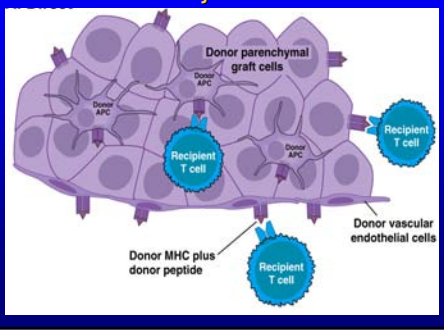
Phases of the alloimmune response

- Antigen recognition
- T cell and B cell activation, differentiation and expansion
- **Effector functions**
- Resolution of the response with residual memory

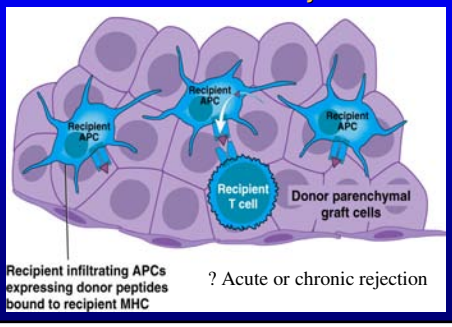
T cell effector mechanisms

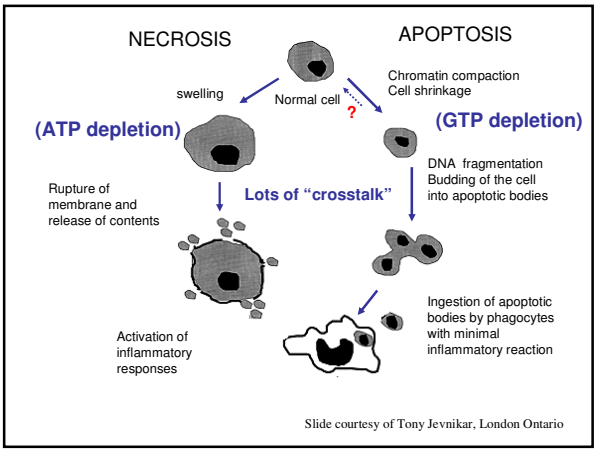
- Primed T cells produce cytokines - amplification of immune response, chemoattraction, etc.
- Delayed Type Hypersensitivity (DTH)
  - macrophage activation and chemoattraction
  - Release of cytokines, INOS/NO, TNF, eicosanoids, others
- Cytotoxicity

T cells responding through the direct pathway may account for acute cellular rejection

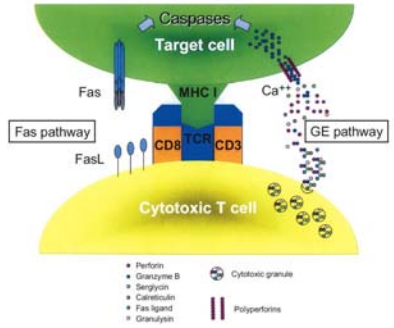


T cells responding through the indirect pathway can contribute to acute and chronic rejection



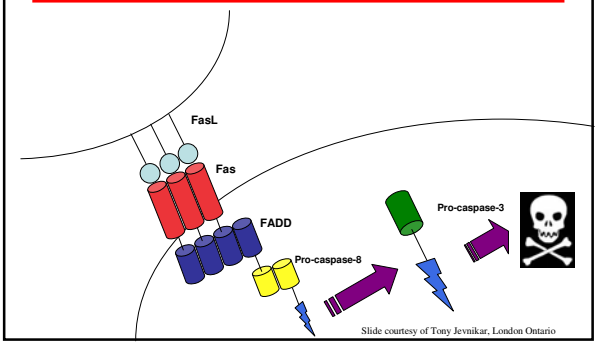


The "classic" model of transplant injury by T cell effectors



Rocha et al Immunological Reviews 2003, 196: 51–64

Fas/FasL pathway of injury as a model of receptor mediated cell death



Slide courtesy of Tony Jevnikar, London Ontario

T cells are not the only effectors

- Innate immunity
  - Macrophage activation
  - Neutrophil recruitment
  - Dendritic cell maturation
  - Adaptive (Th1) immunity enhancement
- B cells and antibodies

- There are also graft derived protective mechanisms (HO1, IDO, etc)

Slide courtesy of Tony Jevnikar, London Ontario

Phases of the alloimmune response

- Antigen recognition
- T cell and B cell activation, differentiation and expansion
- Effector functions
- **Resolution of the response with residual memory**

Resolution and Memory

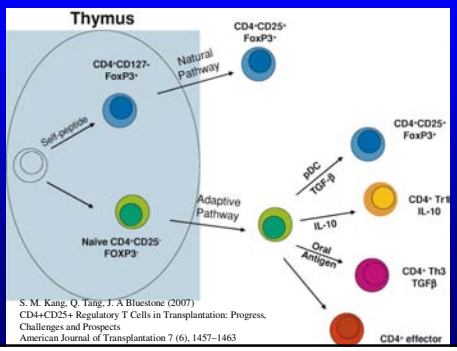
- Down regulation of the induced immune response must occur
- A few antigen specific cells are spared and these are memory cells
- Memory cells have lower activation thresholds than naïve cells and can respond rapidly to previous "seen" antigens
- Memory is important for protection against pathogens
- Anti donor memory T cells are a barrier to transplantation

What are Tregs?

Regulatory T cells

- Regulatory T cells inhibit other lymphocytes (defined by their function)
- Multiple phenotypes
  - CD4+CD25+ (natural, induced)
  - CD8+
  - CD4/CD8 double negative
  - others
- FoxP3 is key transcription factor and most reliable marker
- Human IL-7 receptor ($CD127^{lo}$) expression

CD4 Treg



S. M. Kang, Q. Tang, J. A Bluestone (2007)
CD4+CD25+ Regulatory T Cells in Transplantation: Progress, Challenges and Prospects
American Journal of Transplantation 7 (6), 1457–1463

Regulatory cells

- Inhibit T cell responses as a normal control mechanism, to prevent autoimmunity
- Are induced by transplantation and possibly augmented by certain immunosuppressants, i.e. thymoglobulin (Sayegh and colleagues)
- Activation/induction may require immunoregulatory cytokines (IL-10, TGFbeta)

Treg-antigen specificity

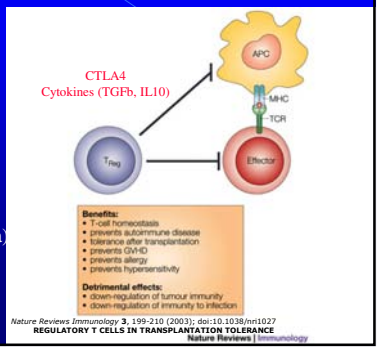
- nTreg self reactive
- iTreg
  - Direct
  - Indirect
- Evidence indicates indirect Treg reactivity is required for tolerance

Treg-mechanisms of action

Block T cell activation in lymph nodes and can also regulate at the graft site

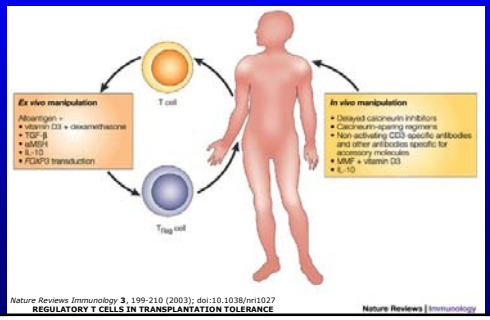
Inhibit other T cells through secretion of immunoregulatory cytokines (IL-10, TGFbeta)

Inhibit other T cells by blocking APC activation



Nature Reviews Immunology **3**, 199-210 (2003); doi:10.1038/nri1027
REGULATORY T CELLS IN TRANSPLANTATION TOLERANCE
Nature Reviews | Immunology

Potential approaches to using Treg in transplantation



Nature Reviews Immunology **3**, 199-210 (2003); doi:10.1038/nri1027
REGULATORY T CELLS IN TRANSPLANTATION TOLERANCE
Nature Reviews | Immunology

Summary
Phases of the alloimmune response

- Allorecognition
- T cell activation-role of costimulation
- T cell differentiation and expansion followed by wide circulation in periphery
- Primed T cells and antibodies accumulate at graft site
- Effector functions of T cells and antibodies result in organ pathology
- Resolution of the immune response with immunologic memory

Thank you

Fellows Symposium on Transplantation Medicine

Friday, September 23
3:25 pm - 3:50 pm

B Cells and Antibodies: From Bench to Bedside

Milagros D. Samaniego, MD

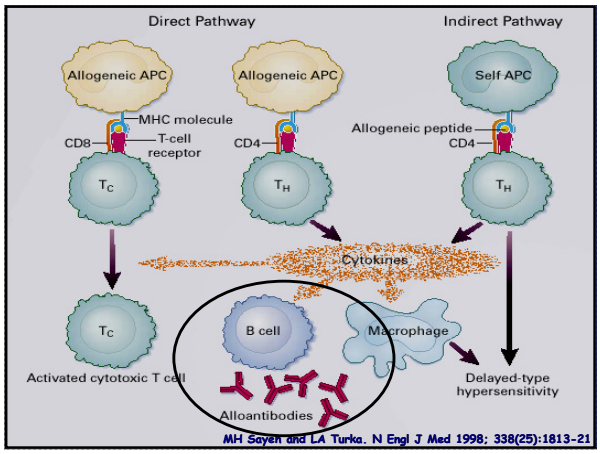
September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

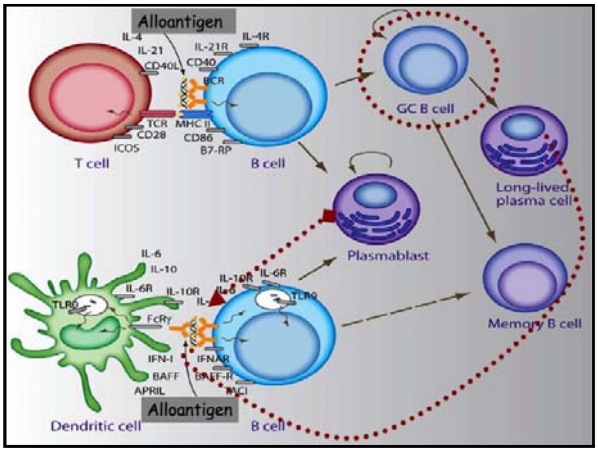
AST Fellows Symposium on Transplantation Medicine September 23-25, 2011

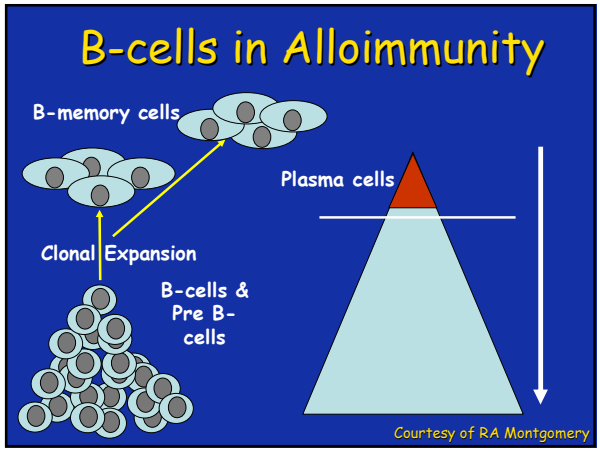
B-cells: Old Problem, New Biology

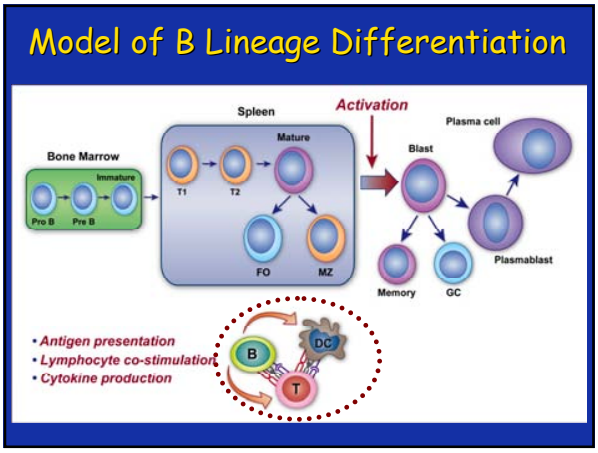
Millie Samaniego, MD
Professor of Medicine
Medical Director, Kidney and
Kidney-Pancreas Transplant Program

University of Michigan
Medical School



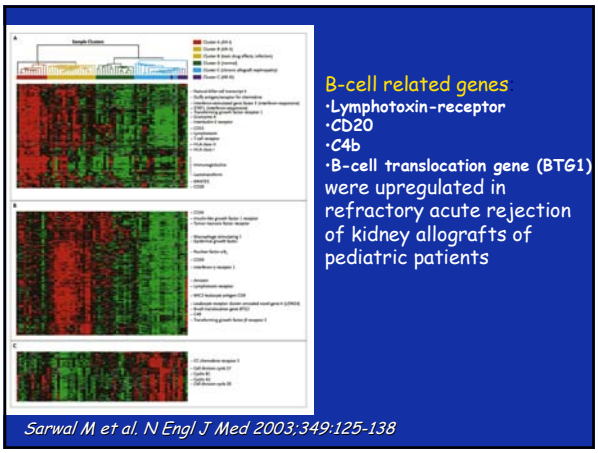


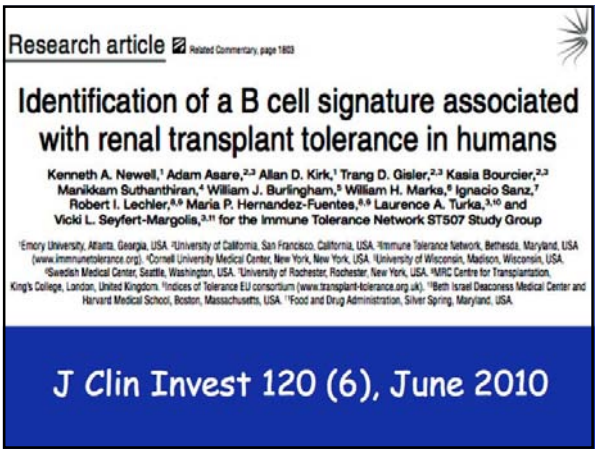


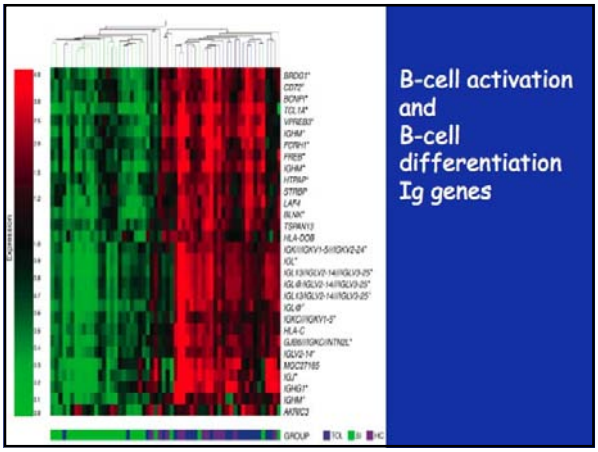


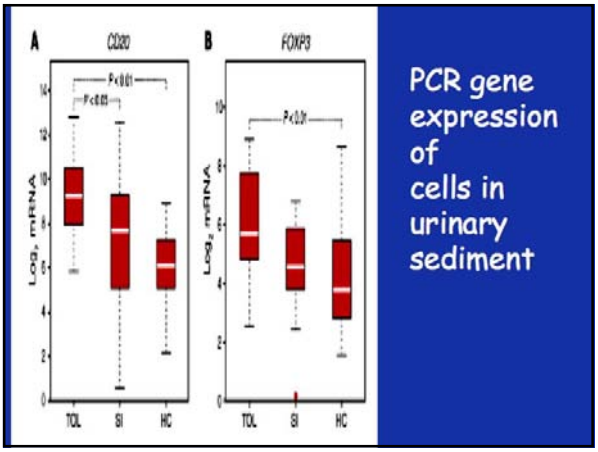
B-cells in Allograft Injury Effector Role

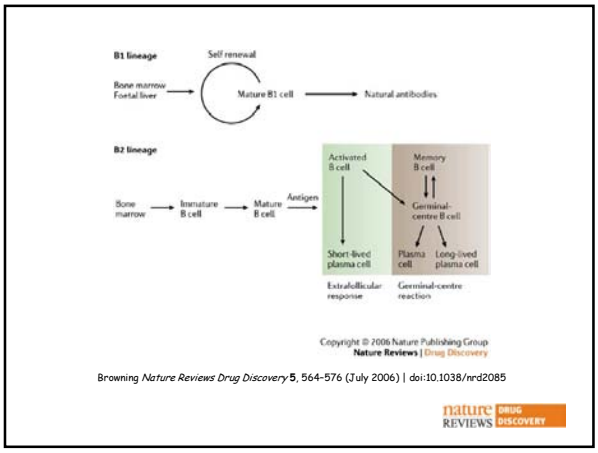
- Sarwal et al (NEJM 349; 2, 2003):
  - The presence of dense $CD20^+$ B-cell infiltrates is associated with both glucocorticoid resistant acute allograft rejection (P=0.01) and graft loss (P<0.001) in pediatric patients
  - No correlation between $CD20^+$ infiltrates and C4d deposition (P=1.0)
  - C1r,s and C4b was noted in some biopsies
  - No testing for donor specific antibody was performed

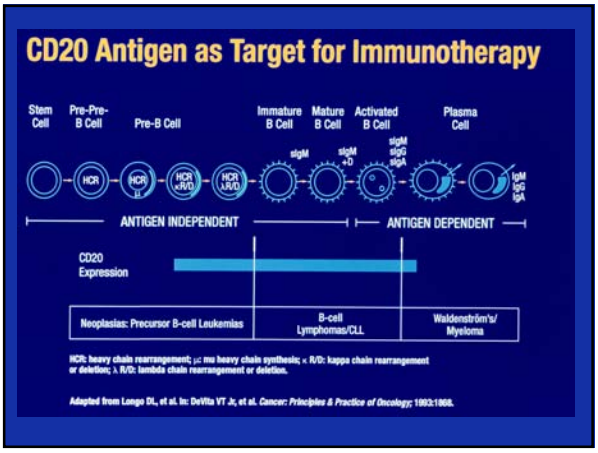

B-cell related genes
·Lymphotoxin-receptor
·CD20
·C4b
·B-cell translocation gene (BTG1)
were upregulated in refractory acute rejection of kidney allografts of pediatric patients
Sarwal M et al. N Engl J Med 2003;349:125-138

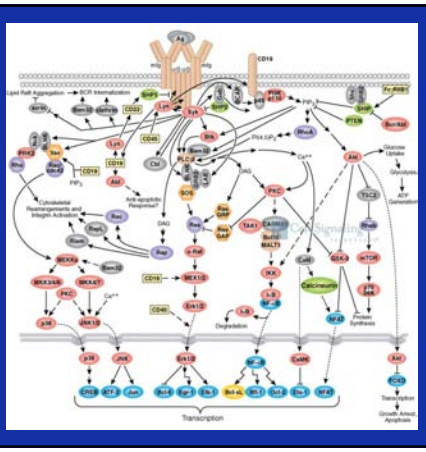

Research article
Identification of a B cell signature associated with renal transplant tolerance in humans
J Clin Invest 120 (6), June 2010


B-cell activation and B-cell differentiation Ig genes

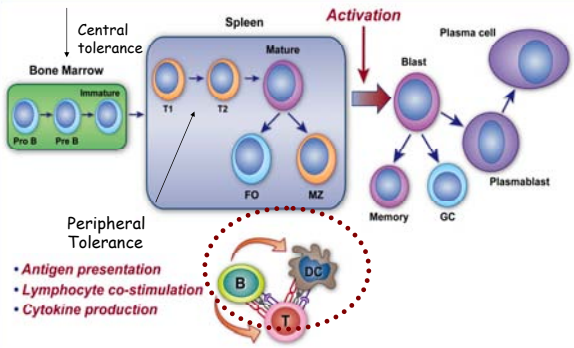

PCR gene expression of cells in urinary sediment


B1 lineage
Self renewal
Mature B1 cell
Natural antibodies
B2 lineage
Antigen
Germinal-centre B cell
Short-lived plasma cell
Long-lived plasma cell
Extrafollicular response
Germinal-centre reaction
Browning Nature Reviews Drug Discovery 5, 564-576 (July 2006) | doi:10.1038/nrd2085


CD20 Antigen as Target for Immunotherapy
Stem Cell
Pre-Pre-B Cell
Pre-B Cell
Immature B Cell
Mature B Cell
Activated B Cell
Plasma Cell
ANTIGEN INDEPENDENT
ANTIGEN DEPENDENT
CD20 Expression
Neoplasias: Precursor B-cell Leukemias
B-cell Lymphomas/CLL
Waldenström's/ Myeloma

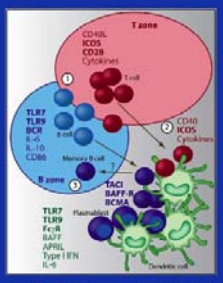


Model of B Lineage Differentiation



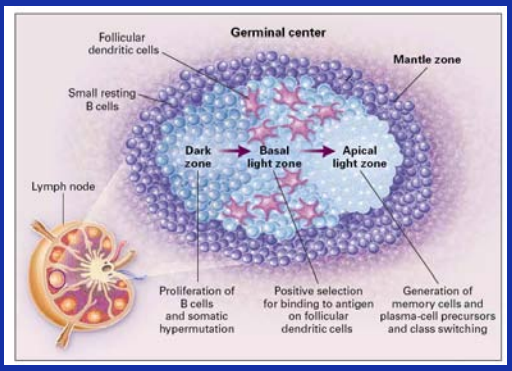
Extrafollicular Pathways of B-cell Activation

1) T-cell and B-cell interactions
   BCR recognition
   TLR7 or TLR 9 signaling
2) Migration of activated B-cells to the T-zone-red pulp border
   Costimulatory signals
   Microenvironment survival signals provided by dendritic cells
   Differentiation into Plasmablasts
3) Generation of B-cell memory ??



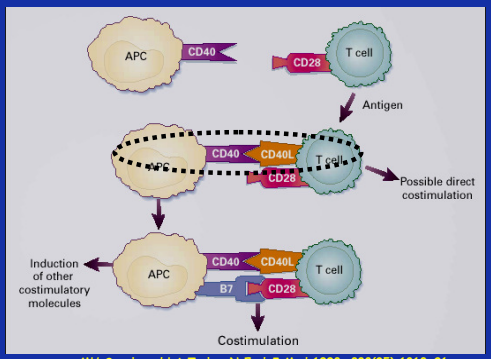
Shlomchik MJ: Immunity 28, Jan 2008

The Germinal Center Reaction



P Delves. N Engl J Med, 2000

Costimulatory Signals and GC Response



MH Sayeh and LA Turka. N Engl J Med 1998; 338(25):1813-21

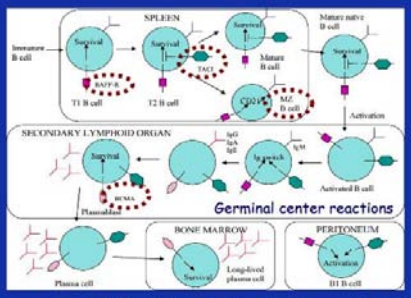
The Blys of APRIL: TNF family of Proteins

- Blys (CD257) and APRIL are key cytokines produced by dendritic [myeloid] cells and Mac/ϕ (?) that regulate:
  - The maturation, proliferation and survival of B-cells
  - B-cell dependent antigen presentation
  - CD40-CD154 independent antibody class switching recombination

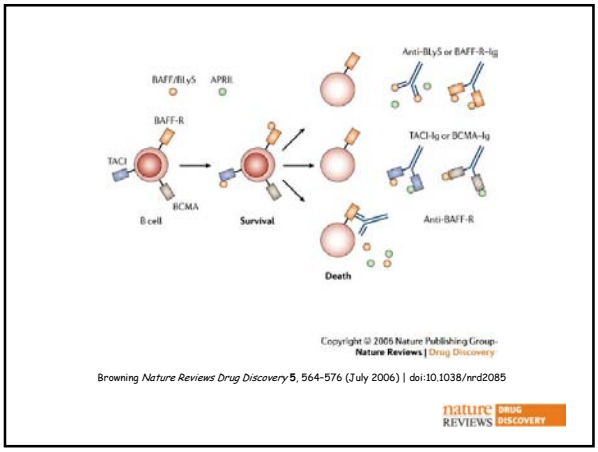
BAFF (Blys): B cell Activating Factor

- B cell survival factor
- Lowers threshold of B cell activation via BCR
- Receptors:
  - BAFF-R (B cells)
  - BCMA (plasma cells)
  - TACI (monocytes)
- Expressed by monocytes, neutrophils, activated T-cells, stromal cells

APRIL and BAFF (Blys) Receptors In B-cell Development



Bossen C and Schneider P: Semin Immunol 18 (2006)



Browning *Nature Reviews Drug Discovery* 5, 564-576 (July 2006) | doi:10.1038/nrd2085

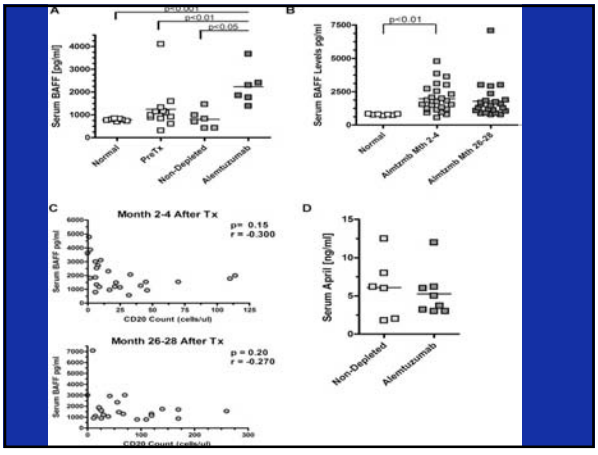
nature REVIEWS DRUG DISCOVERY

BAFF Is Increased in Renal Transplant Patients following Treatment with Alemtuzumab

Bloom, Chang, Pauly, Kwun, Fechner, Hayes, Samaniego, Knechtle

- 22/24 Alemtuzumab-treated renal transplant recipients increased BAFF at 6, 12, 24 mo.
- BAFF-R down-regulated on CD19+ cells
- BAFF mRNA transcripts 7x increased in CD14+ cells
- Addition of BAFF to MLR enhanced B cell activation to alloantigen

Am J Transplant 2009, 9: 1835-45



BAFF and Alloantibody

- BAFF lowers B cell activation threshold
- Association between elevated levels after Alemtuzumab treatment and increased alloantibody in patients
- BAFF targeting to prevent alloantibody?
  - Belimumab
  - TACI-Ig

B-cell stage	Inhibition of:			
	Anti-CD20	BAFF	BAFF+APRIL	CD40L
B1	?	–	–	?
Pro/Pre	–	–	–	–
Immature	√	–*	–*	–
Follicular	√	√	√	√[§]
Marginal zone	√	√	√	–
Germinal centre	–?[‡‡]	–?[‡‡]	?	√[§]
Memory	?	?	?	√[§]
Plasma cell	–[§]	–[§]	?[¶]	–[§]

*BAFF is required at the late transitional (T2) step during maturation. [‡]Anti-CD40L or antagonistic anti-CD40 will block both T-dependent primary and secondary responses. [§]Interference with memory or extrafollicular B cell responses will reduce the numbers of short-lived plasma cells. [¶]Plasma cells might require APRIL–BCMA signalling for survival. [‡‡]The effect on germinal-centre reactions remains unclear in primates. APRIL, a proliferation-inducing ligand; BAFF, B-cell activating factor; BCMA, B-cell maturation factor; CD40L, CD40 ligand.

Browning *Nature Reviews Drug Discovery* 5, 564-576 (July 2006) | doi:10.1038/nrd2085

nature REVIEWS DRUG DISCOVERY

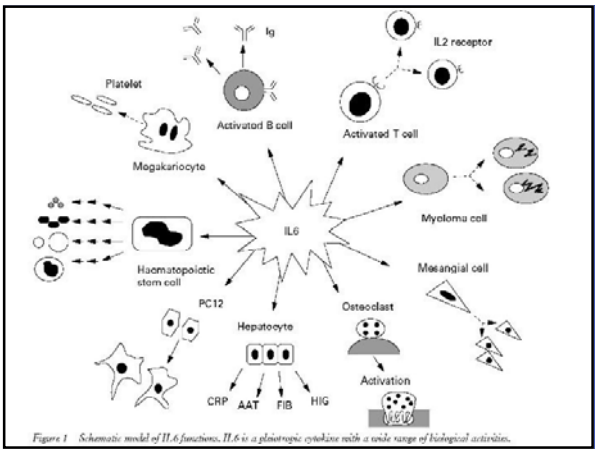


Figure 1 Schematic model of IL-6 functions. IL-6 is a pleiotropic cytokine with a wide range of biological activities.

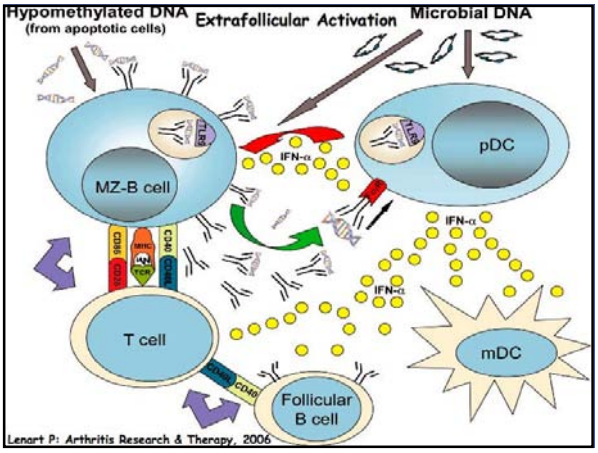
Innate Immunity and B-cell Responses

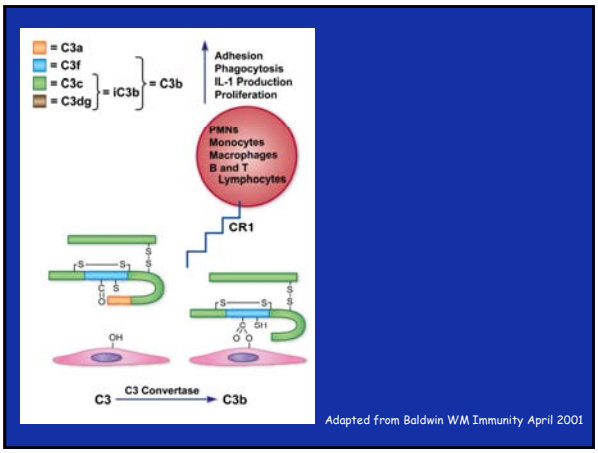
Toll Like Receptors (TLRs)

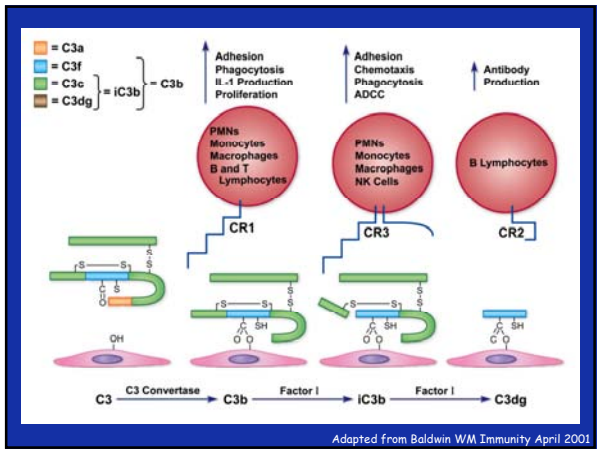
- Special recognition molecules on cell surface [cell cytoplasm]
- Recognize pathogen-associated molecular patterns (PAMPs) (LPS) - Microbial DNA
- Recognize endogenous ligands released from damaged cells: Damage-associated molecular patterns (DAMPs) - Hypomethylated DNA
- Hyaluronic acid, heparin sulfate, fibrinogen, heat shock proteins
- Bridge between innate and adaptive immunity

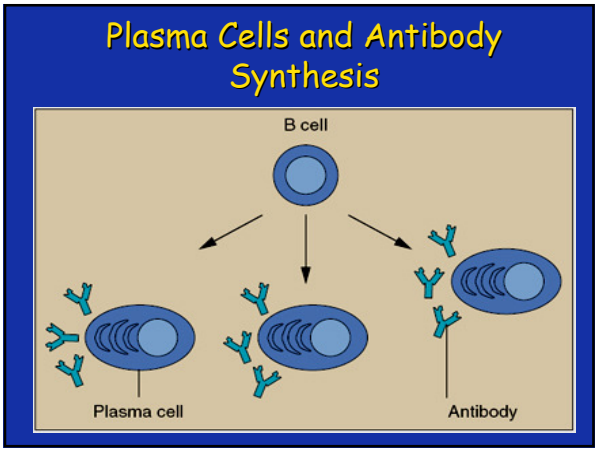
Toll Like Receptors (TLRs)

- In humans TLR-7/-8 and -9 are only expressed in B-cells and plasmacytoid dendritic cells (pDCs)
- These TLRs have been linked to the pathogenesis of human and murine lupus
- Likely play a role in molecular mimicry and alloantibody production following viral and bacterial infections



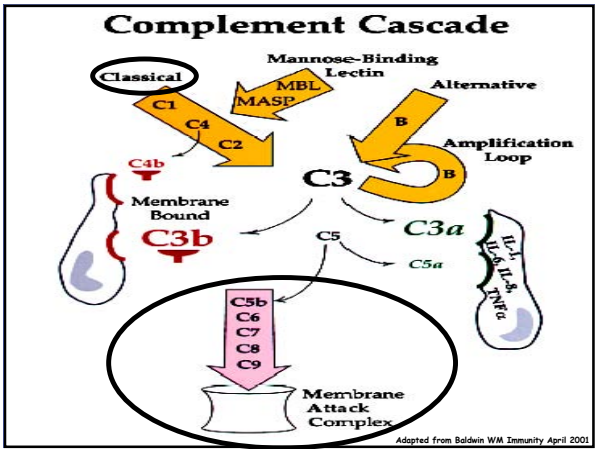






Mechanisms of Antibody-Mediated Injury

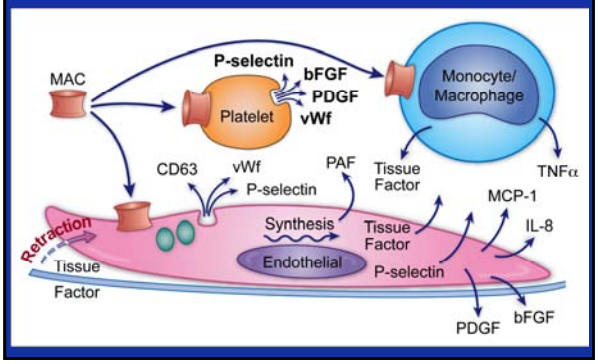
- Local activation of the Complement System
  - Mac (C5b-9)-mediated injury
    - Lytic
    - Sub-lytic



Sub-lytic MAC Injury

A Link between Alloantibody-Mediated Injury and Fibrogenesis?

Mac-related Sub-lytic Activation: The Common Link [CAMR]



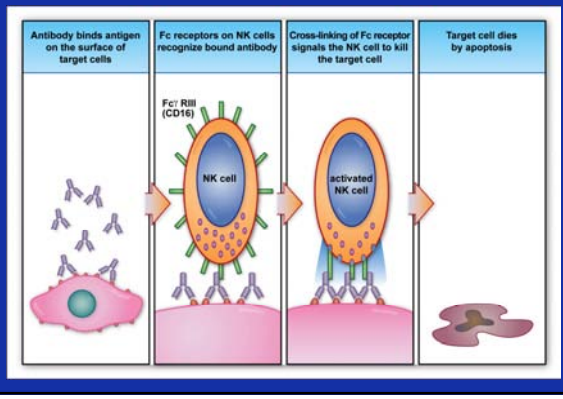
Mechanisms of Antibody-Mediated Injury

- Complement Independent Injury
  - FcR interactions
  - Antibody-cell dependent cell cytotoxicity (ADCC)

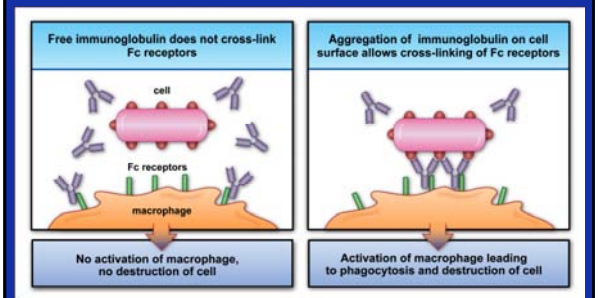
Fcγ Receptors

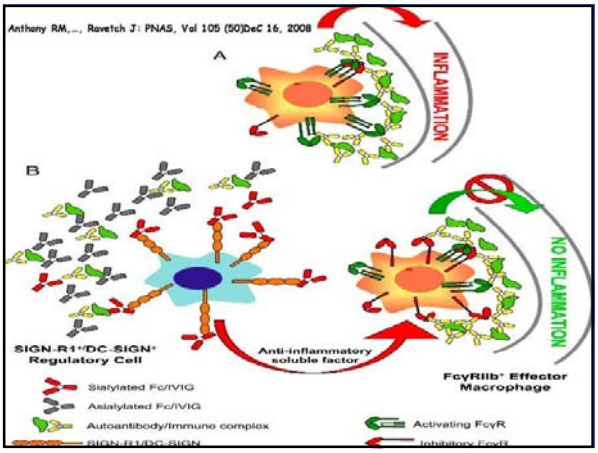
Receptor	FcγRI (CD64)	FcγRII-A (CD32)	FcγRII-B2 (CD32)	FcγRII-B1 (CD32)	FcγRIII (CD16)
Structure	α 72kDa; γ	α 40kDa; γ-like domain	ITIM	ITIM	α 50-70kDa; γ or ζ
Binding Order of affinity	IgG1 $10^8\ M^{-1}$ 1) IgG1 = IgG3 2) IgG4 3) IgG2	IgG1 $2 \times 10^8\ M^{-1}$ 1) IgG1 2) IgG3 > IgG3' 3) IgG4	IgG1 $2 \times 10^8\ M^{-1}$ 1) IgG1 = IgG3 2) IgG4 3) IgG2	IgG1 $2 \times 10^8\ M^{-1}$ 1) IgG1 = IgG3 2) IgG4 3) IgG2	IgG1 $5 \times 10^6\ M^{-1}$ IgG1 = IgG3
Cell type	Macrophages Neutrophils* Eosinophils* Dendritic cells	Macrophages Neutrophils Eosinophils Platelets Langerhans' cells	Macrophages Neutrophils Eosinophils	B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells
Effect of ligation	• Uptake • Stimulation • Activation of respiratory burst induction of killing	• Uptake • Granule release (eosinophils)	• Uptake inhibition of stimulation	• No uptake • Induction of stimulation	• ADCC (NK cells)

ADCC-1



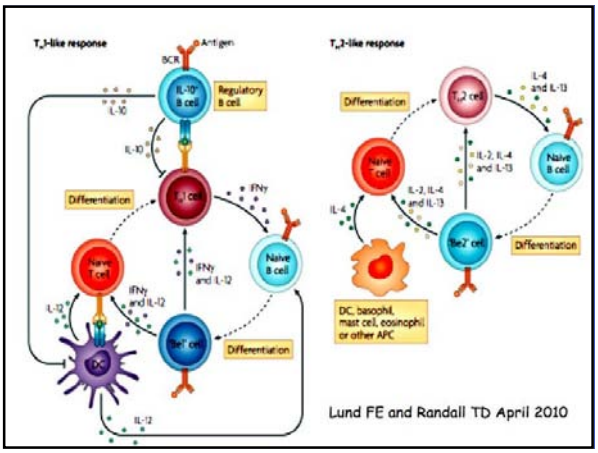
ADCC-2





Antibody-Independent Immune Effects of B-cells

- B-effector cells
  - Modulation of T-cell responses
    - Cytokine production
    - Antigen presentation [low level of antigen]
    - Costimulation
      - 1ary and 2ary T-cell responses
      - Generation of T-cell memory
- Generation and function of T-regulatory cells
  - Late B-cell depletional therapy
  - TGF-β3
- B-regulatory cells (B10)



Summary-1

- The role of B-cells in alloimmune injury remains unclear
- Possible mechanisms of injury include cytokine production, costimulation and antigen presentation
- Links between B-cells and innate immunity can explain enhanced alloantibody production following viral and bacterial infections

Summary-2

- Alloantibodies are proven effectors of acute and chronic allograft injury
- Alloantibody induced injury involves both complement dependent and complement-independent mechanisms

Fellows Symposium on Transplantation Medicine

Friday, September 23
4:20 pm - 5:30 pm

Indications for SOT: Kidney and Pancreas in Adults and Pediatric Recipients

Breakout Session

Vikas R. Dharnidharka, MD, MPH, Robert Gaston, MD and Jeremy Goodman, MD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

Indications for solid organ transplantation: kidney and pancreas

Robert S. Gaston, MD
Vikas Dharnidharka, MD, MPH
Jeremy Goodman, MD

Case #1

A 43-year-old woman with stage IV CKD is referred to a nephrologist for evaluation

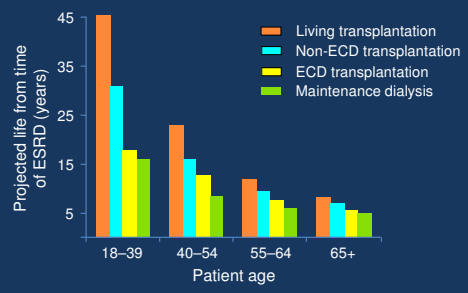
- Type 1 diabetes since age 12
- Retinopathy with well-preserved visual acuity
- Works full time with active social life

Early AM nausea and easy fatigability

- Serum creatinine 4.2 (eGFR = 14)
- No vascular access

2

Projected life expectancy from the time of ESRD



ECD = expanded criteria donor
ESRD = end-stage renal disease

Schold et al, CJASN 1: 532, 2006

Case #1 (cont)

In further discussion with the patient, she expresses the desire for transplant as treatment for her kidney failure. She also inquired about and expressed interest in having a pancreas transplant to treat her diabetes.

She has 2 presumably healthy siblings who have expressed interest in donating a kidney; her children, aged 21 and 23 years, have also expressed interest.

4

Comparing transplant options in diabetes

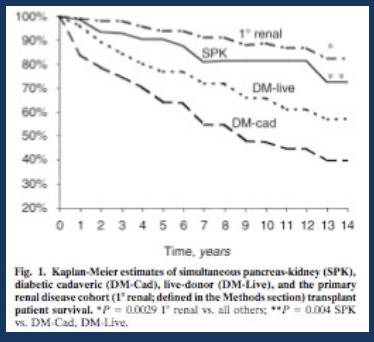


Fig. 1. Kaplan-Meier estimates of simultaneous pancreas-kidney (SPK), diabetic cadaveric (DM-Cad), live-donor (DM-Live), and the primary renal disease cohort (1° renal; defined in the Methods section) transplant patient survival. *P = 0.0029 1° renal vs. all others; **P = 0.004 SPK vs. DM-Cad, DM-Live.

Becker BN et al, *Kidney Int* 57: 2129, 2000

5

Case #1 (cont)

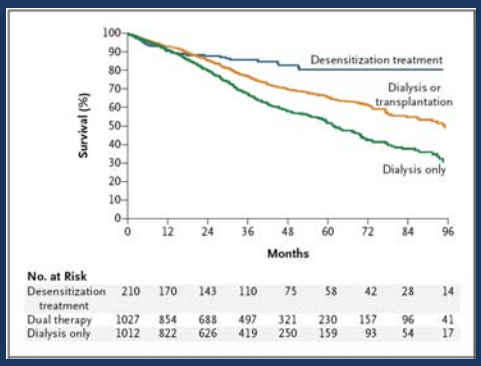
Blood and tissue typing performed:

- ABO type B
- PRA 42%
- 2 siblings both blood type A
- (+) flow cytometry crossmatch with both children

6

Vikas R. Dharnidharka, MD, MPH,
Robert Gaston, MD, and
Jeremy Goodman, MD
www.a-s-t.org

Survival benefit of desensitization



Montgomery R et al, *N Engl J Med* 365: 318, 2011

7

Case #1 (cont)

With this information

- Patient placed on the waiting list for simultaneous kidney/pancreas transplant
- AV fistula created

8

Case #2

A 71-year-old man with ESRD is referred for transplant evaluation

- Started hemodialysis 11 months ago
- Type 2 diabetes mellitus, hypertension, CAD (previous MI, CABG and subsequent PTCA), hyperlipidemia, hypothyroidism, CHF
- Tolerating hemodialysis well
  - One episode of AV graft thrombosis treated with percutaneous thrombectomy

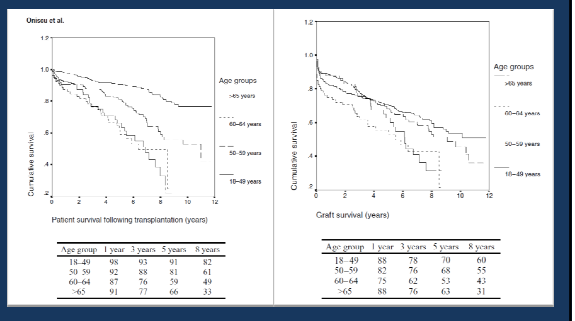
9

Listing Conference Options

- Add to deceased donor waiting list and encourage patient to find a living donor
  - ± expanded criteria donor
- Accept patient for a live donor transplant only
- Decline patient for transplantation

10

How Old is Old for Transplantation?



American Journal of Transplantation 2004; 4: 2067–2074

11

Renal Transplantation in Elderly Patients Older Than 70 Years of Age: Results From the Scientific Registry of Transplant Recipients

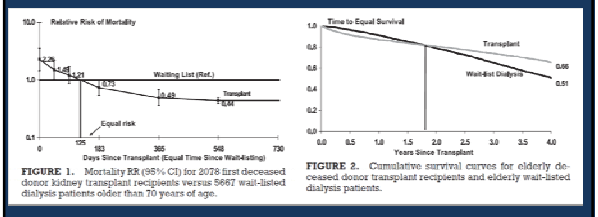


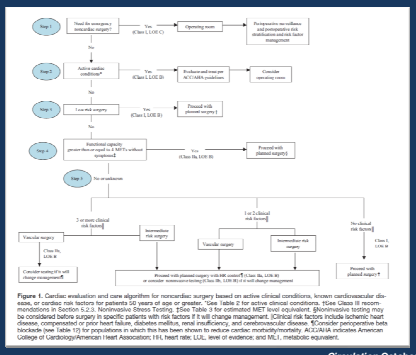
FIGURE 1. Mortality RR (95% CI) for 2078 first deceased donor kidney transplant recipients versus 5667 wait-listed dialysis patients older than 70 years of age.

FIGURE 2. Cumulative survival curves for elderly deceased donor transplant recipients and elderly wait-listed dialysis patients.

Transplantation 2007;83: 1069–1074

12

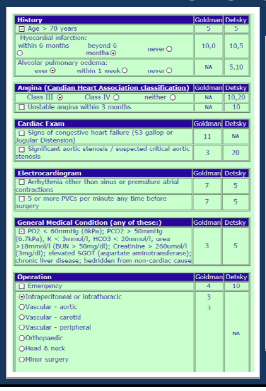
Risk Stratification

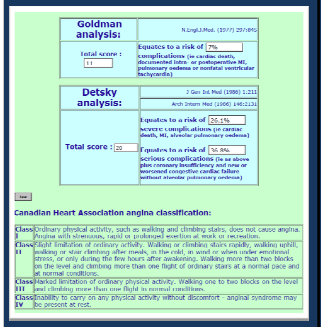


Circulation October 23, 2007

13

Risk Stratification





http://www.vasgbi.com/riskdetsky.htm, accessed 8/25/11

14

What If?

- The patient is having problems with dialysis…
- The patient lives alone and has no support network…
- The patient's living donor is his 23-year-old great-grandson…

15

Case Discussion # 3

Joseph is a 7 month old white infant with posterior urethral valves at birth, renal failure since birth, on maintenance peritoneal dialysis

He has a G-tube for overnight feeds, has maintained growth at 5th percentile

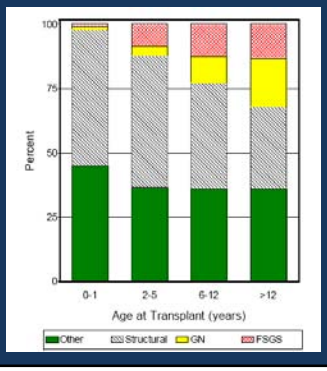
Normal developmental milestones so far

Questions

- How does diagnosis differ in pediatrics versus adults? Age distribution of recipients?
- Continue dialysis or refer for transplant?
  - Relative survival and quality of life in children
- Allocation issues
- How early can you transplant a kidney? Best time?
  - Size and surgical issues
  - Developmental issues
- Outcomes by age?

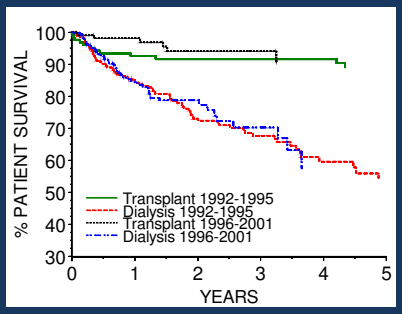
Primary Diagnosis by Age

- Structural lesions (congenital, urological) account for a third of ESRD in children
- Glomerulopathies are more common in older children
- About 5-10% are unknown ESRD cause
- Compare to adults, where diabetes and hypertension are the two leading causes of ESRD



NAPRTCS 2008

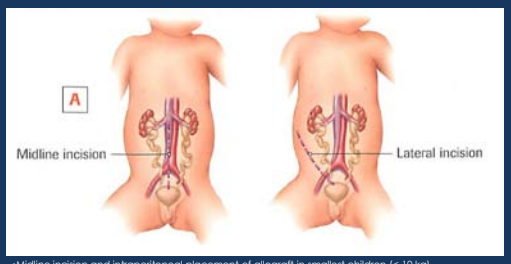
Transplantation vs. Dialysis Infant Patient Survival



Current UNOS Policies-Pediatric Kidney (October 2005)

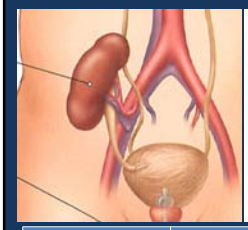
- Children < 18 years age: priority for donors < 35 years age
- Prevent expanded donor kidney from going to children
- Elimination of time goal policies that did not work
- Elimination of additional pediatrics points except for zero HLA mismatch

Surgical issues-1



- Midline incision and intraperitoneal placement of allograft in smallest children (< 10 kg)
  - Kidney can migrate later, biopsy is more difficult
- Standard oblique flank incision and extraperitoneal placement in older children > 30 kg, same as adults

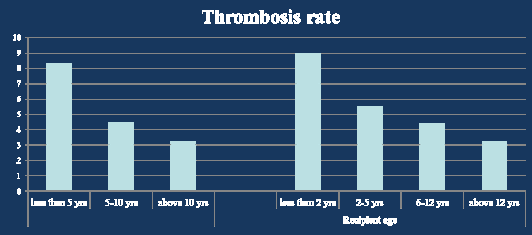
Surgical issues-2



- Strict attention to intravascular volume at time of clamp release and immediate post-operatively
- Risk of thrombosis

	Adults	Children
Vascular anastomosis	Iliac vessels	Aorta and IVC
Blood volume	5000 ml	800 ml
Blood flow	1000 ml/min (renal artery)	330 ml/min (aorta)

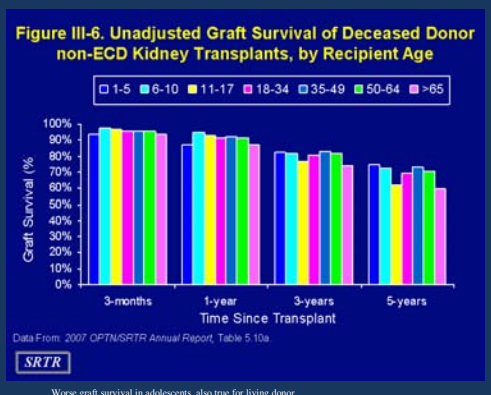
Surgical issues-3



- Practice changed: avoid small kidneys to small recipients; perform *en bloc* instead (superior results)

Singh et al, Transplantation, 1997

Dharnidharka et al. AJT, 2006



Worse graft survival in adolescents, also true for living donor

Fellows Symposium on Transplantation Medicine

Friday, September 23
4:20 pm - 5:30 pm

Indications for SOT: Liver

Breakout Session

Kimberly Brown, MD, Roy D. Bloom, MD and Shawn J. Pelletier, MD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

Indications For Liver Transplantation

Liver Transplantation Definition

- **Treatment for patients with progressive, irreversible liver disease in whom conventional forms of medical therapy no longer offer prospects for prolonged survival**

Liver Transplantation: Milestones

- 1963 First Liver Transplant by Dr. Thomas Starzl
- 1979 Introduction of Cyclosporine: one year survival improves from 45 to 80%
- 1983 NIH Conference: "Liver transplantation is a therapeutic modality for end-stage liver disease."
- 1989 First Successful Living-Related Liver Transplant
- 2000 Longest Liver Transplant Patient Dies: 28 years
- 2000 DHHS "Final Rule" policy effective (de-emphasizes waiting time, emphasizes mortality risk)
- 2001 Validation of new "MELD" model for allocation
- 2002 MELD implemented
- 2005 "Share 15" implemented

Liver Transplantation: Challenges

- Patient Selection
  - Who really benefits?
- Organ Availability
  - Final Rule
  - Too Many Too Few
  - MELD
  - Pushing the Envelope
- The Burdens of Success
  - Medical Consequences
  - Recurrent Disease

Patient Selection

Does everyone with cirrhosis need a new liver?

Liver Transplantation Indications

- Viral hepatitis
- Inherited Liver Ds
- Autoimmune
- Cholestatic
- Alcohol
- Biliary Atresia
- Recurrent disease with graft failure following liver transplantation
- Fulminant failure
- Primary non-function
- Tumors
- Benign Disease

Roy Bloom, MD, Kimberly A. Brown, MD, and Shawn J. Pelletier, MD

Indications for Liver Transplant

- Acceptable
  - Advanced chronic liver disease with decompensation
  - Fulminant hepatic failure
  - Inherited metabolic liver disease
- Controversial
  - Acute alcoholic liver disease
  - HIV
  - Chronic hepatitis B
  - Unresectable hepatic malignancy
  - Benign Conditions of the Liver

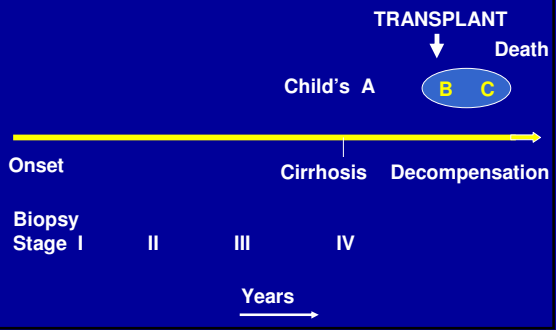
Relative Contraindications to Liver Transplantation

- Age > 65 (long-term survival decreased relative to younger patients)
- Severe malnutrition (BMI < 19-20 associated with decreased survival)
- Morbid obesity (BMI > 40)
- Other organ failure
- Previous upper abdominal surgery
- Poor functional status (can the patients rehab to recovery)

Absolute Contraindications to Liver Transplantation

- Brain death
- Extrahepatic malignancy
- Active untreated sepsis
- AIDS
- Advanced cardiopulmonary disease
- Anatomic anomaly or extensive vascular thromboses precluding transplant
- Active alcoholism or substance abuse
- Unresolved psychosocial issues

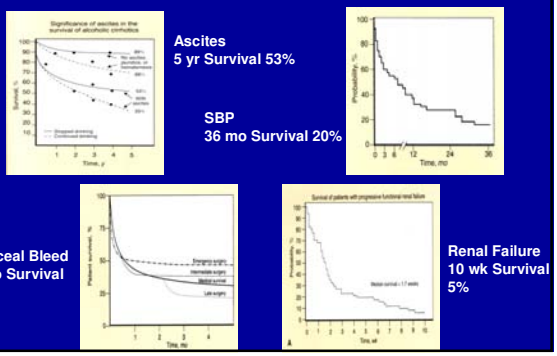
Progression of Liver Disease



Severity of Disease

Clinical	Biochemical
• Refractory ascites	• Prothrombin time
• Variceal bleeding	• Bilirubin
• Encephalopathy	• Albumin
• Spontaneous Bacterial Peritonitis	
• Renal Failure	
• Nutritional status	
• Fatigue, Puritis, Inability to work	

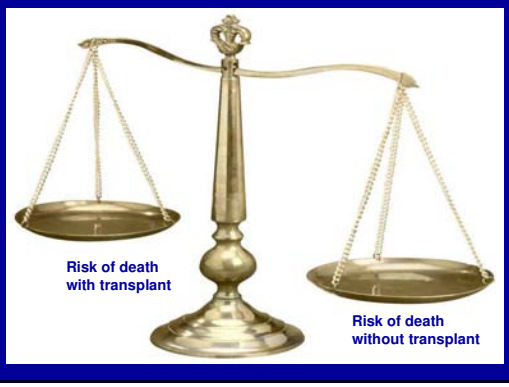
Impact of Clinical Factors on Survival



Factors Contributing to Decompensation

- Fixed
  - Functional (encephalopathy, bleeding, wasting)
    - **Reduced hepatocyte volume**
  - Mechanical (portal hypertension, ascites)
    - **Scar**
- Reversible
  - Functional (eg. Drug, acute fatty liver pregnancy)
    - **Hepatocyte dysfunction**
  - Mechanical
    - **Edema (acute hepatitis due to virus, alcohol)**
    - **Scar (patients treated for hepatitis C, autoimmune)**

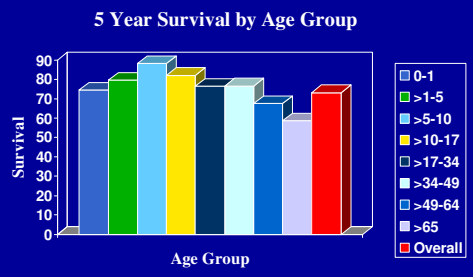
Transplantation: Patient Selection



Factors Increasing the Risks of Liver Transplantation

- Increasing Age
- Renal Failure
- Prior Hepatic Surgery/Transplant
- Cardiac/Pulmonary disease
- Diabetes
- Previous Malignancy
- Hepatitis C

Patient Survival by Age



UNOS 2008

Survival After Liver Transplantation By Diagnosis

Diagnosis	Survival (%) 1 yr	5 yr
Non cholestatic	82.8	66.3
Cholestatic	86	72.8
Acute Liver Failure	74.4	63.3
Biliary Atresia	83.3	75.3
Metabolic	85.9	75
Malignancy	81.9	61.9

SRTR Database
Annual Report 2007

Acute Liver Failure

- Characterized by the development of liver faiure (coagulopathy, jaundice, encephalopathy/coma) in the absence of chronic liver disease
- 5-6% of all liver transplants
- Tylenol leading etiology for ALF
- Idiosyncratic drug reaction leading etiology of sub-fulminent failure

Criteria for Liver Transplantation in Fulminant Hepatic Failure

- King's College Criteria
  - Acetaminophen
    - pH < 7.3 or
    - INR > 6.5 and Creatinine > 3.4
  - Nonacetaminophen
    - INR > 6.5 or
    - Any three of the following
      - 1. Age < 10 or > 40
      - 2. Etiology: NANB, Halothane, Idiosyncratic drug reaction
      - 3. Duration of jaundice before encephalopathy > 7 days
      - 4. INR > 3.5
      - 5. Bilirubin > 17.5

Criteria for Liver Transplantatin in Fulminant Hepatic Failure

- Paul-Brousse Criteria
  - Hepatic encephalopathy and
    - Factor V < 20% in patient younger than 30 yo
    - Factor V < 30% in patient > 30 yo

Patient Selection

- No alternative therapy
- No absolute contraindication to liver transplantation
- Anticipated survival benefit
- Willingness and ability to accept liver transplantation and comply with follow-up care
- Ability to provide for cost of transplant and post transplant care

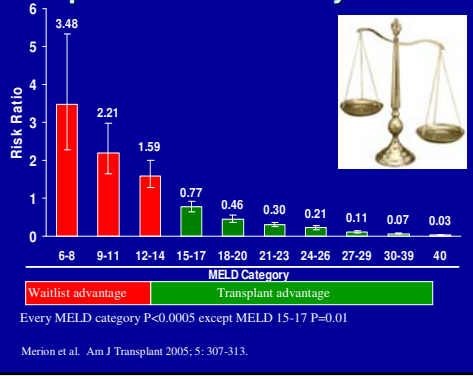
Final Rule

- DHHS (Department of Health and Human Services) issued the "Final Rule" in March 2000
- Replaced the local and regional organ allocation systems with 1 national distribution protocol
- considers the urgency of a recipient patient's need for an organ
- "organs should be distributed over as broad a geographic area as feasible"

MELD

- Prior to MELD, waiting time played a significant role in allocation
- Originally MELD developed to identify predictors of mortality in patients undergoing the TIPS procedure
- 231 patients showed Cr, Bili, INR, disease etiology as predictive
- Sept 2001, MELD elements became mandatory
- Applied model using original MELD parameters to 3,347 patients on OPTN list (4,219 patients in a secondary analysis
- Cr, Bili, INR remained significant but disease etiology did not

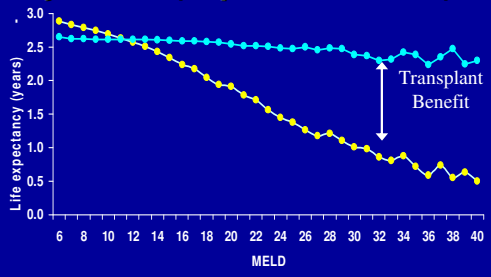
Relative Mortality Rates: Transplant vs. Waitlist By MELD Score



Every MELD category P<0.0005 except MELD 15-17 P=0.01

Merion et al. Am J Transplant 2005; 5: 307-313.

Mean 3-Year Future Lifetimes by MELD (separate models)



Liver Transplant Evaluation

- Hepatology, Surgery
- Psychosocial Team
- Financial Services
- Laboratory
  - Document etiology of liver disease
  - Virology (viral titers)
  - Infectious disease (VDRL, Hepatitis markers, PPD)
  - Exclude tumor (AFP, CEA)
- CXR, EKG, Dobutamine Echo
- Cardiology, Infectious Disease Consults
- Doppler US, CT/MRI of the Abdomen
- Stool Guaiac / Colonoscopy
- EGD to assess for varices
- Mammogram, Pap and Pelvic
- Dental
- Patient Family Meeting and Contract

Multidisciplinary Transplant Committee

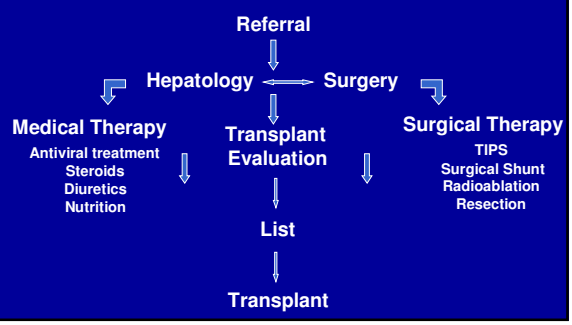
- A Multidisciplinary team of individuals working together to support the patient which includes transplant physicians and nurses and a variety of support services
- Provide an impartial review of information gathered on patients referred for transplant evaluation
- Render a decision on each patient with regards to the information gathered and selection criteria
- Identify areas in which patients may require further evaluation or assistance

Minimal Listing Criteria

- Immediate need for liver transplantation
- Estimated 1 year survival $\leq$ 90%
- Child-Pugh score $\geq$ 7 (B or C)
- Portal hypertensive bleeding or a single episode of spontaneous bacterial peritonitis

Lucey MR, Brown KA, Everson GT et al.
Minimal Listing Criteria for Liver Transplant
Liver Transpl Surg 1997

Summary



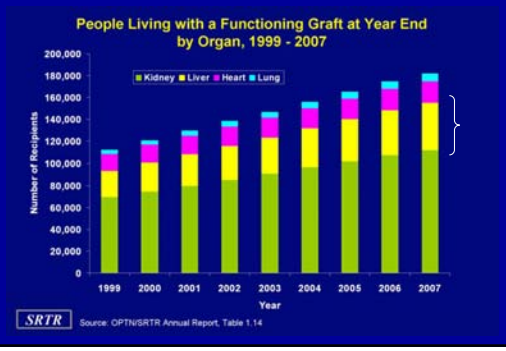
Liver Transplantation: Timing of Referral

- Ideal situation: Patient is referred when clinical or biochemical evidence suggests the patient is likely to develop serious complications within a year
- Remember: After referral, the patient will likely spend 2-4 weeks in the evaluation process and up to several months on the list
- Patients with chemical dependency issues may be asked to exhibit a defined period of abstinence or complete a treatment program in addition to the evaluation and listing times
- THE EARLIER THE BETTER!

The Burden of Success

Challenges for the future

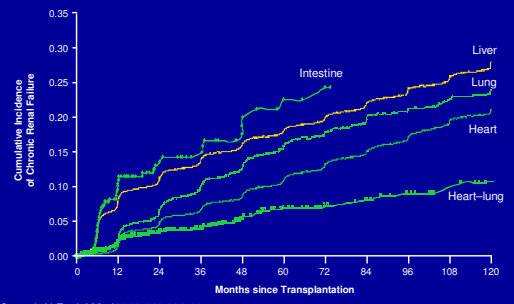
The Burden of Success



Medical Complications

- The only thing we fix with liver transplantation is the liver
- Most diseases patients have actually worsen with transplantation and immunosuppression
  - Diabetes
  - Hypertension
  - Hyperlipidemia
  - Bone Disease
  - Gout
  - Malignancy
  - Renal Disease

Renal Insufficiency Following Solid Organ Transplantation



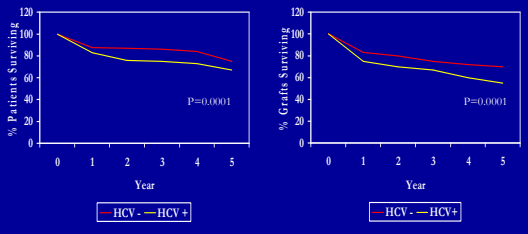
Ojo AO, et al. *N Engl J Med* 2003;349:931-40.

Transplant Diagnosis

	N	%
Hepatitis C/Non-A Non-B*	4,016	**24.8**
Alcoholic liver disease*	3,785	**23.4**
Cryptogenic cirrhosis	1,889	11.7
Primary biliary cirrhosis	1,785	11.0
Primary sclerosing cholangitis	1,602	9.9
Hepatitis B	916	5.7
Autoimmune diseases	868	5.4
Malignancies	648	4.0
Metabolic disorders	605	3.7

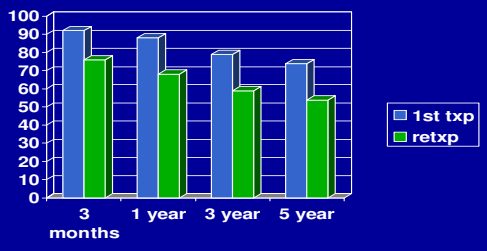
Excludes acute liver failure
*Includes 558 recipients with ALD and Hepatitis C/NANB (>20,000 recipients reported in the UNOS database)

Impact of HCV on Transplant Survival



Forman LM et al. Gastroenterology 2002;122(4): 889-96
UNOS Database

Patient Survival: Effect of Retransplant



Liver Transplantation Summary

- Prolongs life in properly selected patients
- Patient selection remains paramount
- Organ shortage remains a problem
- Expansion of donor pool, maximizing donor consent will continue
- Organ allocation methodology will likely move toward wider distribution to minimize wait list deaths
- Continued focus on maximizing patient outcomes and minimizing effects of immunosuppression

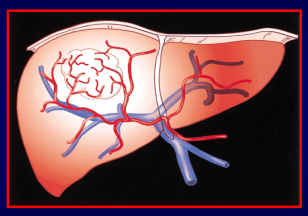
Evaluation and Treatment of Hepatocellular Carcinoma

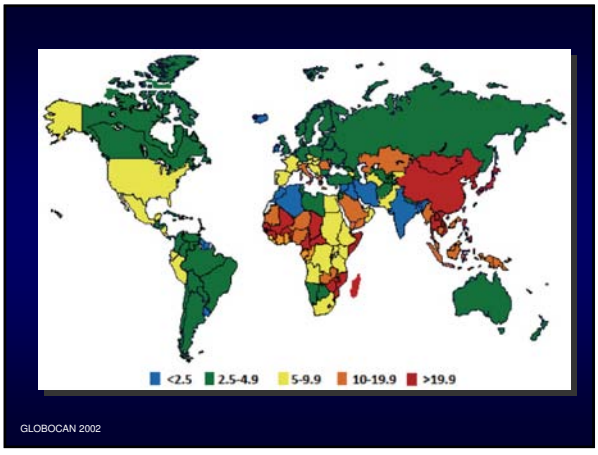
Shawn Pelletier, MD
Section of Transplant Surgery

University of Michigan Health System

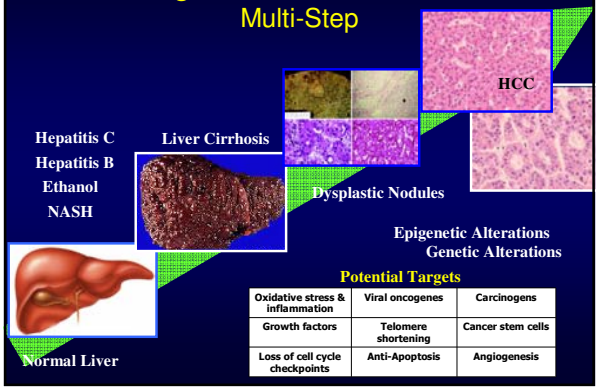
Topics

- Epidemiology
- Early Detection
- Diagnosis
- Treatment

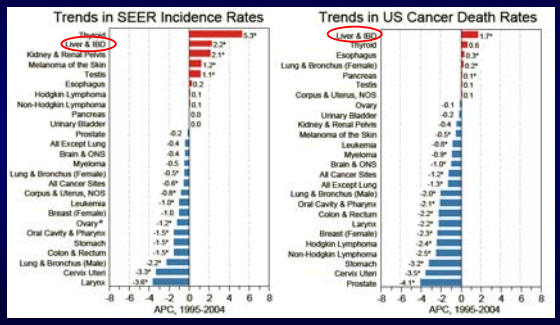




Malignant Transformation
Multi-Step

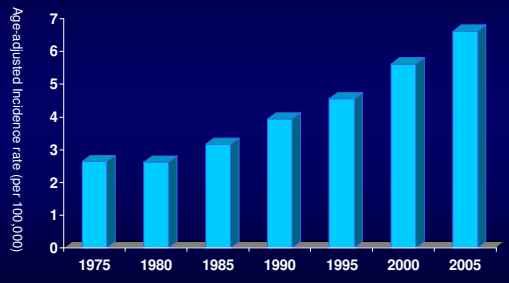


Trends in Incidence and Death Rates 1995-2004



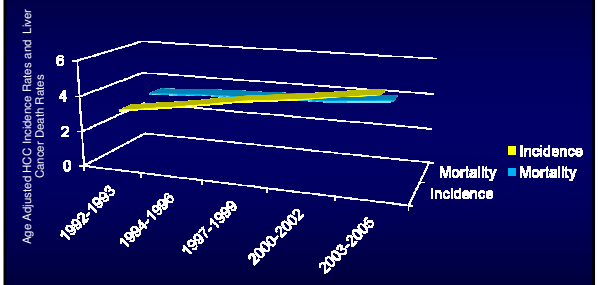
www.seer.cancer.gov

Age-Adjusted Incidence Rates for HCC by Year



www.seer.cancer.gov

HCC Incidence and Mortality



Altekruse, S. JCO. 2009 Mar 20;27(9):1485-91

Overall Survival and Staging of Patients with HCC: SEER Database

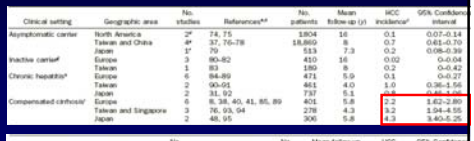
Parameter	92-93	94-96	97-99	00-02	03-04
Survival (%)					
1-yr	25	29	34	40	49
3-yr	11	14	18	24	35
5-yr	8	10	13	20	*
Stage at Diagnosis (%)					
Localized	28	30	33	40	45
Regional	22	26	28	30	29
Distant	22	21	19	18	17

Altekruse, S. JCO. 2009 Mar 20;27(9):1485-91

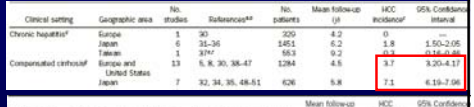
Early Detection

Incidence of Hepatocellular Carcinoma in Cirrhosis

Hepatitis B



Hepatitis C



Alcohol

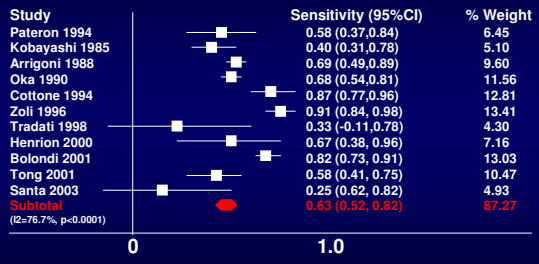
Fattovich G, et al. Gastroenterology 2004;127:S35

Surveillance for HCC Improves Mortality: A Randomized Controlled Trial

	Screened Group	Control Group
Person-years F/U	38,444	41,077
HCC Occurrence		
HCC cases	86	67
Incidence	223.7	163.1
Rate Ratio	1.37 (0.99-1.89)	
Deaths from HCC		
Number	32	54
Mortality Rate	83.2	131.5
Rate Ratio	0.63 (0.41-0.90))	

Zhang BH, et al. J Cancer Res Clin Oncol 2004;130:417

Ultrasound Surveillance in Early HCC: Systematic Review



- AFP improve detection to 70%
- Every 6 months significantly better than 12 months

Singal A, et al. APT 2009

Utilization of Surveillance for HCC: Population-based

Overall (%)	Regular Surveillance* (%)	Inconsistent Surveillance† (%)	No Surveillance (%)
1,873 (100.0)	321 (17.1)	710 (38.0)	842 (44.9)

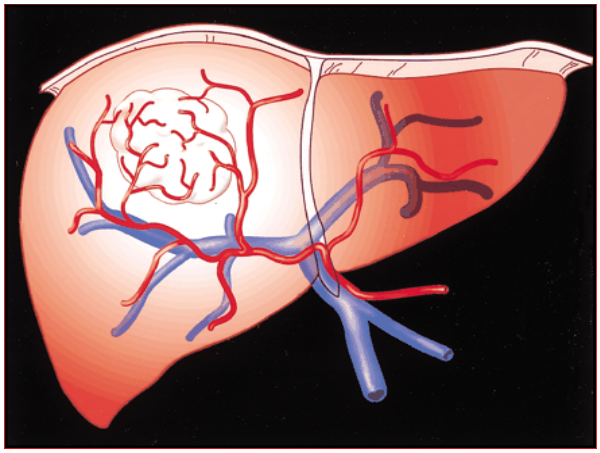
- Regular: 52% US and AFP; 46% AFP only and 2% US only
- GI/Hepatologist or academic affiliation increase likelihood 4.5-fold and 2.8-fold, of regular surveillance

Davial JA, et al. Hepatology 2010

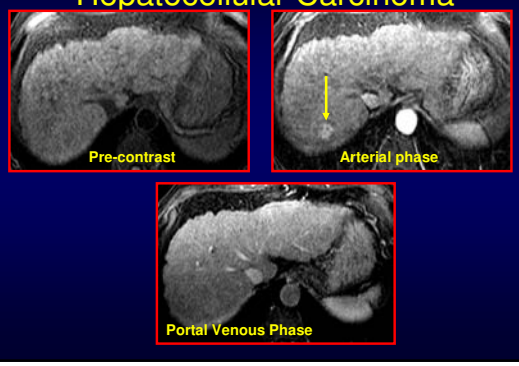
Diagnosis

Evaluation

- Detailed history
  - age, gender, history of cancer, steroid use, exposure (vinyl choride)
  - History of liver disease
- Physical Examination
  - palpable mass, fever, ascites, stigmata of liver disease, bruit in RUQ
- Laboratory data
  - evidence of chronic liver disease, evidence of hematologic disease, tumor markers (CEA, AFP)



Triple Phase Imaging of Hepatocellular Carcinoma

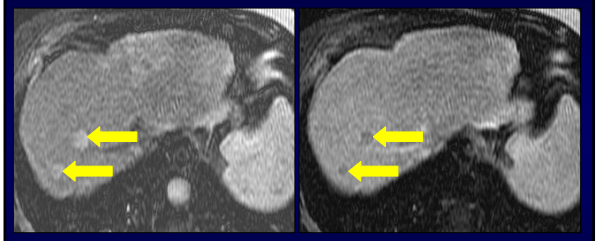


Importance of Contrast Washout of an Arterially Enhancing Mass

Variables	Odds Ratio (95%CI)
All patients (n=124)	
AFP > 20 ng/ml	11.7 (2.3-30.7)
Washout	61 (3.8-73)
< 2 cm only (n=35)	
Washout	6.3 (1.8-13)

Marrero JA, et al Liver Transplant 2004

Contrast Washout in HCC



Arterial Phase | Portal Venous Phase

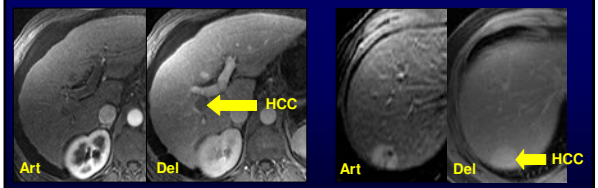
MRI versus CT in Diagnosis of HCC in Cirrhosis

Gold Std	No. Pts	No. Nodules	HCC (n)	CT (%)		MRI (%)	
				Sens	Sp	Sens	Sp
Explant	34	88	54	51	84	61	93
Explant	43	69	13	53	92	77	58
Explant	50	127	76	61	66	76	75
Explant	49	136	77	50	79	70	82

Burrel M, et al. Hepatology 2003;38:1034
Rode A, et al. J Comput Assist Tomogr 2001;25:327
de Ledinghem V, et al. Eur J Gastro Hep 2002;14:159
Libbrecht L, et al. Liver Transpl. 2002 Sep;8(9):749

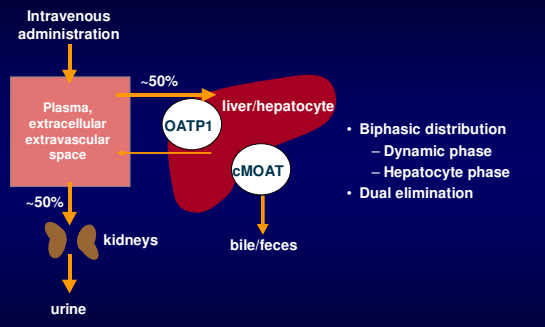
Atypical Hepatocellular Carcinoma

- 85% of HCC > 2cm have "washout"(1)
- Some lesions are atypical
- Biopsy is important for these lesions



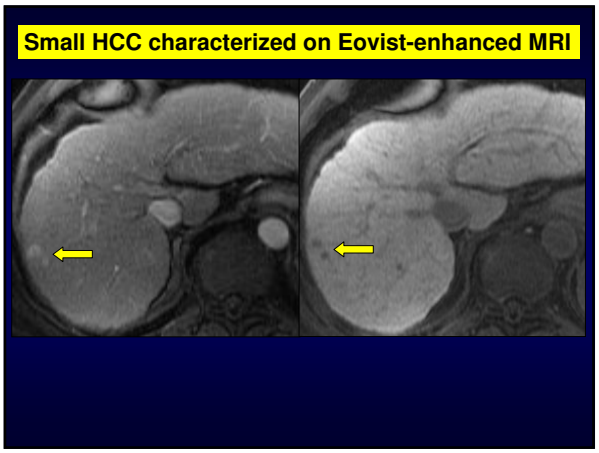
Bolondi L, et al, Hepatology 2002; 42 (1):27-34

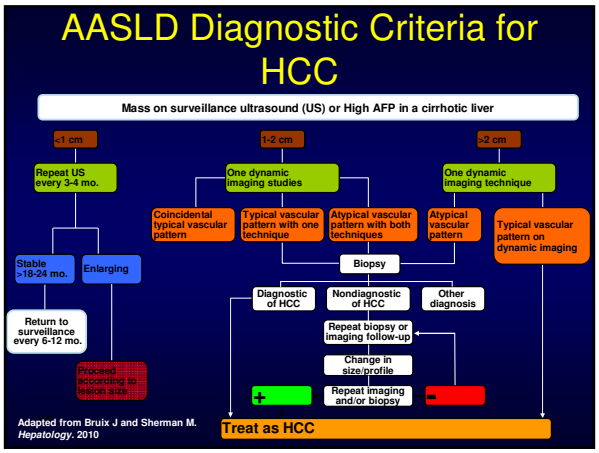
Eovist: Distribution and Elimination



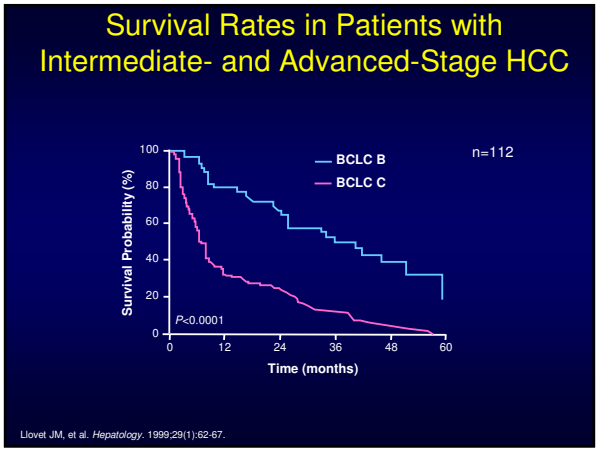
- Biphasic distribution
  - Dynamic phase
  - Hepatocyte phase
- Dual elimination

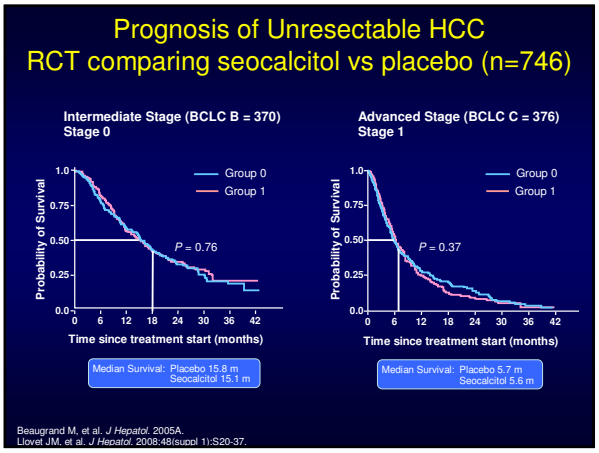
OATP1 = organic anion transporting polypeptide 1 (active, ATP dependent)
cMOAT = canalicular multi-organic anion transporter

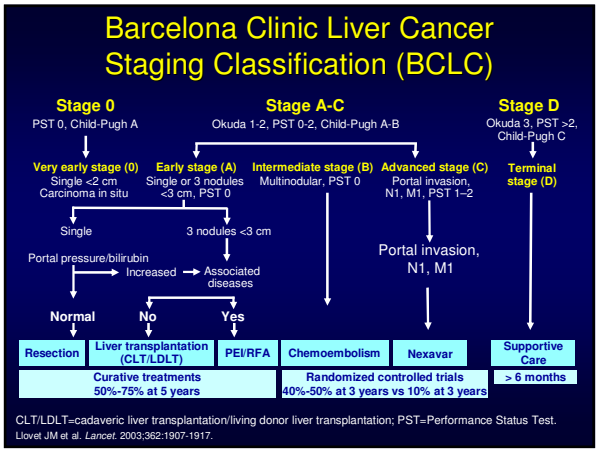

Small HCC characterized on Eovist-enhanced MRI


AASLD Diagnostic Criteria for HCC
Mass on surveillance ultrasound (US) or High AFP in a cirrhotic liver
<1 cm
1-2 cm
>2 cm
Repeat US every 3-4 mo.
One dynamic imaging studies
One dynamic imaging technique
Coincidental typical vascular pattern
Typical vascular pattern with one technique
Atypical vascular pattern with both techniques
Atypical vascular pattern
Typical vascular pattern on dynamic imaging
Stable >18-24 mo.
Enlarging
Biopsy
Return to surveillance every 6-12 mo.
Diagnostic of HCC
Nondiagnostic of HCC
Other diagnosis
Repeat biopsy or imaging follow-up
Proceed according to lesion size
Change in size/profile
+
Repeat imaging and/or biopsy
-
Adapted from Bruix J and Sherman M. Hepatology. 2010
Treat as HCC

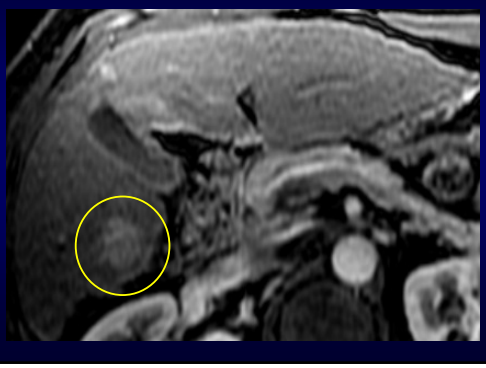

Treatment


Survival Rates in Patients with Intermediate- and Advanced-Stage HCC
n=112
BCLC B
BCLC C
Survival Probability (%)
100
80
60
40
20
0
P<0.0001
0
12
24
36
48
60
Time (months)
Llovet JM, et al. Hepatology. 1999;29(1):62-67.


Prognosis of Unresectable HCC
RCT comparing seocalcitol vs placebo (n=746)
Intermediate Stage (BCLC B = 370)
Stage 0
Advanced Stage (BCLC C = 376)
Stage 1
Probability of Survival
Group 0
Group 1
P = 0.76
P = 0.37
Time since treatment start (months)
Median Survival: Placebo 15.8 m
Seocalcitol 15.1 m
Median Survival: Placebo 5.7 m
Seocalcitol 5.6 m
Beaugrand M, et al. J Hepatol. 2005A.
Llovet JM, et al. J Hepatol. 2008:48(suppl 1):S20-37.


Barcelona Clinic Liver Cancer Staging Classification (BCLC)
Stage 0
PST 0, Child-Pugh A
Stage A-C
Okuda 1-2, PST 0-2, Child-Pugh A-B
Stage D
Okuda 3, PST >2, Child-Pugh C
Very early stage (0)
Single <2 cm
Carcinoma in situ
Early stage (A)
Single or 3 nodules <3 cm, PST 0
Intermediate stage (B)
Multinodular, PST 0
Advanced stage (C)
Portal invasion, N1, M1, PST 1–2
Terminal stage (D)
Single
3 nodules <3 cm
Portal pressure/bilirubin
Increased
Associated diseases
Portal invasion, N1, M1
Normal
No
Yes
Resection
Liver transplantation (CLT/LDLT)
PEI/RFA
Chemoembolism
Nexavar
Supportive Care
Curative treatments
50%-75% at 5 years
Randomized controlled trials
40%-50% at 3 years vs 10% at 3 years
> 6 months
CLT/LDLT=cadaveric liver transplantation/living donor liver transplantation; PST=Performance Status Test.
Llovet JM et al. Lancet. 2003;362:1907-1917.

Very Early HCC

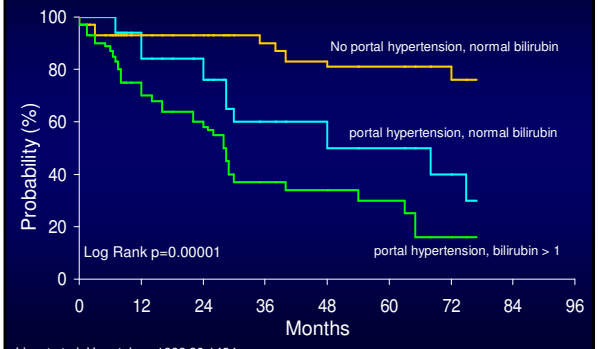


Resection for HCC

Author	N	Survival (%) 1-year	5-year
Takayama '98	52	92	54
Fong '99 (<5 cm)	100	83	42
Llovet '99 (< 5 cm)	35	85	51
Arii '00			
< 3 cm	767	96	54
3-5 cm	587	95	38
Zhou '01	1000		62
Poon '02	161	79	44
Ikai '04			
< 3 cm	2320	83	66
3-5 cm	5956	70	53
5-10 cm	1946	53	37
> 10 cm	819	44	32

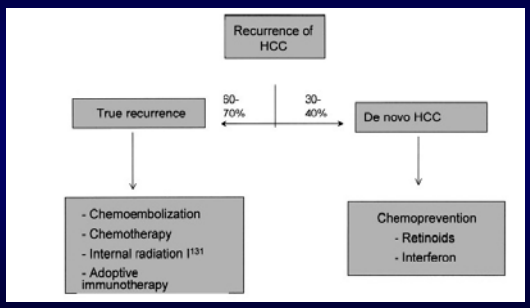
Llovet JM, et al. Semin Liver Dis. 2005;25:181

Survival of HCC with Resection



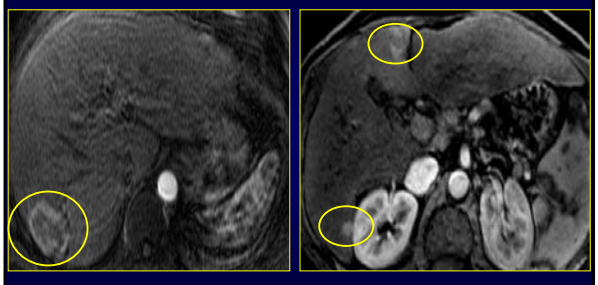
Llovet et al, Hepatology 1999;30:1434

Hepatic Resection 5-Year Recurrence



Llovet JM, et al. Semin Liver Dis. 2005;25:181

Early HCC

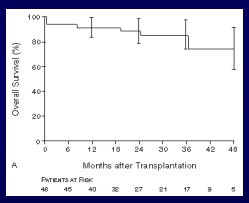


4.7 cm

2.3 cm/1.2 cm

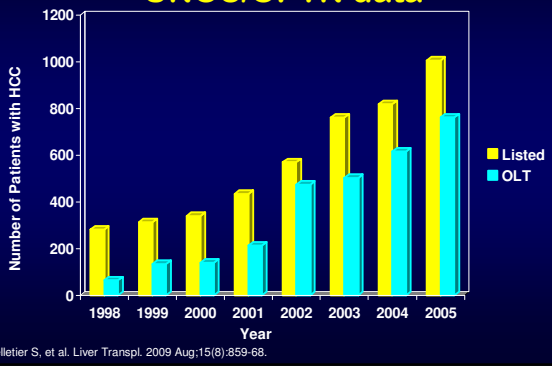
Liver Transplantation for Early Stage HCC

48 patients with unresectable HCC but < 5 cm
Milan Criteria:
Single lesion < 5 cm
< 3 lesions < 3 cm
- **Actuarial survival at 4 yrs 75%**
- **8% recurred**

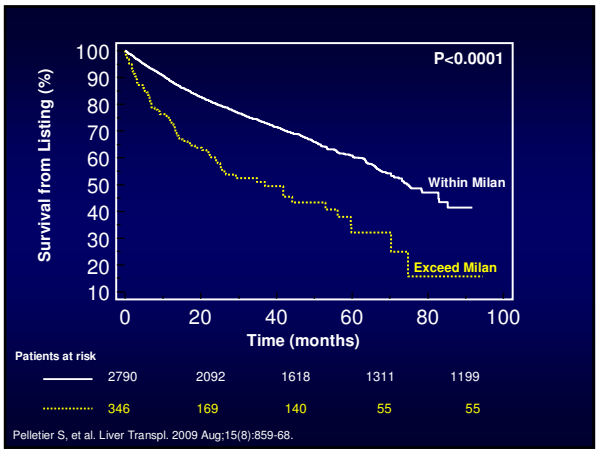


Mazzafero V, et al NEJM 1996; 334:693

Liver Transplantation for HCC: UNOS/OPTN data

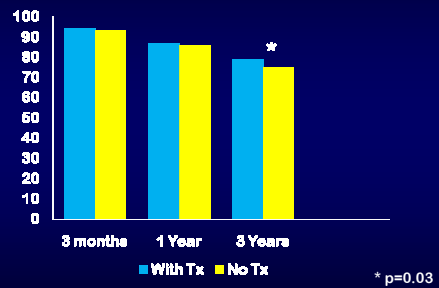


Pelletier S, et al. Liver Transpl. 2009 Aug;15(8):859-68.



Pelletier S, et al. Liver Transpl. 2009 Aug;15(8):859-68.

Post Transplant Survival of Treatment vs. No Treatment



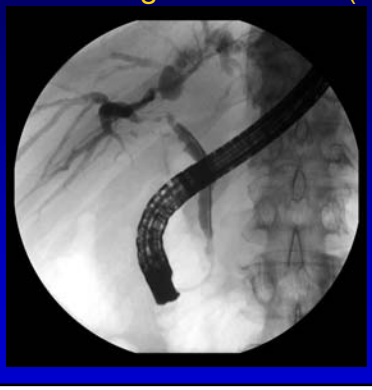
Freeman RB, et al. Am J Transplant 2008; 8(4 Pt 2):958-76.

Conclusions

- HCC is on the rise mostly due to Hepatitis C and Fatty Liver Disease
- There is excellent therapy for patients with HCC
- Curative therapy includes resection, liver transplantation and some patients with RFA
- Nexavar is the treatment of choice for advanced HCC
  - Further studies as adjuvant are needed
  - New agents are being studies

Hilar Cholangiocarcinoma: Resection Versus Transplantation

Hilar Cholangiocarcinoma (CCA)

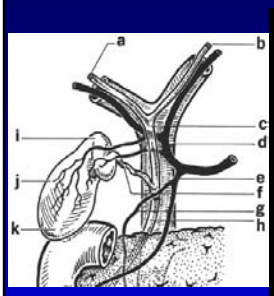


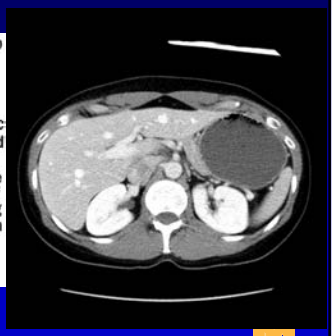
Hilar Cholangiocarcinoma (CCA)

- 1-2 per 100,000 in the U.S.
- >50% of patients greater age 65
- Majority extrahepatic CCA at the hilum
- Risk factors: PSC, choledochal cysts, hepatolithiasis, *Clonorchis*, *Opisthorchis*, other chemicals/toxins?
- PSC: 8 – 20 % incidence

Greenlee et al, CA Cancer J Clin, 2001
Broome et al, Gut, 1996
Nashan et al, Hepatology, 1996

Anatomical Considerations





Resection of Hilar CCA

- Majority of hilar CCA patients unresectable either at evaluation (~25-30%) or exploration (~35%).
- Resection with negative margins (R0) provides best hope for survival
- R0 achievable in 76-80%

Resection of Hilar CCA

- Hepatic Lobectomy and caudate resection, portal lymphadenectomy, bile duct resection
- Vascular resection 11-35%
- R0 achievable in 76-80%
- Mortality 9-10%
- Complications ~40%

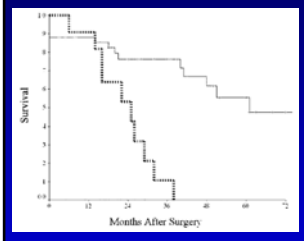
Resection of Hilar CCA

	Hepatic Resection (n = 62)	No Hepatic Resection (n = 18)
Histopathology		
Negative margin	52 (84%)*	10 (56%)*
Tumor size (cm)	2.8 ± 1.5†	2 ± 0.8†
Tumor > 2.5 cm	27 (44%)	3 (17%)
Well differentiated	20 (32%)	10 (56%)
Node positive	16 (26%)	3 (17%)
Papillary tumor	8 (13%)†	6 (33%)†
Perioperative Results		
Estimated blood loss (mL)		
Mean ± SD	1,045 ± 850	622 ± 366
Median (range)	850 (130–7,000)	450 (300–1,200)
Number (%) transfused	32 (52%)	5 (28%)
Total units transfused		
Mean ± SD	5.3 ± 13	2.7 ± 7
Median (range)	2 (0–71)	0 (0–25)
Hospital stay (days)		
Mean ± SD	15 ± 7	13 ± 8
Median (range)	14 (6–49)	12 (7–37)
Complications	42 patients (68%)	9 patients (50%)
Surgical deaths	7 (11%)	1 (6%)
Survival		
Median (mo)	46§	28§
5-year (actuarial)	37%	0%
5-year (actual)	39% (9/23 at risk)	0% (0/7 at risk)

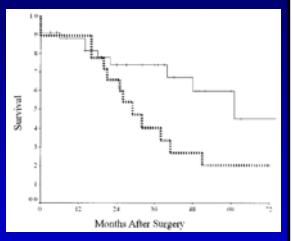
For R0 pts: Concomitant hepatectomy independent predictor of survival

Jarnagin et al, Ann Surg, 2001

Resection Outcomes



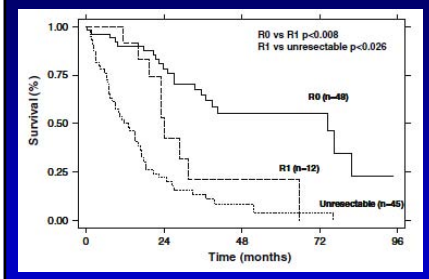
R1 vs. R0



LN+ vs. LN-

Hemming et al, Ann Surg, 2005

Resection Outcomes



LN-, R0 patient survival 85% at 5 years (median not reached)

Rocha et al, J Hepatobiliary Pancreat Surg, 2009

Determinants of Resection Blumgart Staging

- **T1**: +/- unilateral extension to 2nd order biliary radicals
- **T2**: +/- unilateral extension to 2nd order biliary radicals **and** *ipsilateral* PV +/- *ipsilateral* hepatic atrophy
- **T3**: + bilateral extension to 2nd order biliary radicals; **or** *unilateral* extension to 2nd order biliary radicals with *contralateral* PV/lobar atrophy; **or** main/bilat PV

Jarnagin et al, Ann Surg, 2001

Other Determinants of Resection

- Margin of resection
- Future Liver Remnant
  - 25-30% for normal liver function
  - 40% for liver disease (steatosis, fibrosis)
- Adequate Hepatic Compensation
- Freedom from Cholangitis
- Medical Fitness for Major Surgery

Hemming et al, Ann Surg, 2005
Jarnagin et al, Ann Surg, 2001

Initial Liver Transplant (OLT) Outcomes for Hilar CCA

- "Incidental" CCA/OLT outcomes poor
- Early studies poorly controlled (stage, ICC vs. ECC)—no 5 year survivors for ICC
- Goss et al (UCLA): retrospective 10 pts, LN-, hilar CCA < 1cm, 83% 5 yr survival
- Iwatsuki et al (Pitt.): 27 pts, larger tumors, 36% 5 yr survival
- Organ cluster transplantation (Multivisc.) 9 – 38% 5 yr. survival

Goss et al, Ann Surg, 1997
Iwatsuki et al, J Am Coll Surg, 1998
Reviewed in Singal et al, Expert Rev Anticancer Ther, 2009

Neoadjuvant Chemoradiotherapy

Univ. Pitt. survival	9 pts	65% 5 yr
Univ. of Neb.	11 pts	45% 3 yr survival
Mayo Clinic	65 pts	76% 5 yr survival

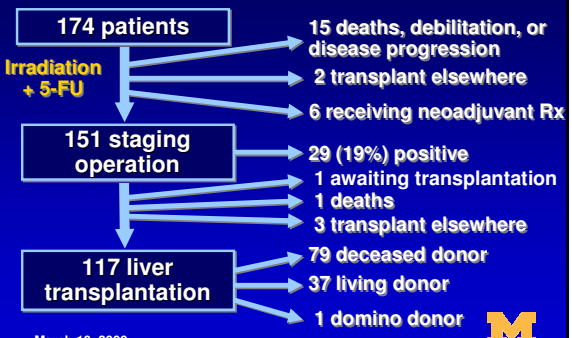
Patients with LN negative disease (assessed by laparotomy)

tumors less than 3 cm

Mayo Clinic Protocol

- External Beam Radiation Therapy (4000 – 4500 cGy)
- Brachytherapy (2000 – 3000 cGy)
- IV 5-FU/PO Capecitabine
- Abdominal Exploration/Staging
- Liver Transplantation

Cholangiocarcinoma Treatment Protocol Results: 1993 – 2009



March 10, 2009

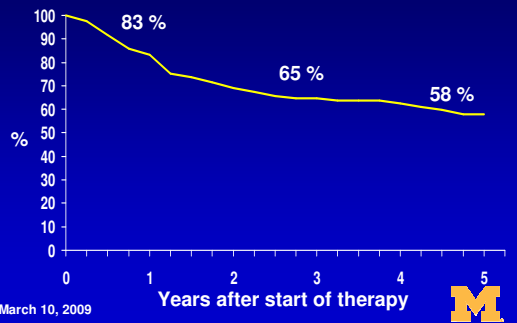
Adapted from Charles B. Rosen, MD

Endoscopic Ultrasound

- EUS (with regional lymph node aspiration) prior to enrollment added in 2002
- Avoids neoadjuvant therapy for many patients destined to fall-out at operative staging
  - 30 – 40% staged positive prior to EUS
  - 10 – 15% stage positive with EUS
- *EUS guided aspiration of the primary tumor causes seeding and should not be done*

Adapted from Charles B. Rosen, MD

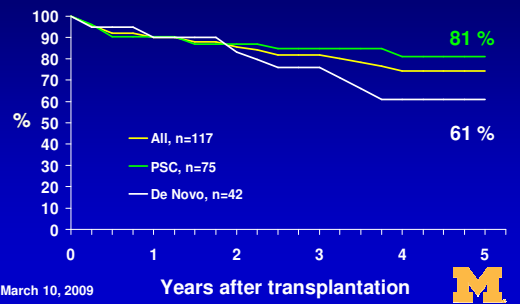
Patient Survival After Start of Therapy 1993 – 2009 n=174



March 10, 2009

Adapted from Charles B. Rosen, MD

Patient Survival After Transplantation 1993 – 2009 n=117



March 10, 2009

Adapted from Charles B. Rosen, MD

Predictors of Recurrence

Clinical Factors

- Older patient age
- Prior cholecystectomy
- CA-19.9 > 100 at transplant
- Visible mass on imaging
- Prolongation of waiting time

Pathological Factors

- *Residual* CCA > 2 cm
- High grade histology
- Perineural invasion

Transplantation 2006; 82:1703

Adapted from Charles B. Rosen, MD

Pathological Confirmation of Diagnosis Explant Pathology and Recurrence

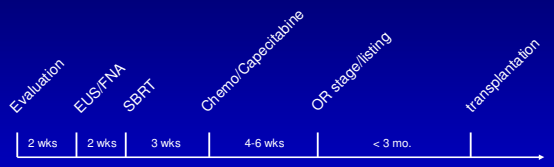
Pathological Confirmation	Number	Residual CCA in Explant*	Recurrence after Transplantation**
No	38	15 (39%)	7 (18%)
Suspicious	22	10 (45%)	1 (5%)
Yes	57	32 (56%)	7 (12%)

No/Suspicious vs Yes (Chi-square): *p=0.13, **p=0.53

March 10, 2009

Adapted from Charles B. Rosen, MD

CCA Protocol



Inclusion Criteria

-Hilar cholangiocarcinoma (CCA) not resectable as determined by surgical criteria and review in the Multidisciplinary Liver Tumor Clinic and Board

-Hilar CCA in the presence of primary sclerosing cholangitis (PSC) such that resection is high risk

-Malignant appearing biliary stricture at hilum *and* CA19-9 > 100 ng/mL, transcatheter biopsy or brush cytology positive for carcinoma, or associated mass on cross-sectional imaging.

Exclusion Criteria

-Intrahepatic metastasis or satellite lesions
-Extrahepatic or lymph node metastasis
-Intrahepatic CCA
-Attempt at prior resection or biliary resection
-Tumor diameter greater than 3 cm.
-*Previous transperitoneal biopsy of the primary tumor (including EUS)*
-Previous chemotherapy or radiation therapy
-Uncontrolled infection

Evaluation and Staging

- 3 phase MRI abd/pelvis or CT abd/pelvis required
- CT chest required
- ERCP or PTC as appropriate, brush cytology or biopsy
- PET to be used *selectively (not as screening)* to clarify lesions identified on other cross-sectional exams
- EUS with FNA of choledochal and hepatic artery lymph nodes or other hilar lymph nodes visualized. *No* transperitoneal or EUS *biopsy* of primary tumor. Performed *before* chemoRT.
- Laparoscopic assisted exploration at completion of RT (4-6 weeks after initiation)
  - biopsy lesions on peritoneal surfaces or liver
  - Hepatic Ultrasound
  - Excisional biopsy of choledochal LN and hepatic arterial LN

Neo-adjuvant Stereotactic Body Radiation Therapy (SBRT) and Chemotherapy

- SBRT in 3-5 fractions every other clinical working day, 10-20 Gy/fraction x 2 weeks (14 days).
- Dose adjustment/individualization based upon known tolerance for liver, stomach, duodenum, heart, spinal cord, kidneys, bowel
- Capecitabine 1330 mg/m2/day in two divided doses po (after meals).

Transplantation

- List for OLT and then apply for MELD exception (22 points) with regional review board once permanent sections from staging operation are negative
- Consider evaluation of potential living donors if allocation of deceased donor liver may be delayed
- CBD margin assessed by frozen section
- Pancreaticoduodenectomy (Whipple) performed if distal CBD margin positive
- Hepaticojejunostomy for biliary reconstruction

Conclusions

- Surgical resection (RO) remains the primary therapy for hilar CCA.
- Neo-adjuvant chemoradiotherapy followed by OLT has become an accepted therapeutic alternative if resection not possible.
- Emerging role for chemoradiotherapy in patients not eligible for resection or OLT?

Simultaneous Liver-Kidney Transplantation

Roy D. Bloom MD
University of Pennsylvania
September 2011

Outline

- Kidney function in the liver txp candidate
- Impact of MELD on SLK transplants
- Liver transplant outcomes in setting of abnormal kidney function
  - *Liver Alone (LTA)*
  - *Simultaneous Liver Kidney (SLK)*
- Selection of candidates for SLK

Assessment of Kidney Function in Liver Transplant Candidates

- Serum creatinine widely used
- Serum creatinine typically overestimates GFR
  - *Poor nutritional status*
  - *Weight loss*
  - *Reduced muscle mass and edema*
  - *Reduced creatinine generation*
- GFR calculating equations not validated

Kidney Function Equations: Inaccurate in Liver Transplant Candidates

Method	GFR<40 ml/min #	GFR	GFR>40 ml/min #	GFR
Iothalamat*	155	22.6	1218	99.4
Cockcroft-Gault	151	46.1	1213	85.5
Rankivell	148	58.0	1198	99.0
MDRD 4	155	44.5	1218	87.8
MDRD 5	155	43.9	1218	90.5
MDRD 6	155	39.0	1218	82.4

1447 OLT recipients, 1984-2001, *iothalamate GFR used as "gold-standard"

Gonwa et al. Liver Transplant. 2004:10:301-9.

Abnormal Kidney Function in Liver Transplant Candidates

- True prevalence unknown
- Common finding in the MELD era
- 3 Patterns of kidney dysfunction
  - *Acute Kidney Injury (AKI)*
  - *Chronic Kidney Disease (CKD)*
  - *Acute Kidney Injury Superimposed on Chronic Kidney Disease (AKI/CKD)*

Acute Kidney Injury in Liver Transplant Candidates

- Fluctuation in renal function common
- Common causes
  - *Hepatorenal syndrome (HRS)*
  - *Hypovolemia/underfilling*
  - *ATN*
- Some reversibility frequent post-txp
- Presence of AKI > 3 mos should be considered CKD, by K/DOQI definition

Chronic Kidney Disease in Liver Transplant Candidates

- Inadequately characterized

Specific renal diagnoses in SLK recipients	%
Diabetes	16.5
Glomerular disease	14.0
Prior renal txp	8.3
Polycystic kidney disease	8.1
Hypertension	6.1
Oxalosis	3.0
AKI and/or HRS	2.0
Not specified	38.5

From SRTR 4/1999 - 8/2004

Gonwa *et al*, Am J Transpl 2006

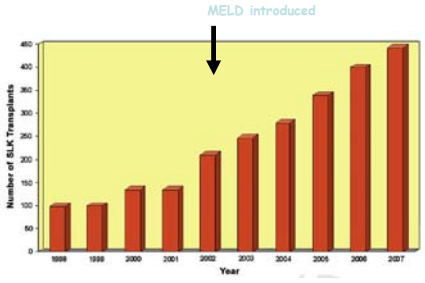
Abnormal Kidney Function is Increased in Liver Candidates in MELD Era

Pre-OLT creat (mg/dl)	% pts pre-MELD	% pts post-MELD	
0-0.99	51.8	46.1	P<0.0001
1-1.99	36.6	38.5	
≥ 2.0	7.9	10.0	
Dialysis	3.7	5.3	

Pre-MELD 1999-2002, n=11010; Post-MELD 2002-04, n=13163, data from SRTR

Gonwa et al. Am J. Transplant. 2006; 6: 2651

Increasing Number of SLK in USA in MELD Era



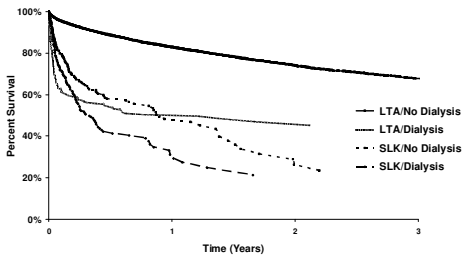
From www.unos.org, 2009

Pre-transplant Kidney Dysfunction and OLT Outcomes in MELD Era

Factors to Consider

- Waiting list outcomes
- Severity
  - *dialysis requirement*
  - *duration*
- Effect of MELD on SLK outcomes
- Patients with HRS
- Rate and extent of CKD progression post-txp

Higher Mortality on Waiting List Among SLK Candidates

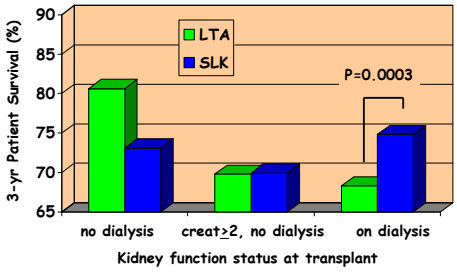


n=28736 pts on liver waitlist 2/02-6/05

Eason J *et al*, Am J Transpl 2008

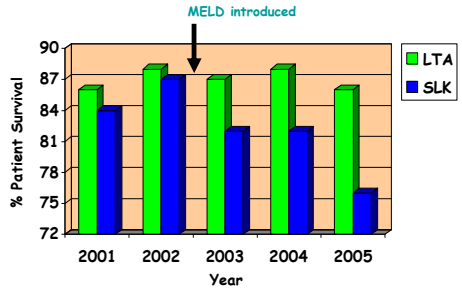
Survival Benefit of SLK Occurs Only in Patients on Dialysis Prior to Transplant

n=24,173 liver recipients, transplanted 1999-2004 (pre and post-MELD)



Adapted from Gonwa T *et al*, Am J Transpl 2006

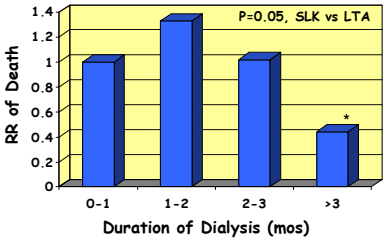
Declining SLK Outcomes in MELD Era



Matched-control analysis, SLK and LTA recipients matched for donor age, race, cause of death, recipient MELD and dialysis status

Adapted from Locke JE *et al*, Transplantation 2008

Pre-transplant Dialysis Duration and Survival After SLK



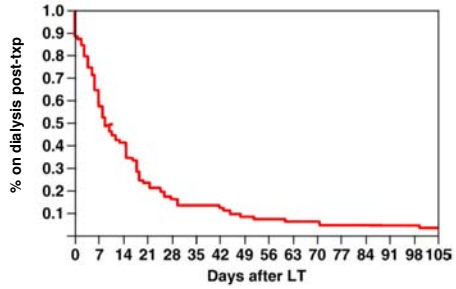
Matched-control analysis, SLK and LTA recipients matched for donor age, race, cause of death, recipient MELD and dialysis status

Adapted from Locke JE *et al*, Transplantation 2008

Limitations with Comparing SLK and LTA Outcomes in MELD Era

- Only retrospective studies
- Lack of:
  - *Appropriate control groups*
  - *Standardized selection criteria for SLK*
  - *Information on pre-OLT kidney function*
  - *Data on pre-txp comorbidity*
- Renal outcomes after LTA not well characterized

Most HRS Patients on Dialysis Discontinue this Modality After LTA

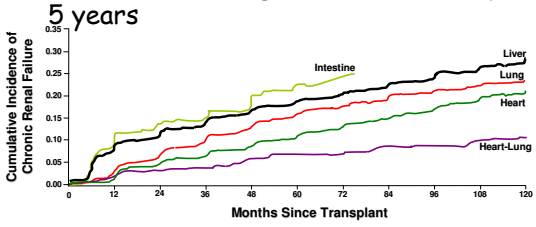


n=80 pts with HRS on dialysis < 4 wks

Ruiz R *et al*, Arch Surg 2006

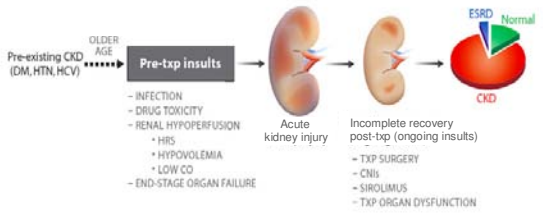
Cumulative Incidence of CKD among Nonrenal Organ Recipients

- Some level of CKD is invariable
- Prevalence of stage 4-5 CKD: 18% by 5 years



Ojo AO, et al. NEJM. 2003; 349(10): 931-40

Factors Contributing to CKD After Non-Renal Organ Transplantation



Most pts have CKD after organ transplantation

From Bloom RD, J Am Soc Nephrol, 2007

Kidney Transplant is Uncommon in First Year Post Liver Transplant Alone

Txp Group by listing and txp type	n	Listed for kidney (%)	Kidney txp (%)
Listed LTA, txp LTA	7198	0.42	0.03
Listed SLK, txp LTA	53	1.89	3.77
Listed SLK, txp SLK	387	1.81	0

Data source: SRTR

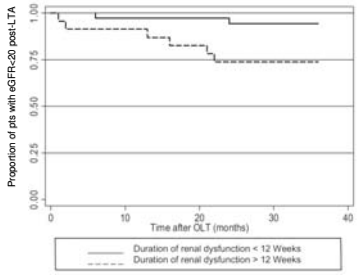
Davis C *et al*, Am J Transpl 2007

Progression to Stage 5 CKD in First Year After Liver Transplant

- <5% OLT patients require kidney listing
- 1/3 patients listed for sequential kidney were on dialysis at time of OLT
- SLK does not safeguard kidney graft function early post-txp

From Eason et al. Am J. Transplant. 2008; 8: 2043
From Davis et al. Am J. Transplant. 2007; 7: 1702

Duration of Pre-LTA Kidney Dysfunction* Predicts Advanced CKD 3 Years Post-txp



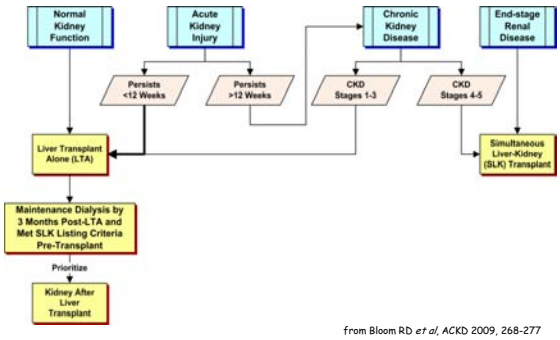
*Defined by serum creat>1.5 mg/dl for 2 or more weeks

Bahirwani R *et al*, Liver Transplantation 2008

SLK Allocation in MELD Era – Summary of Issues

- Inadequate characterization of pre-kidney function has limited the establishment of uniform criteria
- 3 mos duration of severe kidney disease is tipping point for worse outcomes after LTA
- CKD defined by impaired kidney function for ≥3 mos
- Should restrict SLK to pts with stage 4-5 CKD
  - *No clear benefit with earlier CKD stages*
  - *Selects pts with lowest likelihood of renal recovery*

Proposed Algorithm for SLK vs LTA in Liver Candidates with Impaired Kidney Function



from Bloom RD *et al*, ACKD 2009, 268-277

Benefits of this Approach

- Avoids unnecessary depletion of kidneys from pool
- Minimizes jeopardy to >90,000 pts listed for kidneys alone
- Maximizes kidney txp outcome (organ utility)
- LTA recipients who remain dialysis dependent for 3 mos post-txp are not penalized

Kidney Biopsy in Liver Transplant Candidates

- Pathological abnormalities common[1,2, 3]
- High risk of bleeding complications[2]
- Not shown to be better than creat in predicting:
  - *Post-txp reversibility*
  - *Post-txp kidney function*
  - *Rate of decline of GFR*
  - *Time to ESRD*
- Should be considered a research tool for now

[1]McGuire, Ann Int Med 2006
[2]Wadei et al. Am J. Transplant. 2008; 8: 2618
[3]Tanriover et al. Transplantation. 2008, 86, 1548

Conclusions

- MELD era has seen a surge in SLK
- Impaired kidney function and histological damage are common in liver candidates
- CKD occurs in most recipients of liver transplant alone[1]

[1]O'Riordan, Nephrol Dial Trans, 2006

Conclusions

- Most pts with impaired kidney function <3 mos do not warrant SLK
- Need standardized criteria for SLK candidate selection:
  - prevent misuse of kidneys with SLK
  - Prevent depletion of kidneys for pts with stage 4-5 CKD listed for kidney alone

Fellows Symposium on Transplantation Medicine

Friday, September 23
4:20 pm - 5:30 pm

Indications for SOT: Heart

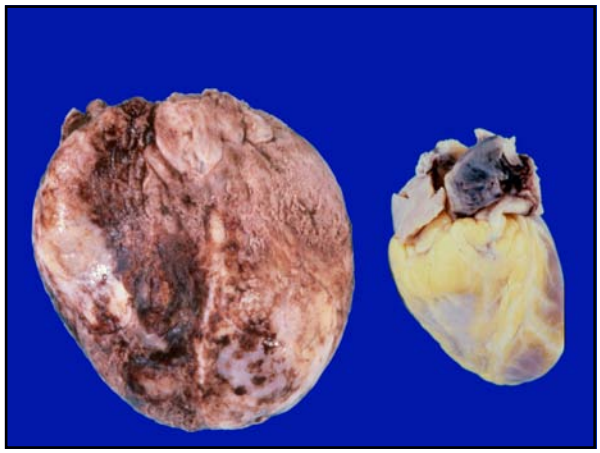
Breakout Session

Maryl R. Johnson, MD, Milagros D. Samaniego, MD and Josef Stehlik, MD, MPH

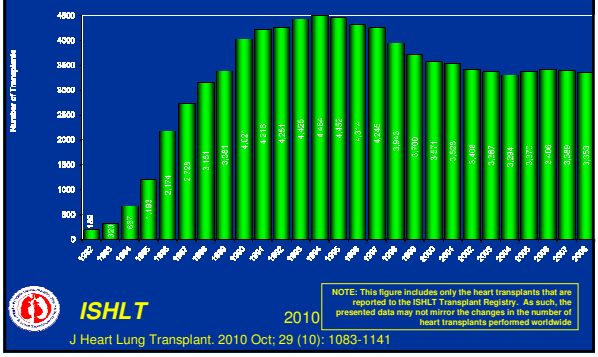
September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

INDICATIONS FOR HEART TRANSPLANTATION

Maryl R. Johnson, M.D.
Professor of Medicine
University of Wisconsin School of Medicine and Public Health
Medical Director, Heart Failure and Transplantation
University of Wisconsin Hospital and Clinics
Madison, WI



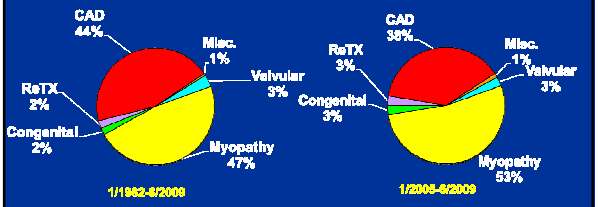
NUMBER OF HEART TRANSPLANTS REPORTED BY YEAR



ISHLT 2010

J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141

DIAGNOSIS IN ADULT HEART TRANSPLANTS



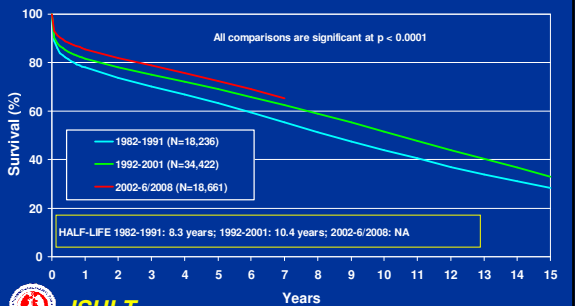
ISHLT 2010

J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141

ADULT HEART TRANSPLANTATION

Kaplan-Meier Survival by Era

(Transplants: 1/1982 – 6/2008)



ISHLT 2010

J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141

INDICATIONS FOR CARDIAC TRANSPLANTATION

- End stage cardiac disease unresponsive to medical/surgical management
  - New York Heart Association class III or IV heart failure
  - End stage ischemic disease which cannot be revascularized
  - Life threatening arrhythmias which are intractable to medical or surgical therapy
- On optimal tolerated medical therapy
- Risk/benefit ratio favors cardiac transplantation

CONSIDERATIONS PRIOR TO TRANSPLANT EVALUATION/LISTING

- Treatment of myocardial ischemia
- Treatment of valvular heart disease
- Optimized medical therapy including:
  - Angiotensin-converting enzyme (ACE) inhibitor (or angiotensin receptor blocker [ARB])
  - β Blocker
  - Aldosterone antagonist
  - Hydralazine and nitrates (if intolerant of ACE inhibitors and [ARBs])
  - Diuretics (as indicated by volume status)
- Prevention of sudden death by implantation of implantable cardioverter-defibrillator

Johnson et al, AST Primer on Transplantation 2011; p 174

CONSIDERATIONS PRIOR TO TRANSPLANT EVALUATION/LISTING (Cont.)

- Restoration of sinus rhythm in patients with atrial fibrillation or atrial flutter, if possible
- Resynchronization therapy in patients with left ventricular dyssynchrony
- Optimal treatment of non-cardiac diseases that adversely affect cardiac performance (i.e., thyroid disease, anemia)
- Confirmed abstinence from excess alcohol, smoking, and recreational drug use
- Intensive education and counseling in patients with a history of non-compliance

Johnson et al, AST Primer on Transplantation 2011; p 174

WEIGHING THE RISK/BENEFIT RATIO FOR CARDIAC TRANSPLANTATION

Cardiac Transplantation	Medical Therapy
Predictors of a poor prognosis in CHF	Conditions that affect morbidity and mortality after heart transplantation

PREDICTORS OF A POOR PROGNOSIS IN PATIENTS WITH HEART FAILURE

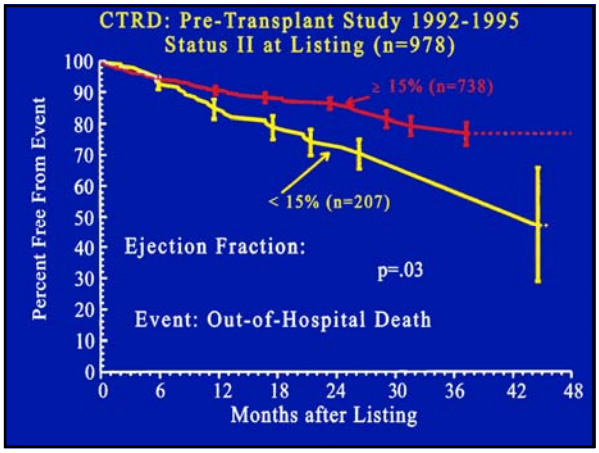
- Low EF
- Abnormal hemodynamics despite optimization of therapy
- Ischemic etiology of heart failure
- Decreased peak VO_2 (or % predicted peak VO_2) on metabolic stress testing
- Ventricular arrhythmias
- Electrolyte abnormalities (i.e., hyponatremia)
- Elevated BNP

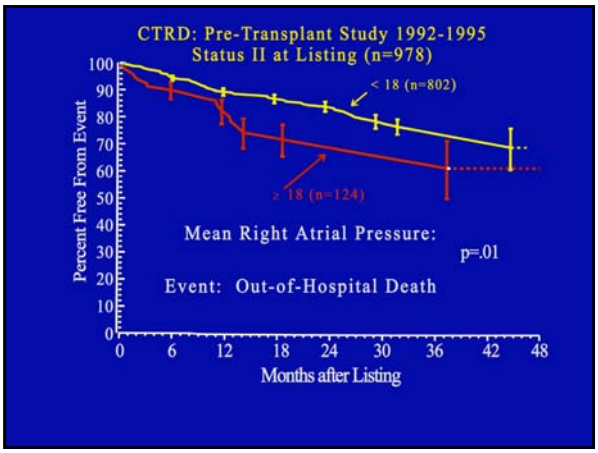
PREDICTORS OF MORTALITY IN V-HeFT I AND V-HeFT II

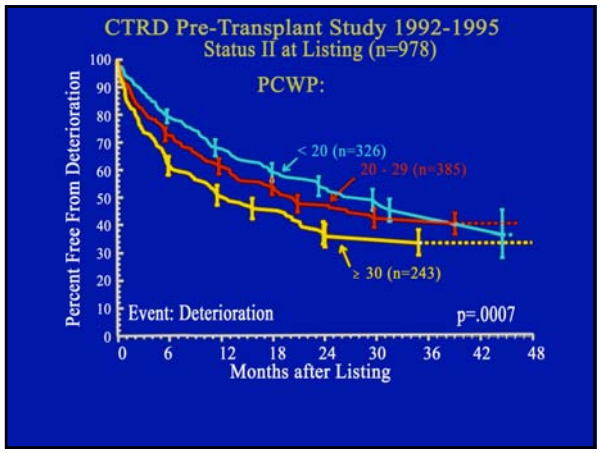
V-HeFT I		V-HeFT II	
Variable	*P*	Variable	*P*
Multivariate Analysis (stratified by treatment)			
LVEF	<0.0001	LVEF	<0.0006
VO_2	<0.005	VO_2	<0.0001
CTR	<0.003	CTR	<0.0012
		VAr	<0.01
		PNE*	<0.061
		PNE**	<0.02

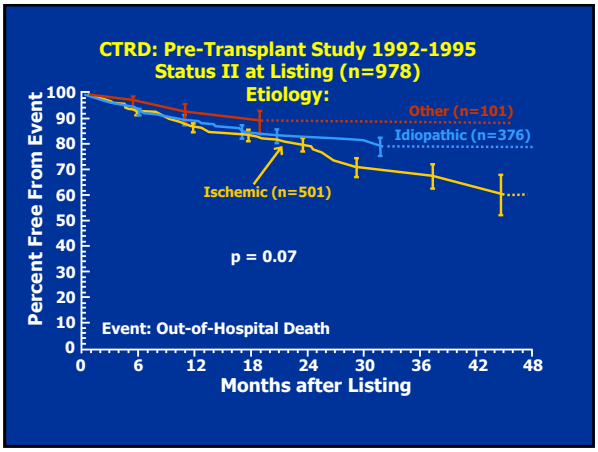
*Continuous variable; **Two groups above or below 700 or 900 pg/ml

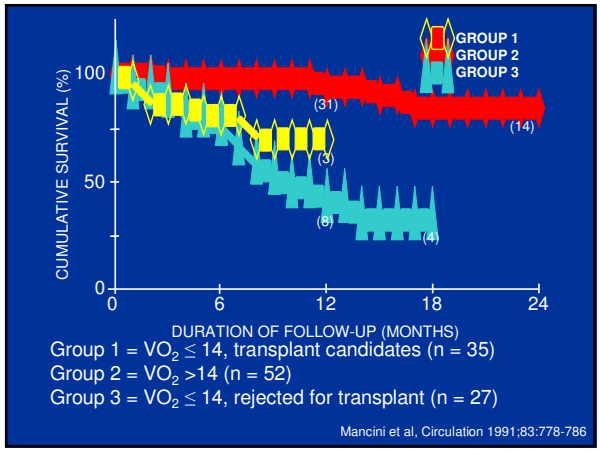
Cohn et al, Circulation 1993;87 (6 Suppl):V-15-16

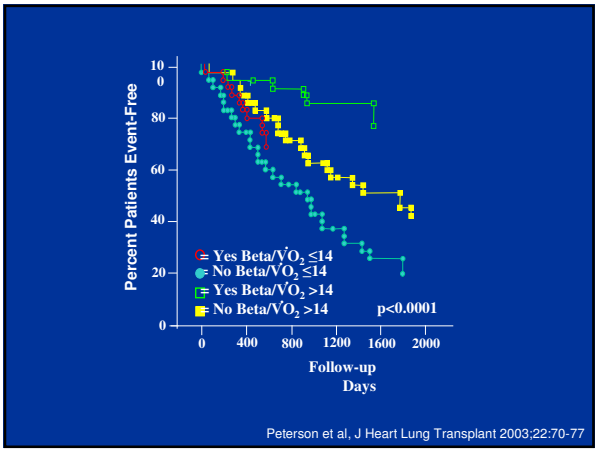












CARDIOPULMONARY STRESS TESTING TO GUIDE TRANSPLANT LISTING

Class I:

1. Maximal CPX has RER >1.05 and achievement of AT on optimal therapy.
2. In pts intolerant of Beta-blocker, peak VO_2 ≤14 ml/kg/min should guide listing.
3. In pts on Beta-blocker, peak VO_2 ≤12 ml/kg/min should guide listing.

Class IIa:

1. In pts <50 years and women, percent predicted VO_2 ≤50% may guide listing.

CARDIOPULMONARY STRESS TESTING TO GUIDE TRANSPLANT LISTING (Cont.)

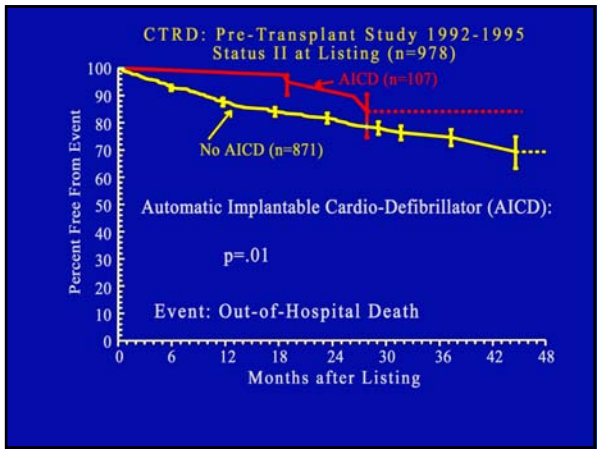
Class IIb

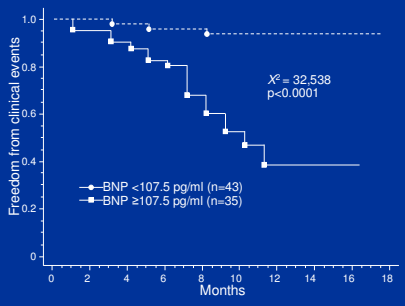
1. If RER <1.05, VE/VCO_2 slope >35 may be considered determinant for listing.
2. If BMI >30 kg/m^2, lean body mass-adjusted peak VO_2 <19 ml/kg/min can be used to assess prognosis.

Class III

1. Pts should not be listed solely based on VO_2 measurement.

J Heart Lung Transplant 2006; 25:1024-42.





Koglin et al, J Am Coll Cardiol 2001;38:1934-41

CTRD: Pre-Transplant Study: 1992-1995 Status II at Listing (n=978)

Risk Factor	P-value
Ischemic etiology	.005
Ejection fraction (lower)	.05
RA mean pressure (higher)	.004
Non use of AICD	.02

HEART FAILURE SURVIVAL SCORE

- LVEF
- Peak VO_2
- Mean arterial blood pressure
- Resting heart rate
- QRS interval
- Serum sodium
- Ischemic etiology

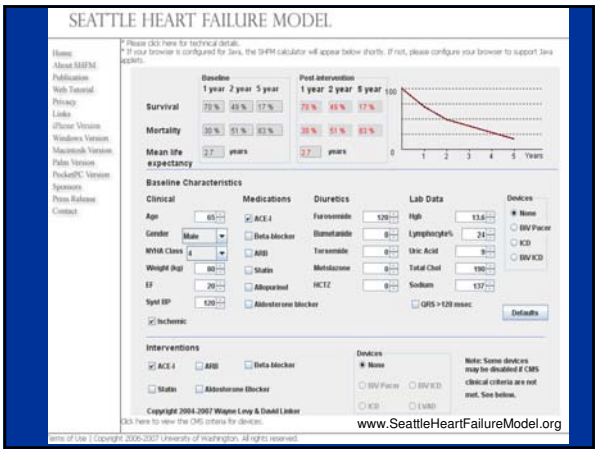
Aaronson et al, Circulation 1997;95:2660-7

HEART FAILURE SURVIVAL SCORE AND MORTALITY

One Year Event Free Survival

	Derivation Sample (n = 268)	Validation Sample (n = 199)
Low risk	93 %	88 %
Medium risk	72 %	60 %
High risk	43 %	35 %

Aaronson et al, Circulation 1997;95:2660-7



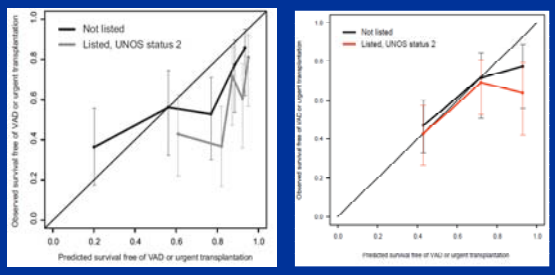
APPLICATION OF SHFM AND HFSS TO ADVANCED HEART FAILURE POPULATION

- 215 consecutive ambulatory patients presented to Cleveland Clinic Advanced HF Committee 2004-2007 (excluded UNOS Status 1, prior transplant, patients on VADs, multiorgan transplant candidates)
- 105 listed UNOS 2, 110 not listed

Gorodeski et al
Circ Heart Fail 2010; 3:706-714

CALIBRATION PLOTS
At 1-Year

SHFM **HFSS**



Gordeski et al
Circ Heart Fail 2010; 3:706-714

USE OF HEART FAILURE PROGNOSIS SCORE TO GUIDE TRANSPLANT LISTING

Class IIb

1. In circumstances of ambiguity (i.e., peak VO_2 >12 and <14 ml/kg/min) a HFSS may be considered to help guide listing for ambulatory pts.

J Heart Lung Transplant 2006; 25:1024-42

POSSIBLE CONTRAINDICATIONS TO HEART TRANSPLANTATION

Condition	Outcomes of Concern
Age > 65 years	Decreased survival benefit
Primary renal insufficiency	Decreased survival, accelerated progression
Hepatic insufficiency	Decreased survival, abnormal pharmacokinetics
Active peptic ulcer disease	Exacerbation with corticosteroids
Chronic inflammatory bowel disease	Increased infectious risk
Pulmonary vascular disease	Right ventricular failure, decreased survival
Chronic lung disease	Decreased survival, functional limitation, infectious risk
Peripheral vascular disease	Functional limitation, accelerated progression, infectious risk
Stroke (recent)	Hemorrhagic transformation

Johnson et al
Primer on Transplantation 2011; p 175

POSSIBLE CONTRAINDICATIONS TO HEART TRANSPLANTATION (Cont.)

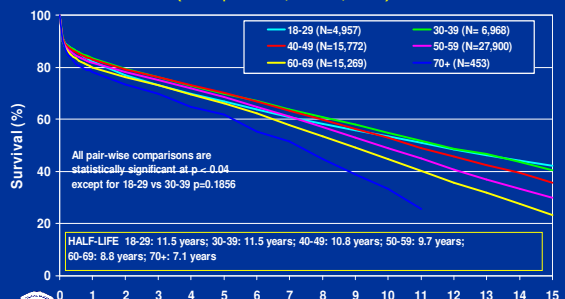
Condition	Outcomes of Concern
Pulmonary embolism (recent)	Hemorrhagic transformation, infection
Malignancy	Premature mortality, accelerated progression with immunosuppression
Infection	Spread with immunosuppression
Diabetes mellitus	Premature mortality, end-organ compromise
Amyloid	End-organ compromise, allograft recurrence
Sarcoid	End-organ compromise, allograft recurrence
Obesity	Decreased survival benefit
Medical non-compliance	Inadequate follow up care, decreased survival
Smoking	Infectious risk, accelerated pulmonary and vascular disease

Johnson et al
Primer on Transplantation 2011; p 175

ADULT HEART TRANSPLANTATION

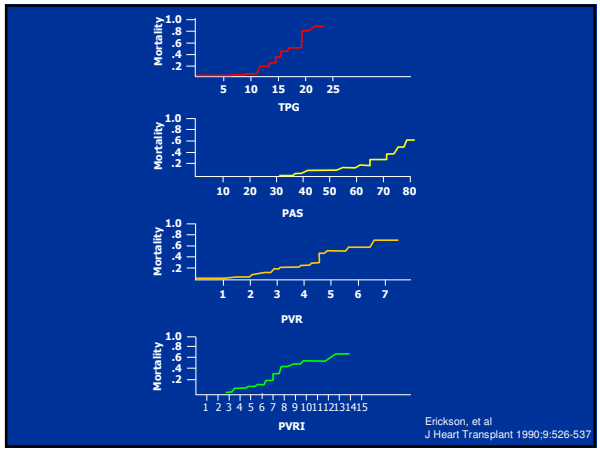
Kaplan-Meier Survival by Age Group

(Transplants: 1/1982-6/2008)



ISHLT 2010

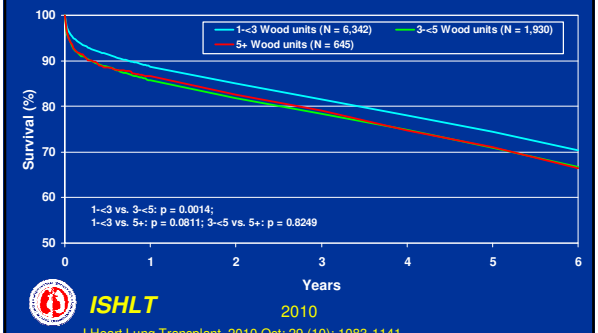
J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141



Erickson, et al
J Heart Transplant 1990;9:526-537

ADULT HEART TRANSPLANTATION

Kaplan-Meier Survival by PVR (Transplants: 1/2002-6/2008)



ISHLT 2010

J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141

IMPORTANT HEMODYNAMIC PARAMETERS TO ASSESS POTENTIAL CARDIAC TRANSPLANT CANDIDATES

- Pulmonary artery hypertension and elevated PVR should be considered as a relative contraindication to cardiac transplantation when the PVR is >5 Wood units or the PVRI is >6 or the TPG exceeds 16 to 20 mm Hg.
- If the PAS exceeds 60 mm Hg in conjunction with any 1 of the preceding 3 variables, the risk of right heart failure and early death is increased.
- If the PVR can be reduced to <2.5 with a vasodilator but the systolic blood pressure falls <85 mm Hg, the patient remains at high risk.

Calculations: transpulmonary gradient (TPG=[PAMP – PCWP]), pulmonary vascular resistance (PVR=[TPG/CO Wood units]), pulmonary vascular resistance index (PVRI=[TPG/CI])

J Heart Lung Transplant 2006; 25:1024-42.

ROLE OF RIGHT HEART CATH IN LISTING FOR HEART TRANSPLANT

Class I:

1. RHC should be performed on all candidates in preparation for listing and annually until transplantation.
2. RHC should be performed at 3 to 6 month intervals in listed patients, especially those with reversible pulmonary hypertension or worsening CHF.
3. A vasodilator challenge should be performed when the PASP ≥50 mm Hg and either the TPG ≥15 or PVR is >3 Wood units.

ROLE OF RIGHT HEART CATH IN LISTING FOR HEART TRANSPLANT (Cont.)

Class I (cont.):

4. If acute vasodilator challenge is unsuccessful, hospitalization with hemodynamic monitoring for 24-48 hours should be performed to assess response to treatment (diuretics, inotropes, vasodilators, NO).

Class IIb:

1. If medical therapy and mechanical unloading with IABP or LVAD doesn't produce acceptable hemodynamics, it is reasonable to consider the pulmonary hypertension irreversible.

J Heart Lung Transplant 2006; 25:1024-42.

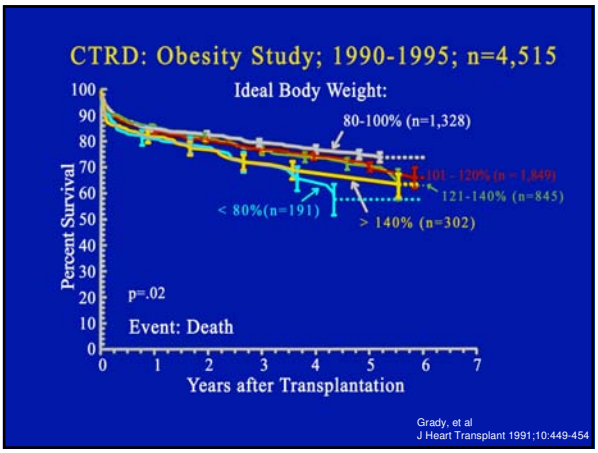
NORMALIZATION OF FIXED PULMONARY HYPERTENSION IN SEVERE HEART FAILURE PATIENTS WITH LVAD PLACEMENT

- Fixed pulmonary hypertension defined as TPG>15, PVR >5, PAd-wedge gradient >7 not reversible with pharmacologic agents
- From 7/03-11/06 8 pts (6 idiopathic, 2 ischemic) with "fixed" pulmonary hypertension (PAs = 66±7; PVR = 7.6 ± 0.7) underwent LVAD placement

	PA systolic (mmHg)	PA diastolic (mmHg)	PA mean (mmHg)	Wedge Mean Pressure (mmHg)	Cardiac Output (L/min)	PVR (Wood Units)
Baseline	66 ± 7	36 ± 1	48 ± 2	24 ± 1	3.1 ± 0.1	7.6 ± 0.7
Post LVAD placement	36 ± 2	14 ± 4	24 ± 2	13 ± 2	5.0 ± 0.6	2.2 ± 0.2
p-value	0.0187*	0.0035*	0.0023*	0.0155*	0.028*	0.0023*

- All successfully transplanted

2007 ATC Abstract 1079 Zolty, et al



BMI EFFECTS ON HEART TRANSPLANT SURVIVAL: SINGLE INSTITUTION VS. NATIONAL EXPERIENCE

- Reviewed 430 pts transplanted at Temple 1992-2002 and 23,113 initial adult transplants reported to UNOS 1996-2006
- At Temple, 20% with BMI >30 had similar survival to cohort with BMI <30. Also similar LOS, infection rate, reoperations. WL times correlated with BMI
- In UNOS data, 20% with BMI >30. BMI not independent risk factor for mortality

Kashem, et al
J Heart Lung Transplant 2009;28:S116

ADULT HEART TRANSPLANTS (1/2003-6/2008)
Risk Factors for 1 Year Mortality

VARIABLE	*N*	*Relative Risk*	*P-value*	*95% Confidence Interval*
Temporary circulatory support*	165	2.73	<0.0001	2.02 -3.68
Diagnosis: Congenital vs. cardiomyopathy	263	2.27	<0.0001	1.71 -3.02
Recipient history of dialysis	294	1.65	<0.0001	1.30 -2.09
Recipient on ventilator at time of transplant	267	1.61	0.0004	1.24 -2.09
Chronic continuous flow device	440	1.33	0.0364	1.02 -1.73
Prior transfusion	2056	1.24	0.0048	1.07 -1.44
Recipient with infection requiring IV drug therapy within 2 weeks prior to transplant	1065	1.24	0.0113	1.05 -1.47
Chronic pulsatile flow device	1621	1.22	0.0211	1.03 -1.45
Not ABO identical	1604	1.19	0.0197	1.03 -1.37
Diagnosis: coronary artery disease vs. cardiomyopathy	4527	1.16	0.0213	1.02 -1.33

* Temporary circulatory support includes ECMO and Abiomed BVS.
NOTE: There were too few temporary continuous flow devices to analyze.

ISHLT 2010 (N=10,547)

J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141

ADULT HEART TRANSPLANTS (1/2003-6/2008)
Risk Factors for 1 Year Mortality

Continuous Factors

Recipient age	**Ischemia time**
Recipient height	**PA diastolic pressure**
Donor age	**Bilirubin**
Donor BMI (borderline)	**Serum creatinine**
Transplant center volume	**PVR**
Weight ratio	

ISHLT 2010

J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141

ADULT HEART TRANSPLANTS (1/2001-6/2004)
Risk Factors for 5 Year Mortality

VARIABLE	*N*	*Relative Risk*	*P-value*	*95% Confidence Interval*
Temporary circulatory support*	100	2.59	<0.0001	1.91 -3.51
Recipient on dialysis at transplant	208	1.63	<0.0001	1.31 -2.03
Diagnosis: Congenital vs. cardiomyopathy	178	1.61	0.0013	1.20 -2.15
Ventilator	195	1.3	0.0426	1.01 -1.68
Previous pregnancy	976	1.28	0.0183	1.04 -1.57
Male recipient/female donor vs. male recipient/male donor	1246	1.25	0.0005	1.10 -1.42
Recipient history of diabetes	1392	1.25	0.0002	1.11 -1.40
Previously cerebrovascular event	382	1.23	0.0335	1.02 -1.49
Recipient with infection requiring IV drug therapy within 2 weeks prior to transplant	768	1.15	0.0716	0.99 -1.33
Chronic pulsatile flow device	1208	1.13	0.0665	0.99 -1.30

* Temporary circulatory support includes ECMO and Abiomed
NOTE: There were too few temporary continuous flow devices to analyze.

ISHLT 2010 (N=7,064)

J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141

ADULT HEART TRANSPLANTS (1/2001-6/2004)
Risk Factors for 5 Year Mortality

Continuous Factors	
Recipient age	Ischemia time
Donor age	Serum creatinine
BMI difference	Transplant center volume
Bilirubin	PA mean pressure
PVR	PRA (borderline)

ISHLT 2010
J Heart Lung Transplant. 2010 Oct; 29 (10): 1083-1141
(N=7,064)

PREOPERATIVE RISK STRATIFICATION SCORE (RSS)

- Analysis of 11,703 adult heart transplant recipients transplanted 2001-2007
- Risks for 1-year graft failure defined and used to define RSS
- Strongest predictors of 1-year graft failure
  - RVAD only
  - ECMO
  - Renal failure
  - Extracorporeal LVAD
  - TAH
  - Advanced age

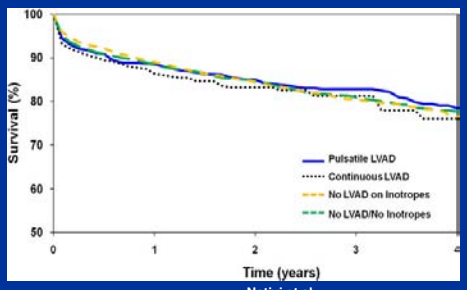
Hong et al
Ann Thorac Surg 2011;92:520-527

OUTCOMES IN PATIENTS BRIDGED TO TRANSPLANT WITH LVADS

- 7457 patients in ISHLT registry 7/04-5/08
  - 880 bridged with pulsatile-flow LVADs
  - 417 bridged with continuous flow LVADs
  - 2,728 on IV inotropes
  - 3432 who required neither inotropes nor LVADs
- Post-transplant outcomes compared

Nativi et al
J Heart Lung Transplant 2011;30:854-61

POSTTRANSPLANT SURVIVAL (TRANSPLANTED 7/04 – 5/08)



Nativi et al
J Heart Lung Transplant 2011;30:854-61

IMPLICATIONS OF COMORBIDITIES ON LISTING FOR HEART TRANSPLANTATION
(Age, Obesity, Cancer, Diabetes, Renal Insufficiency, PVD)

Class I:

1. Patients should be considered for cardiac transplantation if ≤70 years of age.
2. Pre-existing neoplasms are diverse and collaboration with oncologists should occur to stratify pts for risk of tumor recurrence. Transplant should be considered when risk is low based on tumor type, response to therapy, and negative metastatic workup. Amount of time to wait to transplant after neoplasm remission varies and no arbitrary time should be used.

IMPLICATIONS OF COMORBIDITIES ON LISTING FOR HEART TRANSPLANTATION
(Age, Obesity, Cancer, Diabetes, Renal Insufficiency, PVD)
(Cont.)

Class IIa:

1. BMI >30 kg/m^2 or percent ideal body weight >140% are associated with poor outcome. It is reasonable to recommend weight loss to these goals before listing.
2. Diabetes with end-organ damage other than non-proliferative retinopathy or poor glycemic control (HbA_{1C} >7.5) despite optimal effort is a relative contraindication.
3. Renal function should be assessed using eGFR or CrCl on optimal medical therapy. Abnormal renal function should prompt further evaluation (ultrasound, proteinuria, renal arterial disease). eGFR <40 ml/min is a relative contraindication.

IMPLICATIONS OF COMORBIDITIES ON LISTING FOR HEART TRANSPLANTATION (Age, Obesity, Cancer, Diabetes, Renal Insufficiency, PVD) (Cont.)

Class IIb:

1. Carefully selected pts >70 may be considered; for such pts an alternative type program may be pursued.
2. Clinically severe symptomatic cerebrovascular disease not amenable to revascularization may be considered a contraindication. PVD not amenable to revascularization may be considered a relative contraindication if its presence limits rehabilitation.

J Heart Lung Transplant 2006; 25:1024-42.

PSYCHOSOCIAL CONSIDERATIONS IN DEFINING TRANSPLANT CANDIDACY

Class I:

1. Psychosocial assessment should be performed before listing.
2. Education on the importance of tobacco cessation and reduction in second-hand exposure should be performed before transplant and in an ongoing manner.

Class IIa:

1. It is reasonable to consider active smoking a relative contraindication.
2. Mental retardation or dementia may be regarded as relative contraindications.

PSYCHOSOCIAL CONSIDERATIONS IN DEFINING TRANSPLANT CANDIDACY (Cont.)

Class IIb:

1. A structured rehab program may be considered for pts with recent (24 months) alcohol abuse.

Class III:

1. Pts who remain active substance abusers (including alcohol) should not receive heart transplantation.

J Heart Lung Transplant 2006; 25:1024-42.

EVALUATION FOR CARDIAC TRANSPLANTATION

- Cardiac testing
  - EKG
  - Echocardiogram
  - Left heart cath with coronary angiography
  - Right heart catheterization
  - Cardiopulmonary exercise test
  - BNP
  - TSH
- General health screening
  - Blood tests
    - CBC with diff, platelets, retics
    - BUN, Cr, electrolytes, Mg^{++}, glucose

EVALUATION FOR CARDIAC TRANSPLANTATION (Cont.)

- Liver panel, LDH
- INR, PTT
- ESR
- Ca++, PO_4
- Prealbumin
- Fasting lipid panel
- Fe, TIBC
- Hemoglobin A1C (if diabetic)
- PSA (males only)

– Urine tests
- UA
- Urine for cotinine
- 24 hour urine for creatinine clearance and protein

– Stool guaiac
– CXR

EVALUATION FOR CARDIAC TRANSPLANTATION (Cont.)

– Carotid and lower extremity arterial Dopplers (if CAD or >50 years)
– Pulmonary function tests
– Gallbladder ultrasound
– Colonoscopy (if ≥50 years)
– Clinical nutrition consult
– Ophthalmology consult (if >50 years or diabetic)
– Social work consult
– Mammogram (females >40 only)
– Gynecology exam (females only)
– DEXA scan
– Chest CT (if >40 years, h/o smoking, or prior chest surgery)
– Psychological/psychiatric evaluation (selective)
– Financial evaluation/counseling

EVALUATION FOR CARDIAC TRANSPLANTATION (Cont.)

- Infectious disease screening
  - Blood tests
    - Hepatitis A Ab
    - Hepatitis B panel
    - Hepatitis C Ab
    - HIV 1 & 2 Ab screen
    - VDRL
    - CMV Ab (IgG/IgM)
    - Toxoplasma Ab
    - EBV Ab panel
    - HSV
    - Varicella zoster titers
    - Fungal serologies (blasto, cocci, histo)

EVALUATION FOR CARDIAC TRANSPLANTATION (Cont.)

  - Dental exam
  - Panorex of the mandible
  - PPD and anergy battery or quantiferon – TB gold
  - Infectious disease consult
  - Administer Pneumovax, H flu vaccine, influenza vaccine, tetanus toxoid, varicella vaccine (if titers negative), Hepatitis B series (if Hep B Ab negative), Hepatitis A vaccine (if Hep A Ab negative), Zoster vaccine for age >60 and varicella positive
- Typing/immunologic screens
  - ABO typing and Ab screen
  - PRA
  - HLA typing

RECOMMENDED SCHEDULE FOR HEART TRANSPLANT EVALUATION

		Repeat			
Test	Baseline	3 months	6 months	9 months	12 months (and yearly)
Complete H & P	X				
Follow-up assessment		X	X	X	X
Weight/BMI	X	X	X	X	X
Immunocompatibility					
ABO	X				
Repeat ABO	X				
HLA tissue typing	Only at transplant				
PRA and flow cytometry	X				
• >10%	Every 1-2 months				
• VAD	Every 1-2 months				
Transfusion	2 weeks after transfusion and then every month x 6 months				
Assessment of heart failure severity					
Cardiopulmonary exercise test with RER	X				X
Echocardiogram	X				X
Right heart catheter (vasodilator challenge as indicated)	X		X		X
ECG	X				X

RECOMMENDED SCHEDULE FOR HEART TRANSPLANT EVALUATION (Cont.)

		Repeat			
Test	Baseline	3 months	6 months	9 months	12 months (and yearly)
Evaluation of multi-organ function					
Routine lab work (BMP, CBC, LFT)	X	X	X	X	X
PT/INR (More frequent per protocol if on VAD or Coumadin)	X	X	X	X	X
Urinalysis	X	X	X	X	X
GFR (MDRD quadratic equation)	X	X	X	X	X
Untimed urine sample for protein excretion	X	X	X	X	X
PFT with arterial blood gasses	X				
CXR (PA and lateral)	X				X
Abdominal ultrasound	X				
Carotid Doppler (if indicated or >50 y)	X				
ABI (if indicated or >50 y)	X				
DEXA scan (if indicated or >50 y)	X				
Dental examination	X				X
Ophthalmologic examination (if diabetic)	X				X

RECOMMENDED SCHEDULE FOR HEART TRANSPLANT EVALUATION (Cont.)

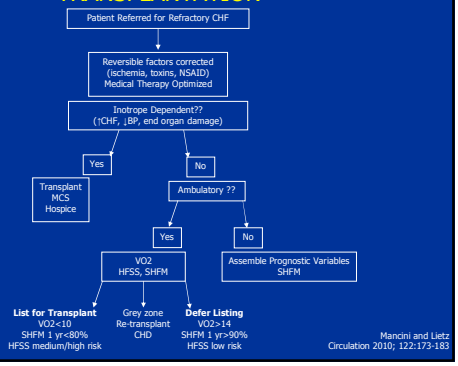
		Repeat			
Test	Baseline	3 months	6 months	9 months	12 months (and yearly)
Infectious serology and vaccination					
Hep B surface Ag	X				
Hep B surface Ab	X				
Hep B core Ab	X				
Hep C Ab	X				
HIV	X				
RPR	X				
HSV IgG	X				
CMV IgG	X				
Toxoplasmosis IgG	X				
EBV IgG	X				
Varicella IgG	X				
PPD	X				
Flu shot (q 1 year)	X				
Pneumovax (q 5 years)	X				
Hep B immunizations: 1_2_3_	X				
Hep B surface AB (immunity)	6 weeks after third immunization				

RECOMMENDED SCHEDULE FOR HEART TRANSPLANT EVALUATION (Cont.)

		Repeat			
Test	Baseline	3 months	6 months	9 months	12 months (and yearly)
Prevention and malignancy					
Stool for occult blood x 3	X				X
Colonoscopy (if indicated or >50 y)	X				
Mammography (if indicated or >40 y)	X				X
GYN/Pap (if indicated ≥18 y sexually active)	X				X
PSA and digital rectal exam (men >50 y)	X				X
General consultations					
Social work	X				
Psychiatry	X				
Financial	X				
Neuro/psych (if applicable)	X				

J Heart Lung Transplant 2006; 25:1024-42

SELECTION OF CANDIDATES FOR HEART TRANSPLANTATION



ONGOING CHALLENGES IN THE SELECTION OF CANDIDATES FOR HEART TRANSPLANTATION

- Improving outcomes with medical and surgical therapy for CHF
  - Beta-blockers
  - Resynchronization therapy
  - LVADs
- Donor shortage
- Prolonged waiting times
- Status 2 candidates rarely transplanted

UNOS STATUS CRITERIA (As of 6/29/11)

Status 1A Admitted to listing transplant center with at least one of the following:

1. Mechanical circulatory support
   a. LVAD and/or RVAD-Status 1A granted for 30 days at any time after device implantation, once physician determines patient is clinically stable (patient need not be hospitalized at the listing transplant center)
   b. Total artificial heart (if discharged may be listed as status 1A for 30 days at any time after discharge)
   c. IABP
   d. ECMO

UNOS STATUS CRITERIA (cont)

Status 1A (cont)

2. Mechanical circulatory support with device-related complication (thromboembolism, device infection, mechanical failure and/or life-threatening ventricular arrhythmias); (patient need not be hospitalized at listing center)
3. Mechanical ventilation
4. Continuous infusion of single high-dose IV inotrope or multiple IV inotropes with hemodynamic monitoring.
5. Life expectancy < 7 days (by application to Regional Review Board)

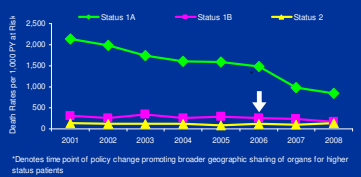
UNOS STATUS CRITERIA (cont)

Status 1B
1. LVAD and/or RVAD
2. Continuous IV inotropes
3. Exception

Status 2 All others

Status 7 Temporarily unsuitable for transplantation

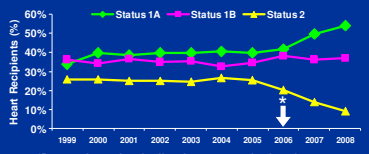
ANNUAL DEATH RATES PER 1,000 PATIENT-YEARS ON THE HEART WAITING LIST BY STATUS, 2001-2008



*Denotes time point of policy change promoting broader geographic sharing of organs for higher status patients

Source: *2009 OPTN/SRTR Annual Report*, Table 11.3.

STATUS OF HEART TRANSPLANT RECIPIENTS, 1999-2008



*Denotes time point of policy change promoting broader geographic sharing of organs for higher status patients

Source: *2009 OPTN/SRTR Annual Report*, Table 11.4.

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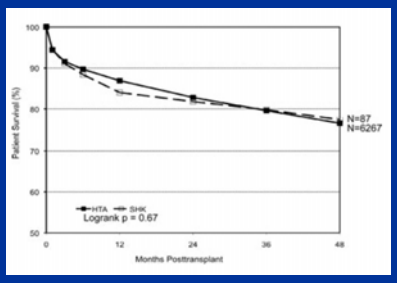
A. ISHLT Guidelines for Heart Transplantation
1. Management of transplant candidates
2. Listing criteria for heart transplantation
3. Heart rhythm and VAD considerations

www.ishlt.org
or
J Heart Lung Transplant 2006;25:1001-1056.

B. Mancini D and Lietz K. Selection of Cardiac Transplantation Candidates in 2010. *Circulation* 2010;122:173-183.

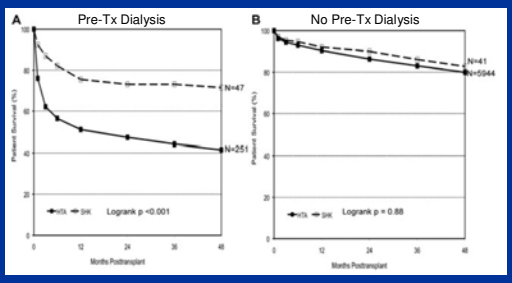
C. Johnson MR et al. Heart Transplantation. In: Primer on Transplantation, Third Edition. Wiley-Blackwell, West Sussex, UK, 2011, pp 171-204.

OPTN/UNOS DATA 1/98-1/07 PATIENT SURVIVAL



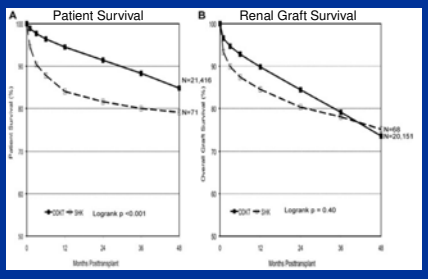
Gill et al, Am J Transplant 2009;9:844-52

OPTN/UNOS DATA 1/98-1/07



Gill et al, Am J Transplant 2009;9:844-52

OPTN/UNOS DATA 1/98-1/07



Gill et al, Am J Transplant 2009;9:844-52

Fellows Symposium on Transplantation Medicine

Friday, September 23
4:20 pm - 5:30 pm

Indications for SOT: Lung

Breakout Session

Mark L. Barr, MD and Scott M. Palmer, MD, MHS

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

Lung Transplant Recipient Selection 2011

Scott M. Palmer, MD, MHS
Associate Professor,
Duke Lung Transplant Program

- **General Guidelines**
- Disease Specific Criteria
- LAS
- Recent Data/Trends

General Guidelines

- Lung transplantation could be considered in any patient with advanced lung disease
- Most common indications:
  - COPD, CF, IPF, IPH, sarcoid
- Lung transplant can significantly improve FEV1, oxygenation, QOL and survival
- Lungs tend to perform worse than most other commonly transplant solid organs
- Careful recipient selection is critical!

Approach to Lung Transplant Evaluation

- Multi-disciplinary screening process
  - Medicine, surgery, psychology, financial, SW, coordinators
- Objective tests include:
  - PFTs, ABG, 6MWD, Chest CT
  - Cardiac catheterization, GFR study, other studies
- Objective testing goals:
  - Assess disease severity
  - Identify any contraindications to transplantation
- Subjective evaluation
  - Assess motivation and compliance, depression
  - Risk relapse in prior smokers or other substance abuse

Jonathan B. Orens MD et al. **The Journal of Heart and Lung Transplantation** Volume 25, Issue 7 , July 2006, Pages 745-755

"Absolute" Medical Contraindications to Lung Transplantation

- Advanced dysfunction of another major organ system (e.g., heart, liver, or kidney)
- Recent malignancy (>5 years free prudent)
- Non-curable chronic extrapulmonary infection including chronic active viral hepatitis B, hepatitis C, and HIV
- Significant chest wall/spinal deformity

Jonathan B. Orens MD et al. **The Journal of Heart and Lung Transplantation** Volume 25, Issue 7 , July 2006, Pages 745-755

Psychological Contraindications to Lung Transplantation

- Documented medical non-compliance
- Active psychiatric disorder
- Absence of social support system
- Active substance addiction (>6 months free of tobacco)
- Lack of insurance/inability to afford medications
- *Psychosocial factors weigh strongly in overall decision making process!!!*

Jonathan B. Orens MD et al. **The Journal of Heart and Lung Transplantation** Volume 25, Issue 7 , July 2006, Pages 745-755

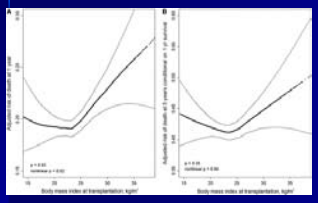
Relative Contraindications to Lung Transplantation

- Older age
  - Older patients have less optimal survival, therefore, recipient age should be a factor in candidate selection
  - We currently have no absolute upper age limit defined
- Unstable clinical condition (e.g. mechanical ventilation)
- Severely limited functional status
- Colonization with highly virulent bacteria, fungi, or mycobacteria
  - Burkholderia cenocepacia
  - Mycobacteria abscessus
- Severe obesity defined as a body mass index (BMI) exceeding 30 kg/m^2
- Severe, symptomatic osteoporosis

Jonathan B. Orens MD et al. **The Journal of Heart and Lung Transplantation** Volume 25, Issue 7 , July 2006, Pages 745-755

Impact of Weight on Survival after Lung Transplantation

Lederer AJRCCM Vol 180. pp. 887-895, (2009)



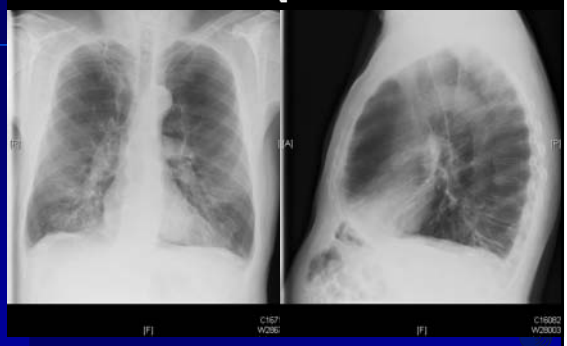
- OPTN registry analysis
- Linear relationship between increased BMI and death once over BMI 25
- Similar effects after multivariable analysis and stratification by main diseases

Transplant Balancing Act

- Individualize risk/benefit ratio to each patient based on their specific relative contraindications
  - *Prognosticate with and without transplant*
- Many unanswered questions about selection…
  - How many relative contraindications is too many
  - Role psychological vs. medical factors in decision
  - Center specific practice variation (e.g. BCC)

- General Guidelines
- **Disease Specific Criteria**
- LAS
- Recent Data/Trends

When to Transplant?

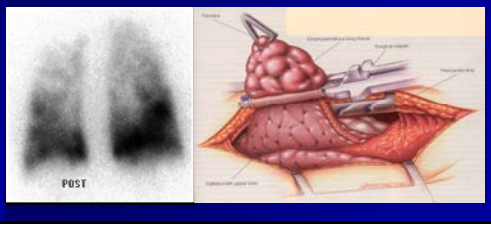


Alternatives to Lung Transplantation in COPD

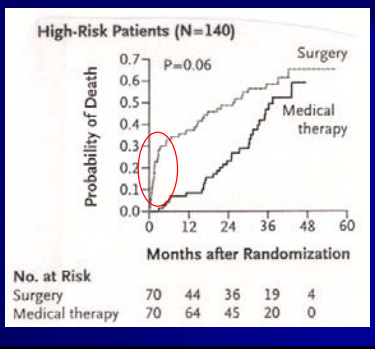
- *Should consider all medical and surgical alternatives to lung transplantation*
- Maximal Medical management
  - Smoking cessation
  - Oxygen therapy
  - Bronchodilators
- Pulmonary rehabilitation
- Consider Lung volume reduction surgery

Lung Volume Reduction Surgery (LVRS)

- Introduced in the 1950s but abandoned because of mortality
- Better techniques rekindled interest in the 1990s
- Rationale: removed diseased lung to reduce hyperinflation and improve diaphragmatic function

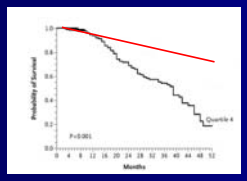


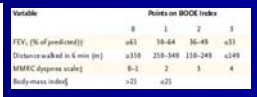
LVRS Exclusion: FEV1 < 20% AND either homogeneous disease or DLCO < 20%



Prognostication in Advanced COPD: BODE Index

- **BODE: Multidimensional index - best prognostic model**





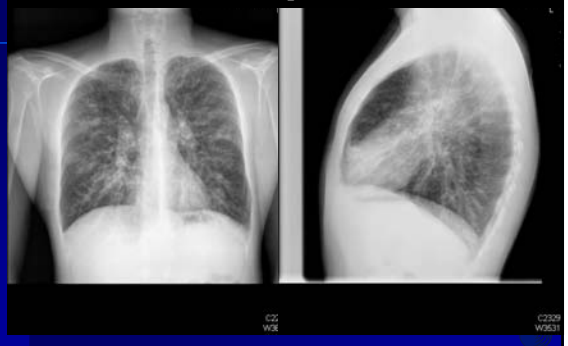
BODE Score of 7 or more identifies COPD patients with 20% chance at 4 yr survival

- **NEJM: Celli et al. 350: 1005 March 4, 2004: BODE Index**

COPD: Guidelines for Transplantation

- Lung transplant for COPD is controversial
  - Early studies suggested QOL not survival benefit
    - Older transplant cohorts with worse survival
    - Predominately single lung transplant performed
    - Less ill patients undergoing transplant
- ISHLT selection guidelines for COPD
  - History of hospitalization for exacerbation associated with acute hypercapnia (Pco2 exceeding 50 mm Hg)
  - Pulmonary hypertension or cor pulmonale, or both, despite oxygen therapy
  - Patients with a high BODE index (7 to 10)
  - FEV1 of less than 20% and either DLCO of less than 20% or homogenous distribution of emphysema

When To Transplant?

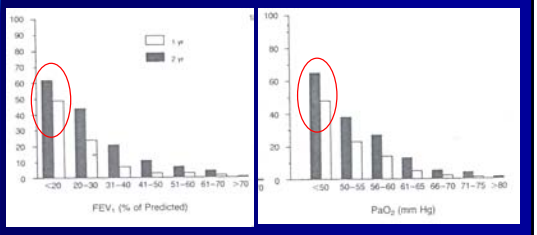


Cystic Fibrosis: Guidelines for Referral

- FEV1 below 30% predicted or a rapid decline in FEV1—in particular in young female patients
- Increasing frequency of exacerbations requiring antibiotic therapy
- Exacerbation requiring ICU stay
- Refractory and/or recurrent pneumothorax
- Recurrent hemoptysis not controlled by embolization
- Oxygen-dependent
- Hypercapnia
- Pulmonary hypertension

Mortality in CF

50% rate of death at 1 years with FEV1 < 20% or pO2 <50

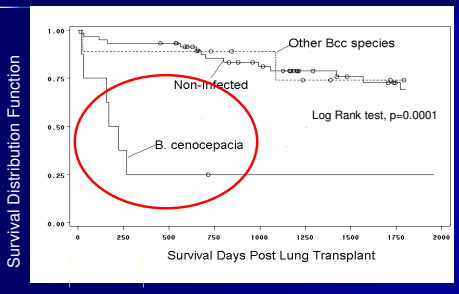


*Kerem et al, NEJM 1992;326:1187

Special Considerations in CF Recipients

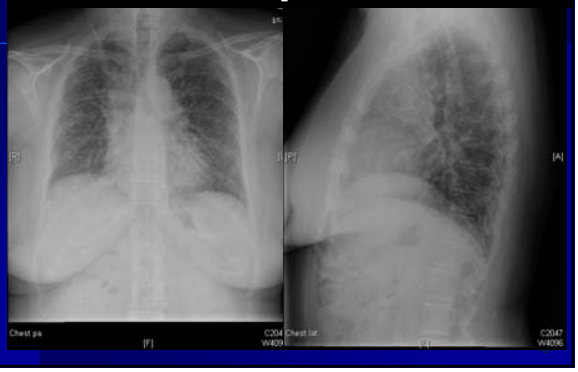
- Septic lung disease
  - Bacterial, mycobacterial, fungal pathogens
  - We exclude *B. cenocepacia (genomovar III)*
- Compliance/maturity
  - Psychological and SW evaluations
- Multiorgan system nature of disease
  - Sinus (occult source of infections)
  - GI (aggressive bowel regimen)
  - Liver disease (lung-liver transplant)

Survival Analysis Based on Pre-Lung Transplant BCC Status at Duke



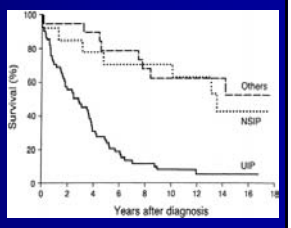
In press AJT

When to Transplant?



ISHLT Guidelines and IPF

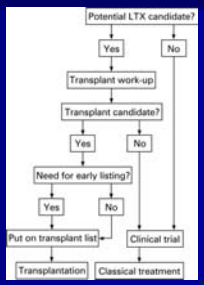
- Histologic or radiographic evidence of UIP and
- A DLCO < than 39%
- A 10% drop in FVC over 6 months of follow-up
- A decrease in pulse oximetry below 88% during a 6-MWT
- Honeycombing on HRCT



Bjoraker, JA, Ryu, JH, Edwin, MK, et al Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* **1998;157**,199-203

Refer IPF Early for Transplant

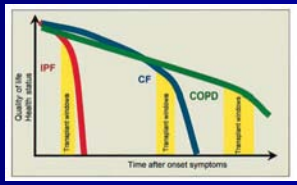
- Refer any patient with IFP early in disease
- There are currently no effective medical therapies for IPF
- Natural history include abrupt declines
- Consider transplant in any patient that requires supplemental oxygen



G M Verleden
Thorax 2008;63:292

Summary: When to Transplant

- "Window of opportunity"
- Aim to transplant when benefit > risk
  - 2 year survival is < 50%
  - not so debilitated that can survive transplant
- Also consider waiting time



Hofer www.smw.ch 137;2007

- General Guidelines
- Disease Specific Criteria
- **LAS**
- Recent Trends/Data

New Organ Allocation in the US: The LAS Score

- Mandated by HHS: need based allocation
- Based on severity of disease, *not waiting time*
- Unique (vs. heart, liver) priority based on
  - risk of death without Lung tx (urgency)
  - probability of post transplant survival (utility)
- Offers improved access to organs for young pediatric and adolescent candidates

How the LAS works

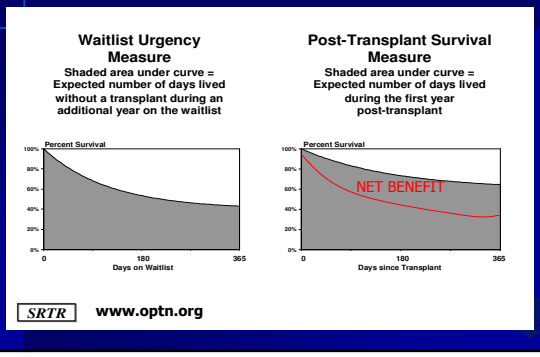
- Assigns number from 0-100 based on clinical factors, native disease
- Pretransplant risk for death considered 2:1 vs. estimated posttransplant survival
- Score is based on differential survival benefit to one year posttransplant
- Now makes urgent evaluation, listing and transplant possible

LAS Diagnostic Groups

- A – Obstructive
  - COPD
  - Alpha-1 Antitrypsin Deficiency
- B – Vascular
  - PPH
  - Eisenmenger's Physiology
- C – Cystic Fibrosis
- D - Restrictive
  - IPF
  - Sarcoidosis with Pulmonary Hypertension

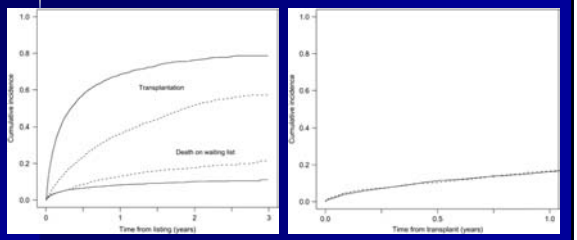
- Age
- Diabetes
- FiO2 at rest
- Diagnosis group
- Serum creatinine
- Assisted ventilation
- 6-minute walk distance
- FVC liter volume & % predicted
- NY Heart Association classification
- Hemodynamics (PAS, PAM, PCWP)

LAS Concept: Net Transplant Benefit



National Impact LAS

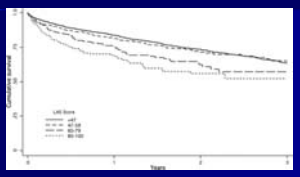
Chen et al. *AJRCCM* Vol 180. pp. 468-474, (2009)



- Cumulative incidence curves comparing transplantation, death on the waiting list and survival before LAS (dashed) and after implementation of the LAS (solid line)

High LAS and Survival

Liu et al. AJT Volume 10, Issue 4, 2010, pages 915–920



Model	1 (≤46)	2 (47–59)	3 (60–79)	4 (80–100)
All recipients				
Unadjusted	1.00 [reference]	1.08 (0.91–1.30)	1.52 (1.21–1.90)	2.03 (1.61–2.55)
Age-adjusted	1.00 [reference]	1.08 (0.90–1.30)	1.52 (1.22–1.91)	2.01 (1.63–2.58)
Multivariate	1.00 [reference]	1.05 (0.87–1.27)	1.50 (1.19–1.89)	2.05 (1.62–2.61)

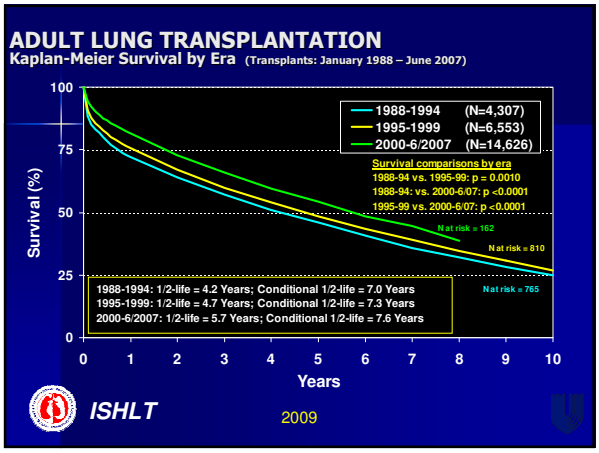
Summary LAS

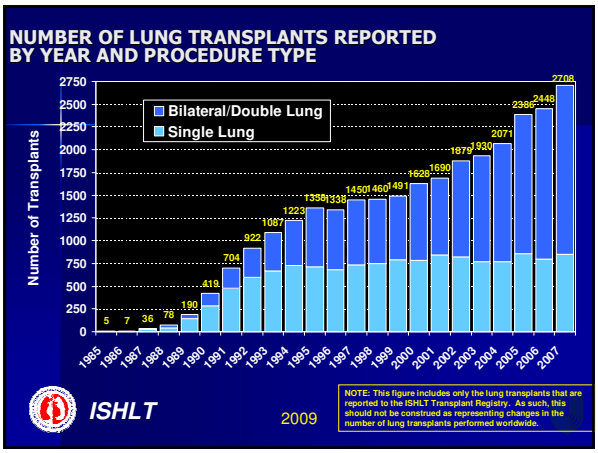
- Shortened waiting times, reduce deaths while waiting
- Contributed to increased total numbers of lung transplants
  - Other factors like increased donors, Donornet
  - LAS improves efficiency of organ allocation
- Contributed increased IPF transplants (sick with highest LAS)
- Created ability to do urgent transplants on ventilator dependent patients

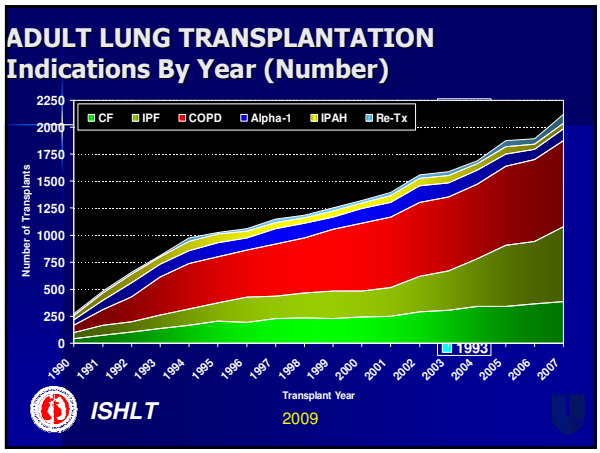
What's ahead for LAS?

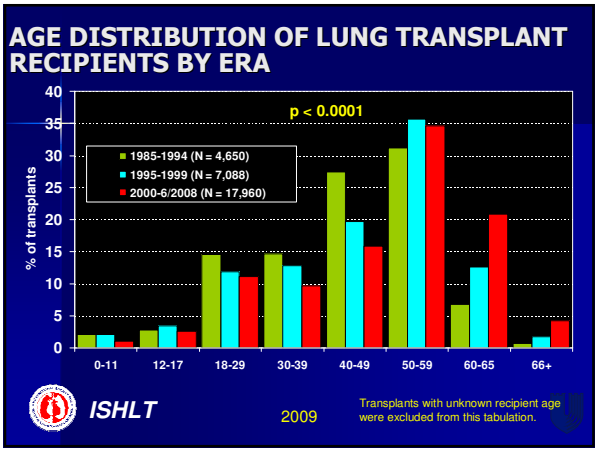
- Factors not considered in model (e.g. pCO2 recently added)
- Certain diseases (e.g. PH) disadvantaged
- Survival benefit only considered to 1 year
- Is high LAS simply another relative contraindication?
- Is there an LAS beyond which successful transplant is not possible?

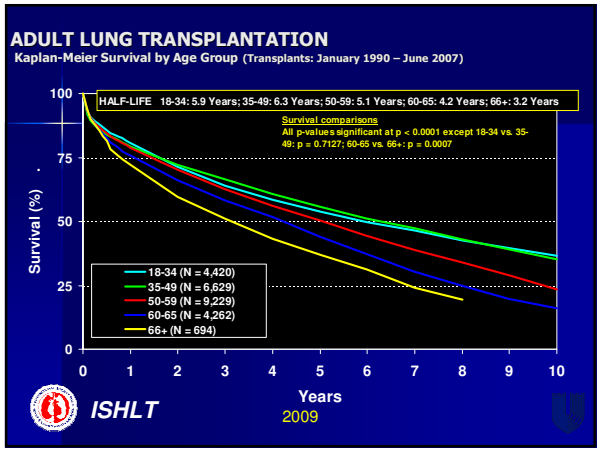
- General Guidelines
- Disease Specific Criteria
- LAS
- **Recent Trends/Data**

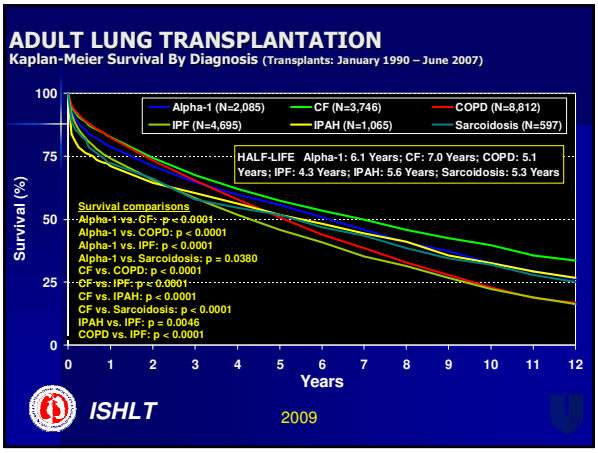


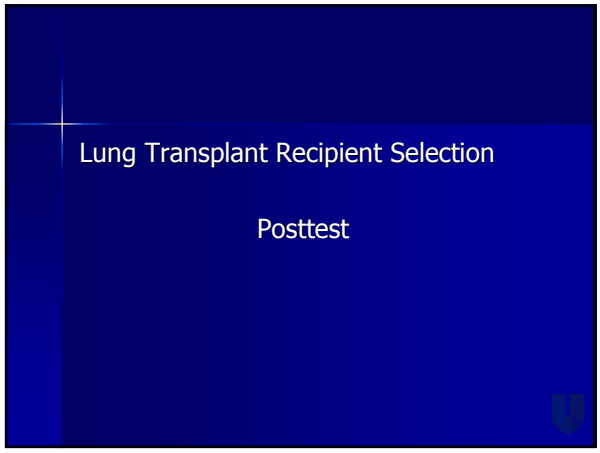

NUMBER OF LUNG TRANSPLANTS REPORTED
BY YEAR AND PROCEDURE TYPE
Bilateral/Double Lung
Single Lung
Number of Transplants
ISHLT
2009
NOTE: This figure includes only the lung transplants that are reported to the ISHLT Transplant Registry. As such, this should not be construed as representing changes in the number of lung transplants performed worldwide.


ADULT LUNG TRANSPLANTATION
Indications By Year (Number)
CF
IPF
COPD
Alpha-1
IPAH
Re-Tx
Number of Transplants
Transplant Year
ISHLT
2009


AGE DISTRIBUTION OF LUNG TRANSPLANT
RECIPIENTS BY ERA
p < 0.0001
1985-1994 (N = 4,650)
1995-1999 (N = 7,088)
2000-6/2008 (N = 17,960)
% of transplants
0-11
12-17
18-29
30-39
40-49
50-59
60-65
66+
ISHLT
2009
Transplants with unknown recipient age were excluded from this tabulation.


ADULT LUNG TRANSPLANTATION
Kaplan-Meier Survival by Age Group (Transplants: January 1990 – June 2007)
HALF-LIFE 18-34: 5.9 Years; 35-49: 6.3 Years; 50-59: 5.1 Years; 60-65: 4.2 Years; 66+: 3.2 Years
Survival comparisons
All p-values significant at p < 0.0001 except 18-34 vs. 35-49: p = 0.7127; 60-65 vs. 66+: p = 0.0007
18-34 (N = 4,420)
35-49 (N = 6,629)
50-59 (N = 9,229)
60-65 (N = 4,262)
66+ (N = 694)
Survival (%)
Years
ISHLT
2009


ADULT LUNG TRANSPLANTATION
Kaplan-Meier Survival By Diagnosis (Transplants: January 1990 – June 2007)
Alpha-1 (N=2,085)
CF (N=3,746)
COPD (N=8,812)
IPF (N=4,695)
IPAH (N=1,065)
Sarcoidosis (N=597)
HALF-LIFE Alpha-1: 6.1 Years; CF: 7.0 Years; COPD: 5.1 Years; IPF: 4.3 Years; IPAH: 5.6 Years; Sarcoidosis: 5.3 Years
Survival comparisons
Alpha-1 vs. CF: p < 0.0001
Alpha-1 vs. COPD: p < 0.0001
Alpha-1 vs. IPF: p < 0.0001
Alpha-1 vs. Sarcoidosis: p = 0.0380
CF vs. COPD: p < 0.0001
CF vs. IPF: p < 0.0001
CF vs. IPAH: p < 0.0001
CF vs. Sarcoidosis: p < 0.0001
IPAH vs. IPF: p = 0.0046
COPD vs. IPF: p < 0.0001
Survival (%)
Years
ISHLT
2009


Lung Transplant Recipient Selection
Posttest

Is this patient appropriate candidate for lung transplant?

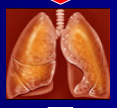
- 58 year old prisoner
- Squamous cell carcinoma
- Malnutrition
- Renal insufficiency

Whose a Better Candidate?

- 31 year old female CF patients with FEV1 of 35%, BMI 22, and working part time
- 25 year old CF patient uses oxygen with activity, pCO_2=58, but prior drug use
- A 70 year man with IPF, intubated 48 hours for acute exacerbation on 100% FiO_2

Recipient Selection is Critical to Successful Posttransplant Outcomes

Poor Candidate



Poor Outcome

Summary: Lung Transplant Recipient Selection

- Recipient selection requires understanding
  - Natural history diverse native lung disease
  - Risk factors for posttransplant success and mortality
- Successful approach to recipient selection will maximize patients life expectancy
- LAS has improved ability to offer lung transplant to those in greatest need

Fellows Symposium on Transplantation Medicine

Friday, September 23
4:20 pm - 5:30 pm

Grant Writing Workshop

Breakout Session

Robert L. Fairchild, PhD
and Peter S. Heeger, MD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org

Grant writing workshop

Peter S. Heeger
Mount Sinai School of Medicine
Robert Fairchild, PhD
Cleveland Clinic

Types of grants

- Why does everyone want an NIH grant? Follow the $. INDIRECT COSTS
- Training
  - usually stipend for salary with a little extra money
  - T32 or individual NRSA (US citizen or green card), foundation grants (NKF, ASN, AST, AHA, JDRF, other, for fellows or grad students)
- Junior faculty
  - K08, K23 (NIH, US citizen or green card), foundation (AST, AHA, ASN, JDRF,other)
  - Salary support plus some relatively small amount for supplies
- R01 NIH
  - Main grant support from NIH
  - generally 250,000 per year max
  - Funding at <15%ile
  - Better if you are a "new investigator"
- AHA grant in aid for faculty
- JDRF for faculty
- U grants
  - Large collaborative projects involving multiple institutions (e.g. CTOT)
- Program projects (PPG)
  - Several funded investigators submit new projects on a theme (multiple R01s) along with core facilities
  - The result of the collaboration is greater than the sum of the parts
- others

Review process

- Review committees consist of experts in the field as well as non experts
- Generally 2 or 3 people read each grant
- If everyone believes the grant is weak, it will not be discussed further (triage)
- Strengths and weaknesses are discussed by these primary reviewers and then the rest of the group can ask questions and chime in
- The whole committee votes and you are given a score
- For NIH grants, 1-9 with lower numbers being better
- NIH only allows one resubmission so it needs to be great

Components of a grant proposal

- Introduction with Specific Aims and Hypothesis
- Background
- Significance
- Innovation
- Preliminary Data
- Experimental Design and Methods
- The best proposals will study something important and previously untested, using novel techniques or novel reagents and using an experimental design that will result in answers regardless of the outcome of the individual experiment. The experiments will be interrelated, but not dependent on one another.
- For animal models in particular, if you propose a set of mechanistic studies based on the possibility that drug x will prolong graft survival, the grant will not be funded. You need to show that drug x prolongs graft survival and then design experiments to understand mechanisms.

Hypothesis

- A TESTABLE hypothesis should be clearly stated
- Drug x will prolong graft survival
- Molecule y is a key mediator of graft injury.
- If true, blocking or removing molecule y will prolong graft survival
- Molecule y is a key mediator of graft injury. It functions by upregulating and activating a set of cell surface molecules and receptors that control cell entry into a graft
- If true, blocking y will prolong graft survival and alter cell surface molecule expression / function and will prevent cell entry into the graft
- If true, blocking molecule y will not be effective if the cell surface molecules are over expressed or are functionally over active, etc

New NIH Scoring: Impact

"Is it worthwhile to carry out the proposed study?"

http://www.niaid.nih.gov/ncn/newsletters/2008/1217.htm#n01

Impact =

significance of the topic
+
the *feasibility* (reality) of your approach
and likelihood it will make a difference in field.

http://funding.niaid.nih.gov/ncn/newsletters/2009/1112.htm#n01

IMPACT: Reviewers will ask:

- Is work making a advancing impact in field (rather than lateral?)
- Are these *key* questions in the field?
- Will results interest many people in the field

Or, rather

- Is this a rehash of a previous project with a new tissue?
- Is this "me too" research?

Significance vs. Innovation

SIGNIFICANCE: the [positive] effect something is likely to have on other things (i.e. the *field*)

INNOVATION: a new and substantially different *way* of doing/considering something, which results in positive change

Significance

- Is this an important problem and will it impact human health?
  - New mechanisms of tolerance
  - Developing a new solution for cold storage of kidneys
  - Studying whether mixed lymphocyte reactions are helpful predictors of incipient rejection in children
- If it is important, it is important to tell the reviewer why this is an important question
  - Mechanisms of graft injury are not fully understood. Defining new molecular mechanisms could lead to novel therapies aimed by…
- Don't overstate it.
  - The results of this study will clearly lead to new therapies that will prolong transplant survival in humans
- Often helpful to give the reviewers the right arguments to help defend your proposal

General Considerations:
Don't overestimate your audience

- Be *explicit* about the significance of the project
- Don't *assume* the reader understands the impact
- Clearly identify key 'gaps in knowledge'

- Clearly identify the impact of doing *this* project
  ('*how much would I want to read the paper?*)

Specific Aims Page:
Where you gain or lose your audience!

- 1-2 sentences: *key problem and importance*
- 1-2 sentences: *key issues to be addressed and how these issues/problems will be addressed*
- 2-4 sentences: *preliminary data and interpretation*
- 1 sentence: *model proposed*
- *Key sentence*: clearly stated overall hypothesis!

Specific Aims

- Succinct and unambiguous questions/goals
- Aims should be *inter-dependent*, not *dependent*
- State what performing each Aim will accomplish
- Conclude: What will be the impact in the field

Background / Rationale

- *Not* an exhaustive literature search
- Build a story to form compelling support for the studies
- Highlight (BOLD) *key* concepts and the issues that remain to be clarified that are germane to your application

Background

- Not a comprehensive review of the literature
- Focused on specific issues relevant to your proposal
- Need to strike a balance based on the expertise of the reviewers
- Note what is known and what is not known. State that you will address what is not known (foreshadow)

Preliminary Results

- *Don't* need to have 'already performed the grant'!
- Key area for supporting feasibility and rationale (*especially* if a new technique or model)
- Preliminary results should be solid and interpretable (including statistics)
- Actual data should be clearly legible to 'aging' reviewers eyes!
(e.g. histology/FACS plots/histograms, etc)

Preliminary data

- A preliminary result
  - Two groups of 2 animals were studied, one KO and one WT, and there were modest differences between the groups. The results need to be repeated and expanded
- Preliminary data
  - 2 groups of 5 animals per group were studied. Results were different and significant. These findings support the proposed mechanistic studies

Preliminary data

- Hypothesis: MR1 and CTLA4Ig induce tolerance by inducing Treg which prevent expansion and migration of T eff cells
  - How would you propose to test this?
  - What preliminary data would be supportive?
- Urinary PCR detection of message for granzyme B is a useful diagnostic test for acute rejection
  - How would you propose to test this?
  - What preliminary data would be supportive?
- If you have a novel technique, novel mouse, novel system, etc. this is where to highlight and explain it

Experimental Approach

- *Emphasize* the rationale
- Clarify and *justify* (defend) the choice of models (e.g. specific animal models)
- *Clearly* describe interpretation of results
- Diagrams/schematics help: a picture can be worth a thousand words

Feasibility!

- Demonstrate that you can do this (yourself and/or with appropriate collaborators/co-investigators)
- Does *not* mean including extensive and tedious methodology
- Key relationship between feasibility and impact!
- Be your *own* best critic! *Clearly* outline pitfalls and *alternative* explanations for results.

Experimental Design and Methods

- Divide into Aims
- Can divide into subaims that are closely related
- Each aim should have a rationale, design, interpretation of outcomes and a discussion of potential problems/alternative

Experimental Design and Methods

- Rationale
  - Why you will do the experiment and a summary of your approach
  - Example 1. Our working model is that molecule x is a key regulator of chemokine receptor expression on T cells. To test this we will (subaim 1) compare chemokine receptor expression on WT and KO T cells, (subaim 2) add back molecule x to KO cells by viral transduction and test receptor expression and (subaim 3) assess in vitro responses to chemokines in each situation using migration assays.
  - Example 2. Our preliminary data indicate that absence of molecule x prolongs graft survival. The goal of the proposed work in this aim is to determine the cellular source of molecule x that mediates the effects. We will make BM chimeric animals using WT and KO mice as donors or recipients to determine if the BM derived cells or nonBM derived cells are required.

Experimental Design and Methods

- Design
  - Specifics of the experimental design including control groups, numbers of animals, statistical methods.
  - Experimental methods can be referenced if they are standard in the lab. If new method, then details are required.
  - Best experiments provide new information regardless of outcome. If possible, don't ask if something happens (may the answer is no), test mechanisms. In the BM chimera example above, the results will provide information either way that will guide the next set of studies (what might they be?)
  - If a clinical study, looking for the strength of a correlation or differences in group outcomes—be sure you have sufficient power
  - Designs should include complementary ways to get at the same question (KO and blocking antibodies as examples)

Experimental Design and Methods

- Interpretation of outcomes
- which of these is better?:
  - We anticipate that the results will confirm our hypothesis
  - If we find "a" then we will conclude "y." if we find "b" we will reach a different conclusion
- what might you do in a follow-up experiment based on the result (tells the reviewer where you are going)?

Experimental Design and Methods

- Anticipated problems and solutions
  - Methodological issues are relatively minor if your lab is experienced
  - Only need to address potential method issues if you are proposing to use a new method
  - More important is problems in interpretation; could there be another explanation to account for your result besides the one you consider?
  - Example
    - depletion of B cells prevents rejection. you conclude that is because B cell make antibody and no antibody is found in the animal. It is also possible that B cells act as APCs and then present alloantigen to T cells and that is the mechanism. How could you test this?

General Conclusions I

- Clearly answer: *So What?*
- Do I have a clear and important question/hypothesis? (descriptive/confirmatory experiments almost always will decrease impact)
- Can I convince the reader that I can *do* this?
- Do *both* 'positive' and 'negative' results have meaning? (difference between *testing* and hypothesis and trying to demonstrate only *one* viewpoint)

General Conclusions II

- 'Cosmetics' matter: Carefully put together and edit!
- Be *explicit* regarding conclusions (experimental or conceptual): *Not* 'results will lead to new directions in the field'……What does that *mean*/

How to go about this difficult process

- One suggested approach (others may be fine)
- Start with your hypothesis/working model
- Design your aims and experiments along with anticipated outcomes
- Let someone experienced look at this to see if you are going about this correctly and asking the right questions
- Designing you experiments first guides what preliminary data you need to support the work
- Add the preliminary data and tell the reader that these support the experiments
- Add the background at the end—only that portion relevant to the proposed work

How to go about this difficult process

- Provide the reviewer with the arguments that he or she can use to support your grant
- Be succinct and not repetitious
- Start early– at least 2 months before the grant is due
- Give it to someone experienced to read with sufficient time to change things based on the responses
- READ THE INSTRUCTIONS
- DON'T FORGET ALL OF THE TRAINING DOCUMENTATION REQUIRED

- Resubmitting Applications