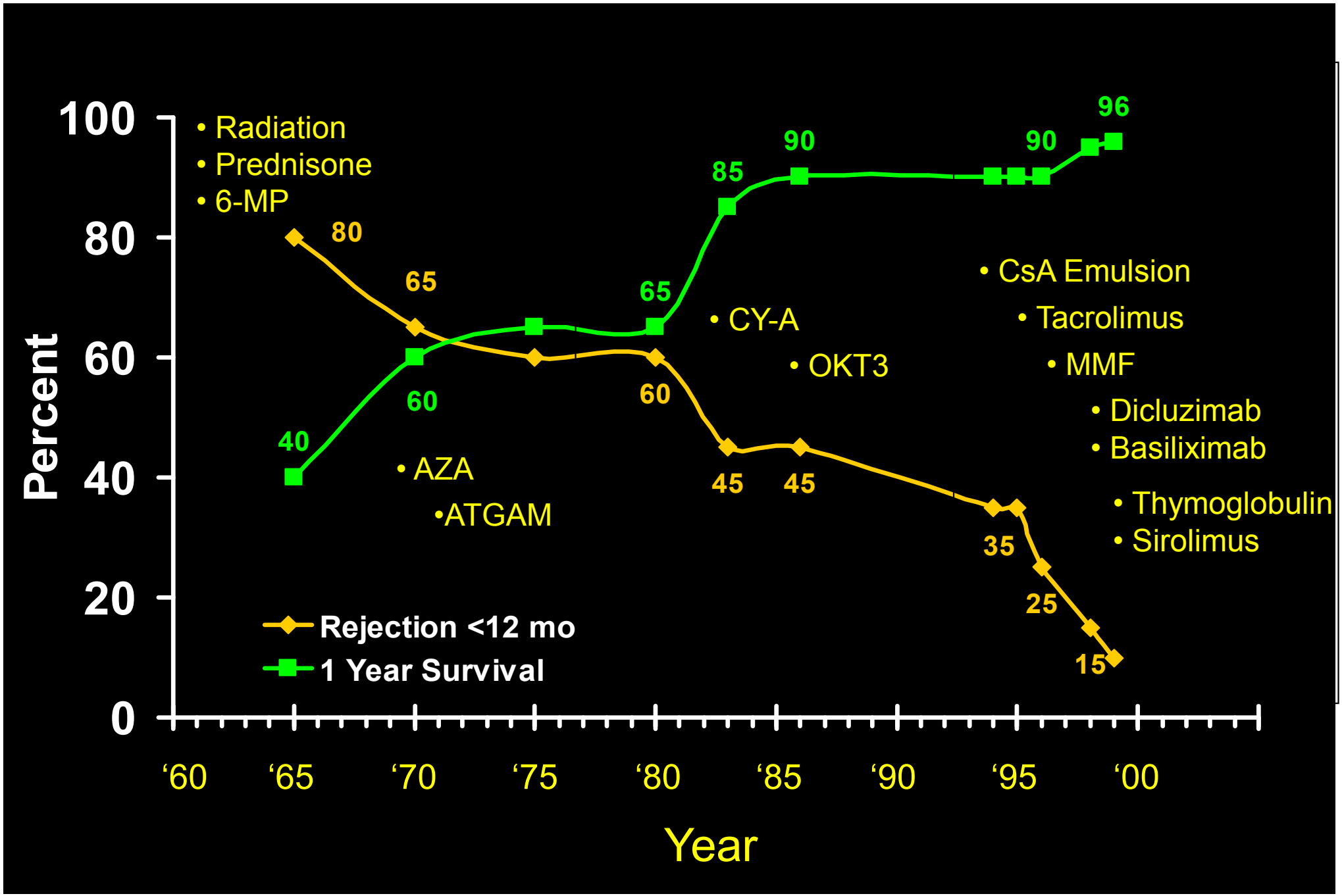
PRETRANSPLANT IMMUNOLOGIC RISK ASSESMENT FOR IMMUNOSUPPRESSIVE MANAGEMENT OF KIDNEY TRANSPLANT RECIPIENTS

Enver Akalin, M.D.
Professor of Clinical Medicine and Surgery
Albert Einstein College of Medicine
Medical Director
Kidney and Pancreas Transplantation
Montefiore Medical Center
Bronx, NY

Rejection rate and graft survival in kidney tx



Adapted from Stewart F, *Organ Transplantation*, 1999

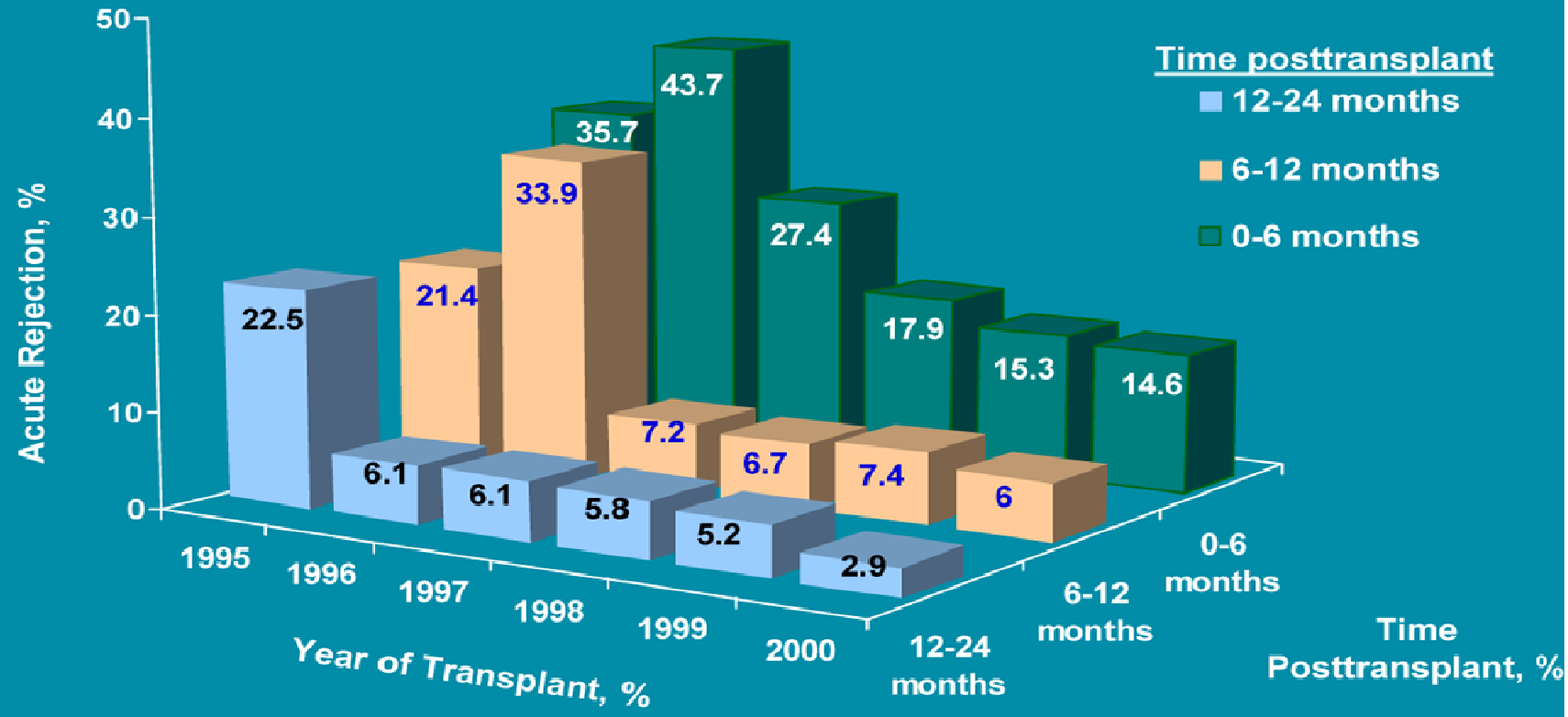
Renal Transplantation Outcomes

	1yr	3yrs	5yrs
Deceased-donor tx			
Graft Survival	89.4%	76.3%	64.7%
Patient Survival	94.8%	88.9%	81.8%
Living-donor tx			
Graft Survival	94.5%	87%	78.4%
Patient Survival	97.6%	94.6%	91%

Short-Term Outcomes Are Improving

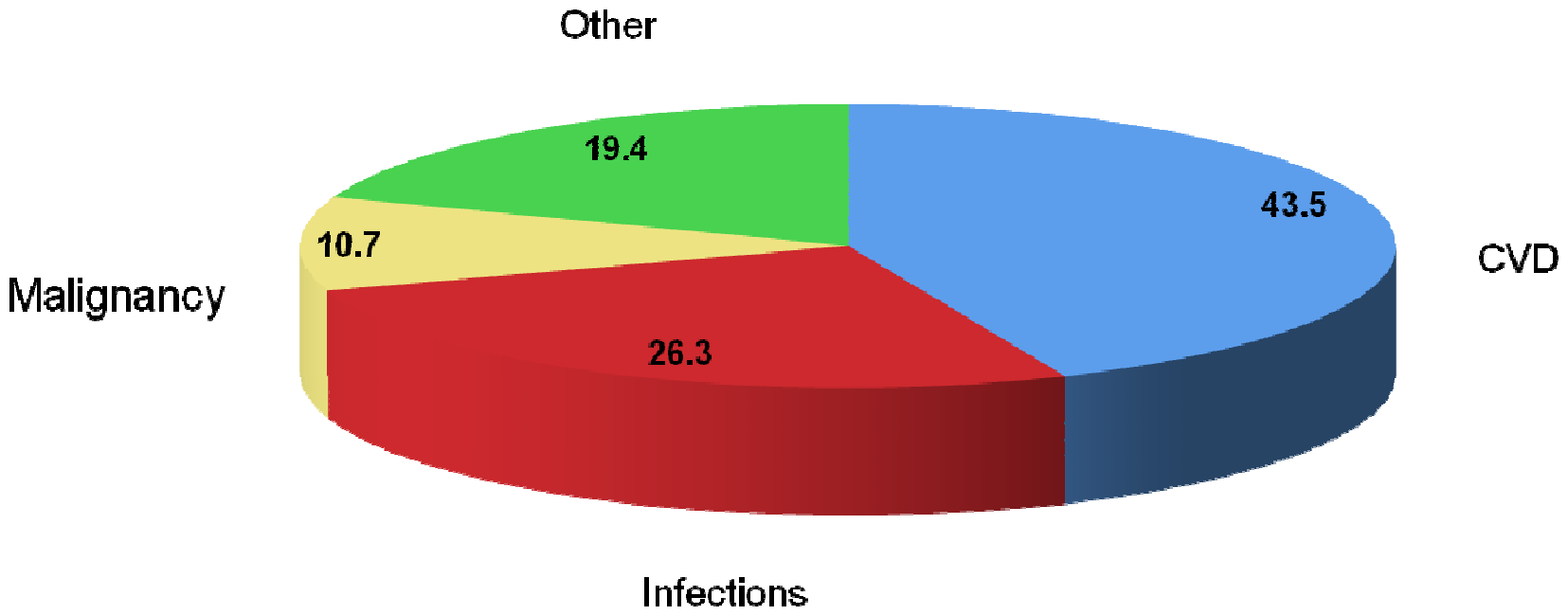
- Decrease in acute rejection may be related to the development of newer (improved) immunosuppressive agents and/or regimens

Incidence of Early Acute Rejection Episodes up to 2 Years Posttransplant



Meier-Kriesche H-U et al. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant*. 2004;4:378-383. Copyright © 2004. Reproduced with permission of Blackwell Publishing Ltd.

CVD: major causes of death (%) in KTR with function



Adult, first-time, kidney-only transplant recipients, 1995–2003, who died with functioning graft (*N*=10,648).
Cause of death obtained from OPTN when available, otherwise taken from the ESRD Death Notification form.

2006 ADR

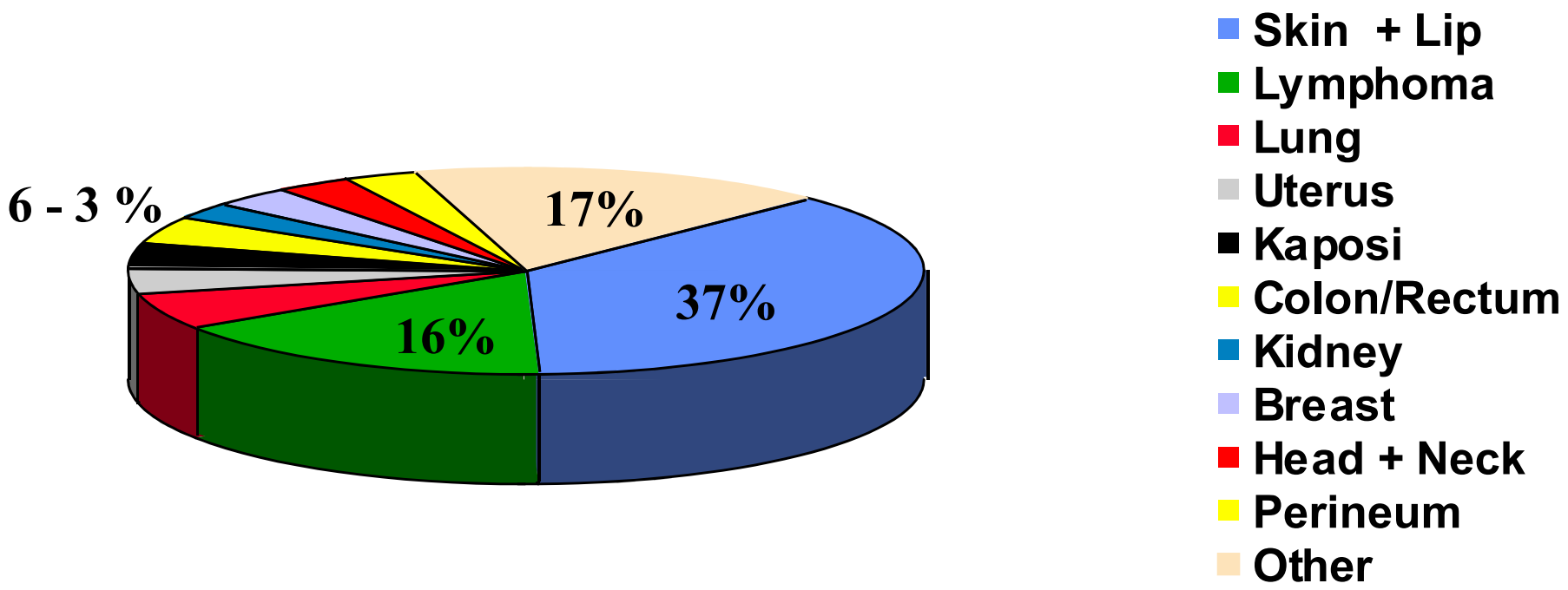
Risk of CVD in Renal Transplant Recipients

- CVD is the most common cause of death in renal transplant recipients[1]
- Approximately 75% to 80% of renal transplant recipients have 1 or more cardiovascular risk factors[2-4]
  - Hypertension
  - Diabetes mellitus
  - Hypercholesterolemia
  - Obesity (BMI >30)
  - Smoking
  - Left ventricular hypertrophy
  - Anemia (HCT <30%)
  - Reduced glomerular filtration rate
  - Proteinuria
  - Inflammation
- The annual rate of CVD-related death in renal transplant recipien is 3.5% to 5%[2]
  - Approximately 50 times greater than the rate in the general populat

CVD=cardiovascular disease; BMI=body mass index; HCT=hematocrit.

1. USRDS 2008 Annual Data Report. http://www.usrds.org/2008/pdf/V2_07_2008.pdf. Accessed February 25, 2009. **2.** Ojo AO. *Transplantation*. 2006;82:603-611. **3**. Fellstrom B et al. *Transplantation*. 2005;79:1160-1163. **4.** Vanrenterghem YFC et al. *Transplantation*. 2008;85:209-216.

Post Transplant Malignancies



Cincinnati Transplant Tumor Registry

Choice of Immunosuppressive Agents May Increase Risk of Selected Posttransplant Complications

- Ideally, immunosuppression should be individualized based on patient r factors and preexisting comorbidities[1,2]
  - Immunosuppression regimens should be selected bearing in mind the poten risks and benefits of each agent

	Calcineurin Inhibitors			
Risk	**CsA**	**Tacrolimus**	**Corticosteroids**	**MPA***
Increased risk of malignancy	+ → ++	+ → ++	+	+ → +
Increased cardiovascular risk	++	++	++	–
▪ **Hypertension**	+++	+	++	–
▪ **Diabetes**	+	+++	+++	–
▪ **Hyperlipidemia**	++	+	++	–
Renal dysfunction	+++	+++	–	–

This table is not a comprehensive list of all posttransplant complications associated with immunosuppressive agents.
All immunosuppressive agents increase risk of infection.
*Gastrointestinal adverse events, anemia, and leukopenia are associated more frequently with mycophenolate therapy.

1. Table adapted from Kirk AD et al. *Transpl Int*. 2005;18:2-14. 2. Giessing M et al. *World J Urol*. 2007;25:325-332.

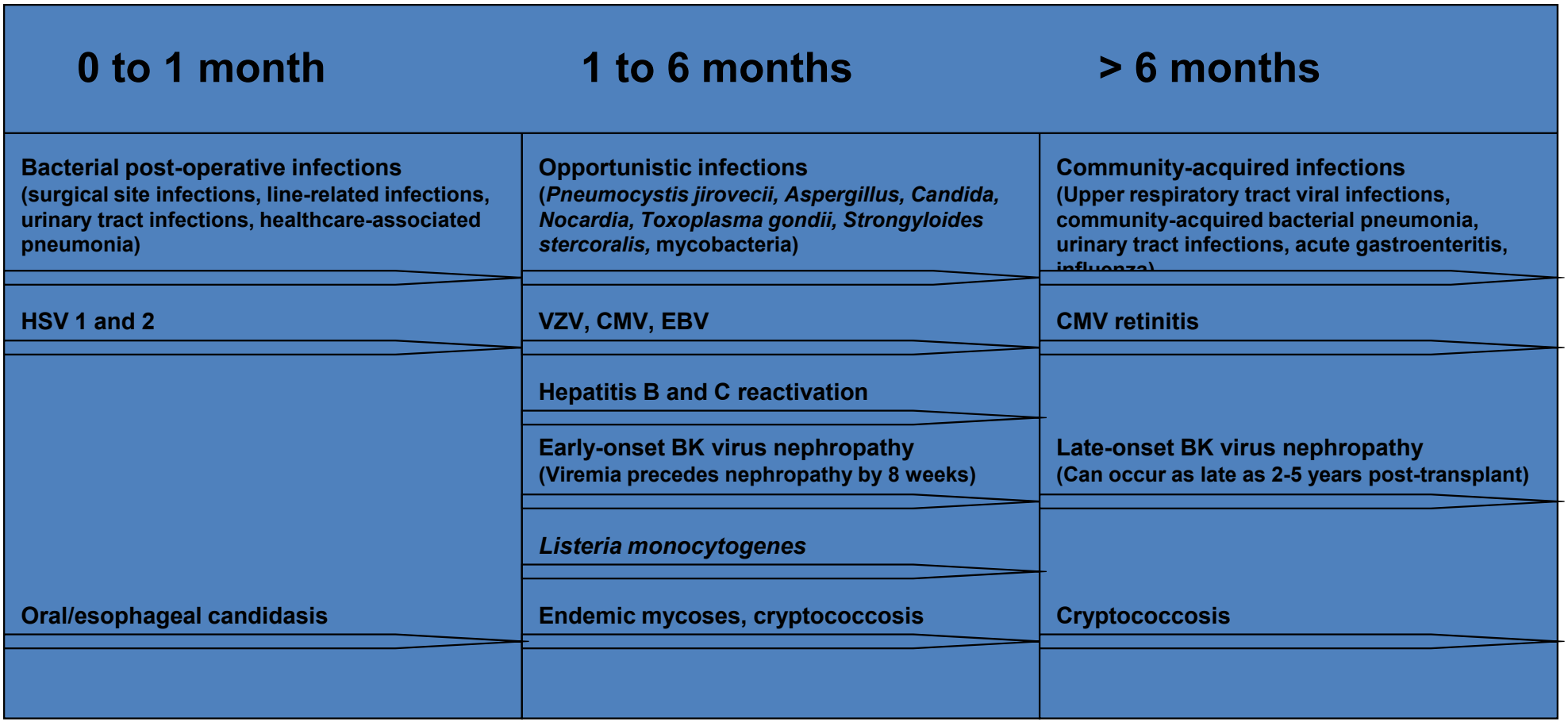
The natural history of chronic allograft nephropathy (Follow-up 119 kidney/pancreas transplant recipients by protocol biopsies up to 10 years)

NEJM 2003;349:2326

Table 2. Cumulative Kaplan–Meier Estimates of the Prevalence of Histologic Diagnoses, According to the Time after Transplantation.

Histologic Diagnosis	1 Yr	5 Yr	10 Yr
		percent	
Chronic allograft nephropathy			
Banff grade I	94.2	100.0	100.0
Banff grade II or III	24.7	65.9	89.8
Calcineurin-inhibitor nephrotoxicity	76.4	93.5	96.8
Arteriolar hyalinosis	62.0	90.3	100.0
Striped fibrosis	33.2	68.3	87.3
Tubular microcalcification	42.7	67.2	78.5

Timing of Infection After Transplant



Trimethoprim-sulfamethoxaxole (6 to 12 months—some centers continue for life)

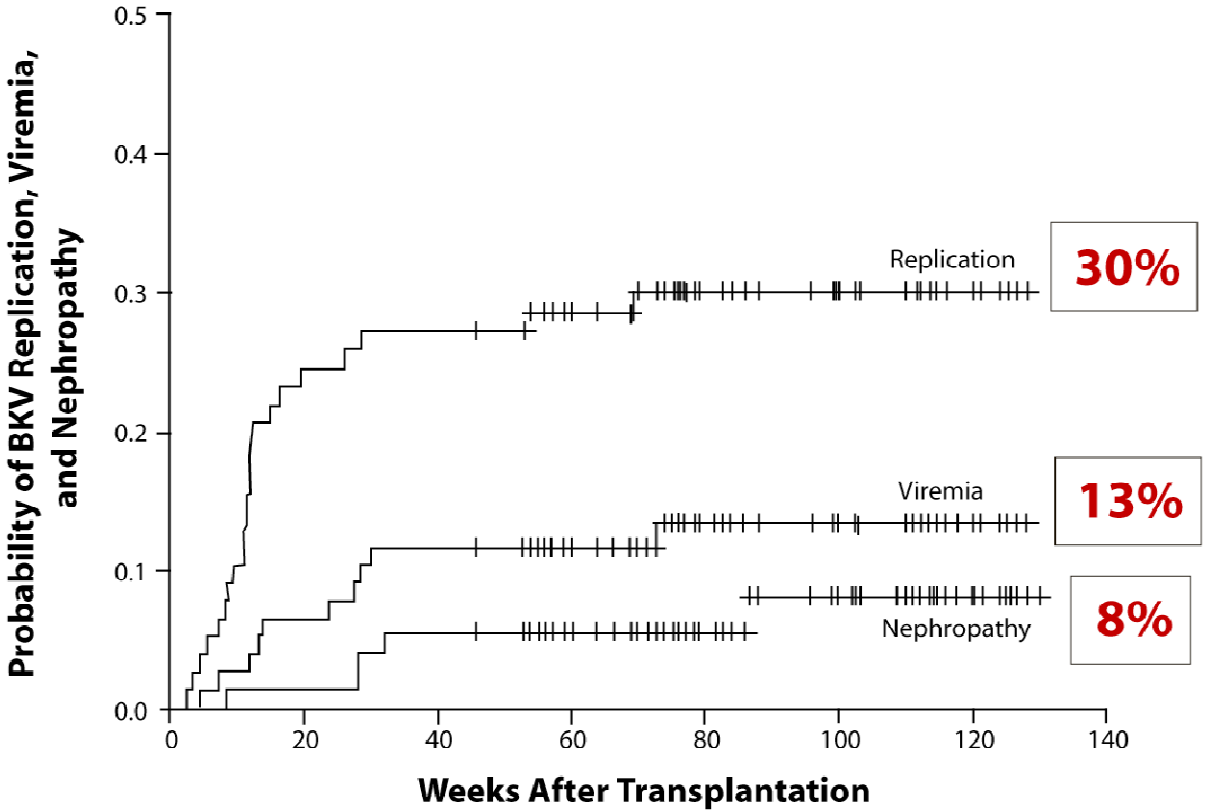
Oral clotrimazole lozenges, nystatin or weekly fluconazole(1 to 3 months)

Oral acyclovir, valacyclovir or valganciclovir (3 to 6 months)

Polyoma Virus Nephropathy

Hirsch HH, et al. *N Engl J Med*. 2002;347:488-496.

- First reported in 1995 and associated with polyomavirus type BK. JC virus (PMLE) and SV 40 in same family
- 90% seroprevalence rate worldwide
- Mainly the disease of kidney tx patients. Association with anti-rejection treatment and the degree of immunosuppression



Balancing Immunosuppressive Treatment

Too Much

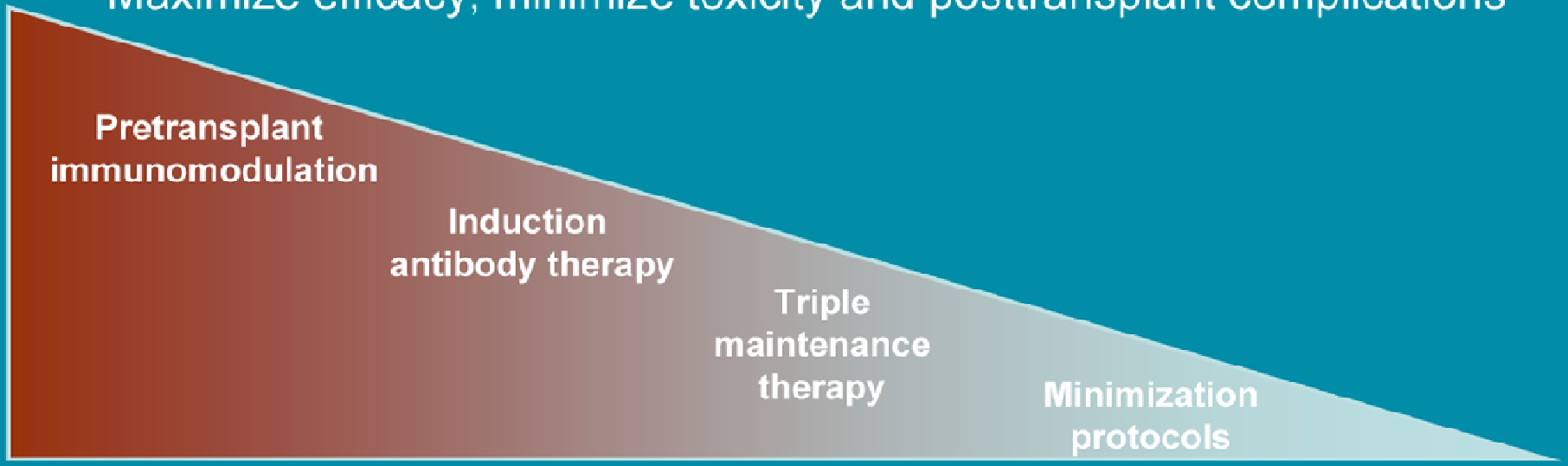
- *Infection*
- *Malignancy*
- *CVD*
- *Nephrotoxic*

Too Little

- *Allograft Rejection*

Individualizing Immunosuppression Therapy

- Overall immunosuppression goals[1,2]
  - Improve short- and long-term survival
  - Maximize efficacy, minimize toxicity and posttransplant complications



- **High immunologic risk**
  - Highly sensitized
  - Nonprimary transplant*
  - African American ethnicity
  - Deceased donor source
  - Poor HLA match
- **Low immunologic risk**
  - Nonsensitized
  - Asian ethnicity
  - Elderly
  - Living donor source
  - Good HLA match

*Loss to acute rejection; HLA=human leukocyte antigen

1. Srinivas TR et al. *Clin J Am Soc Nephrol* 2008;3:S101-S116; **2.** Patel J et al. *Transpl Immunol* 2008;20:48-54.

INCIDENCE OF ACUTE REJECTION IN MULTICENTER AND RANDOMIZED TRIALS REGARDING RAPID STEROID WITHDRAWAL

FREEDOM STUDY

(AJT 2007; 8:307)

Basiliximab induction, EC-MPS, and CsA-ME:

- No steroids (N=112) **36%**
- RSW at d7 (N=115) **29.6%**
- Standard steroids (N=109) **19.3%**

ASTELLAS RSW

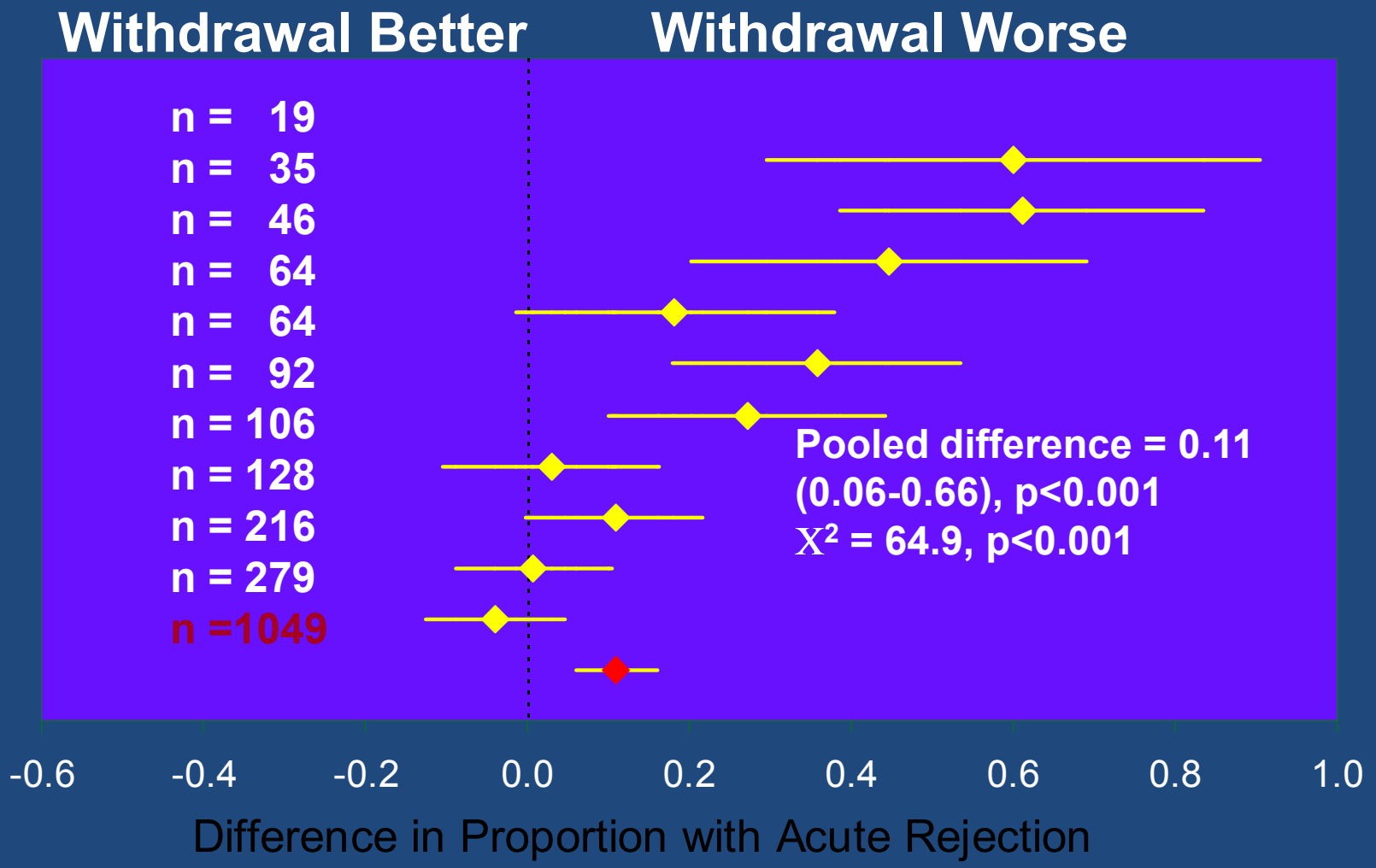
(Ann Surg 2008; 248:564)

Anti-IL2R Ab or Thymo, MMF, tacrolimus:

- RSW at 7 d (N=191) **17.8%**
  - Anti-IL2R Ab **24.2%**
  - Thymo **14.4%**
- Standard steroids (N= 195) **10.8%**
  - Anti-IL2R Ab **11.9%**
  - Thymo **10.3%**

Calcineurin Inhibitor Withdrawal Meta-analysis

Kasiske, et al. *J Am Soc Nephol 2000; 11:1910*



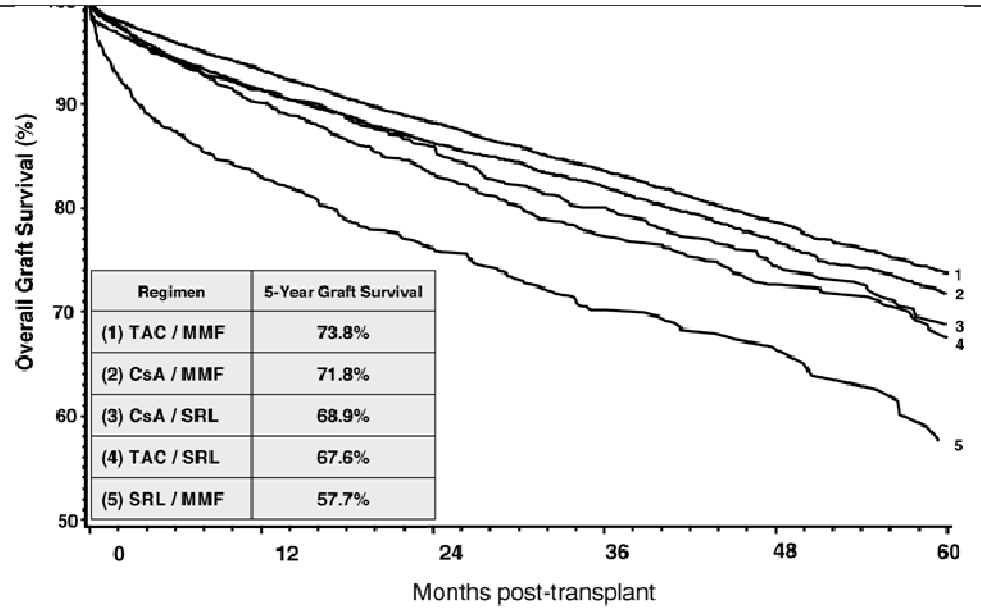
SIROLIMUS USE IN KIDNEY TRANSPLANTATION

Sirinivas et al. AJT 2007 Mar;7(3):586

	Year of transplant					
Regimen	2000	2001	2002	2003	2004	2005
TAC/MMF (%)	41.6	52.1	63.4	67.0	74.5	80.4
CsA/MMF (%)	44.9	29.9	21.6	17.6	12.9	9.4
TAC/SRL (%)	6.1	9.7	7.3	7.9	6.5	4.5
CsA/SRL (%)	6.0	4.1	2.8	3.5	2.9	2.8
SRL/MMF (%)	1.5	4.2	4.9	4.0	3.2	3.0

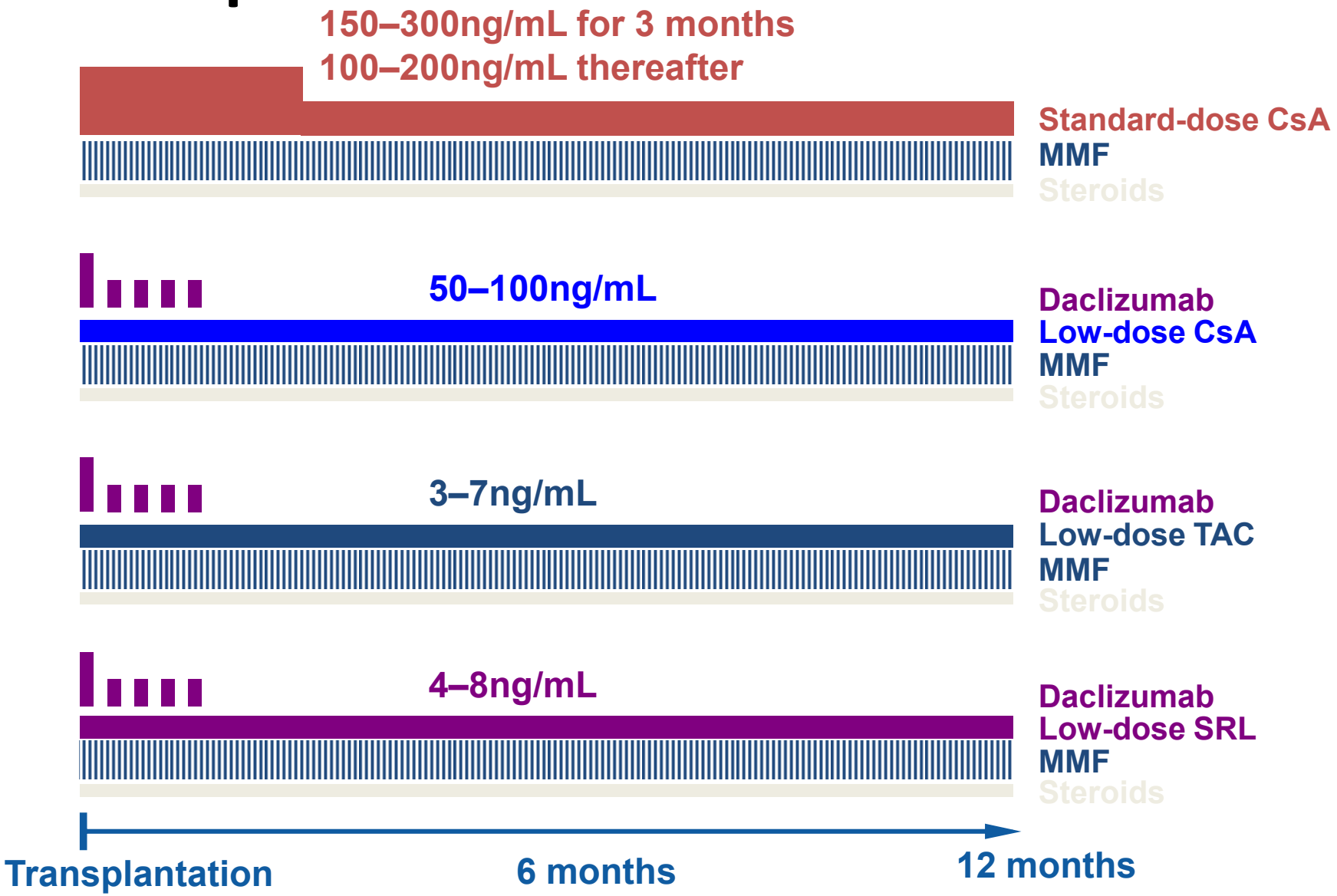
Baseline regimen	AOR	95% C.I.
TAC/MMF	Reference group	–
CsA /MMF	1.16	1.09–1.24
TAC/SRL	0.92	0.82–1.03
CsA/SRL	1.01	0.87–1.17
SRL/MMF	1.53	1.33–1.75

Immunosuppressant regimen	Adjusted hazard ratio for overall graft loss	95% C.I.	Adjusted hazard ratio for patient death	95% C.I.
TAC/MMF	Reference	–	Reference	–
CsA/MMF	1.16	1.10–1.22	1.17	1.09–1.26
CsA/SRL	1.37	1.22–1.53	1.49	1.29–1.72
TAC/SRL	1.38	1.27–1.50	1.33	1.19–1.49
SRL/MMF	2.01	1.83–2.22	1.75	1.53–2.00



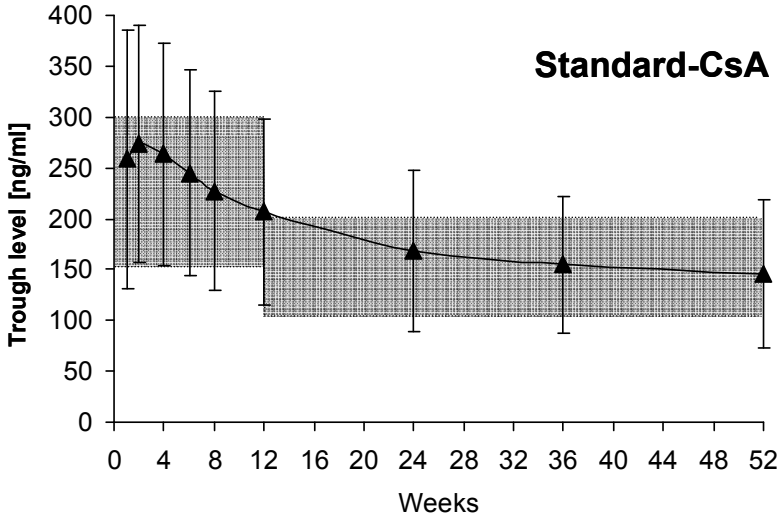
Symphony Study Design

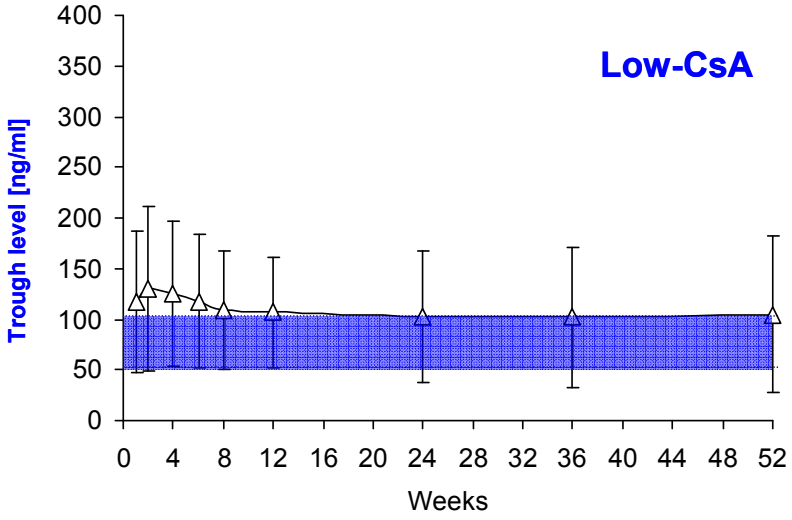
1645 patients at 83 sites in 15 countries

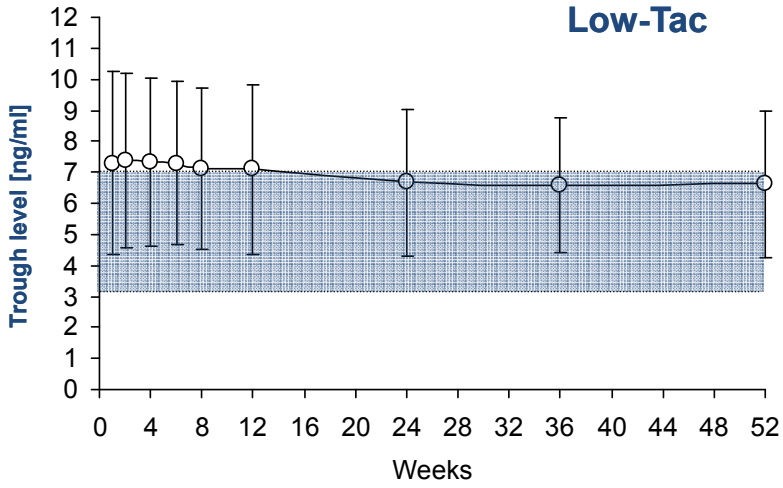


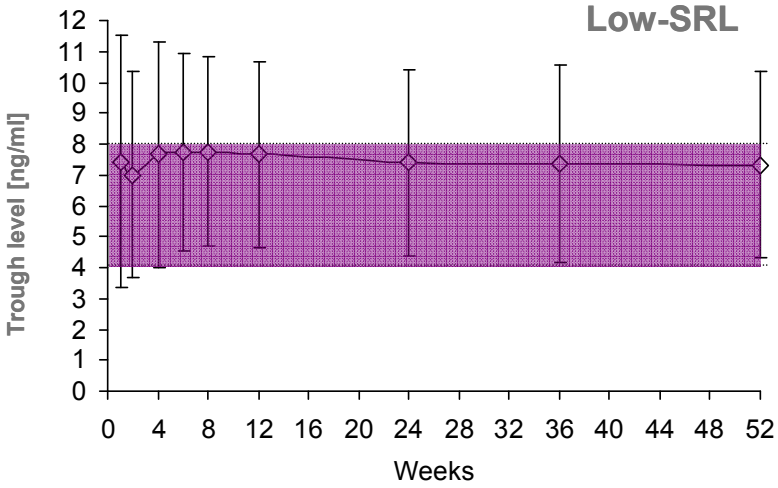
Ekberg H, et al. NEJM 2007; 357: 2562

Mean trough levels were within target ranges





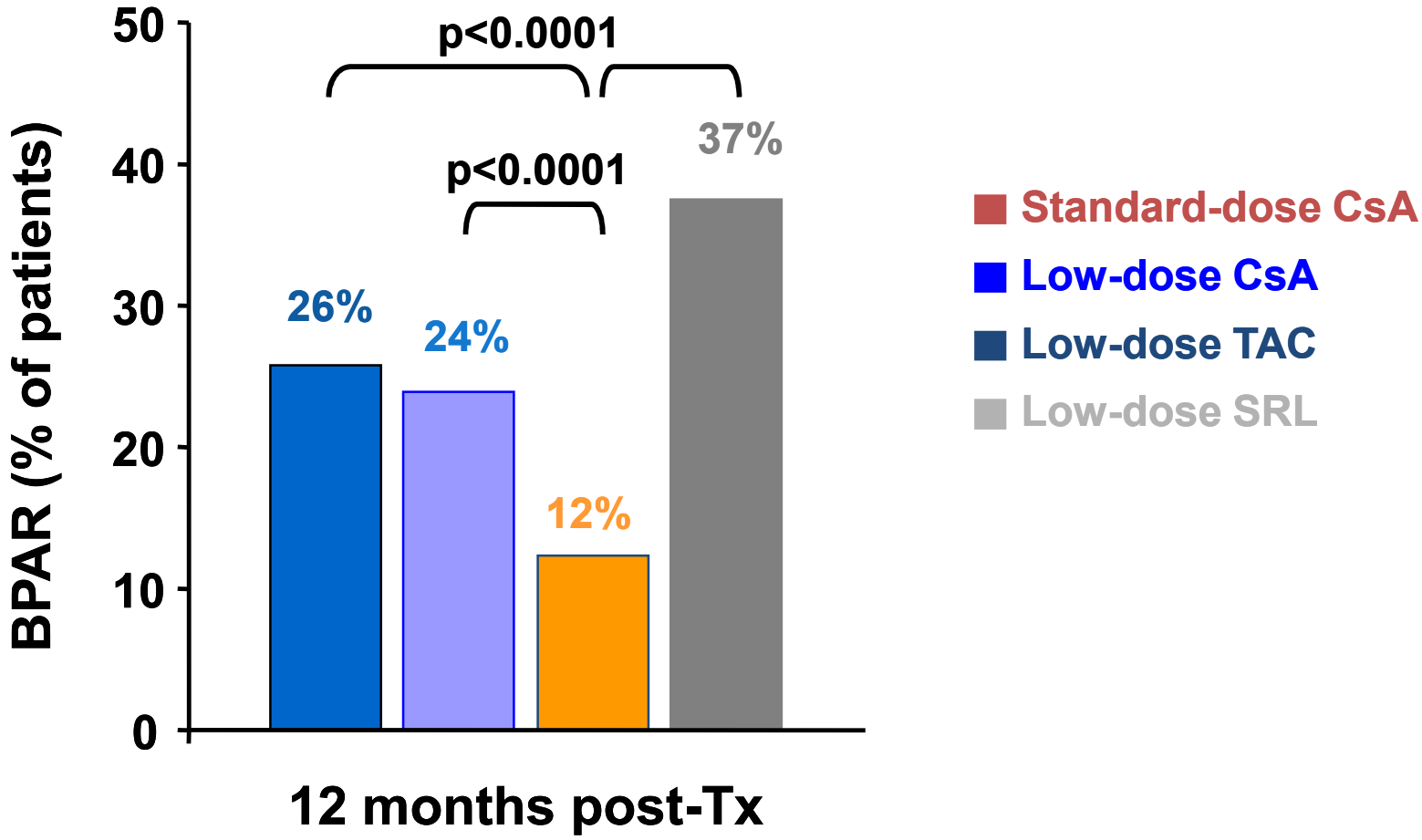




* means ± SD

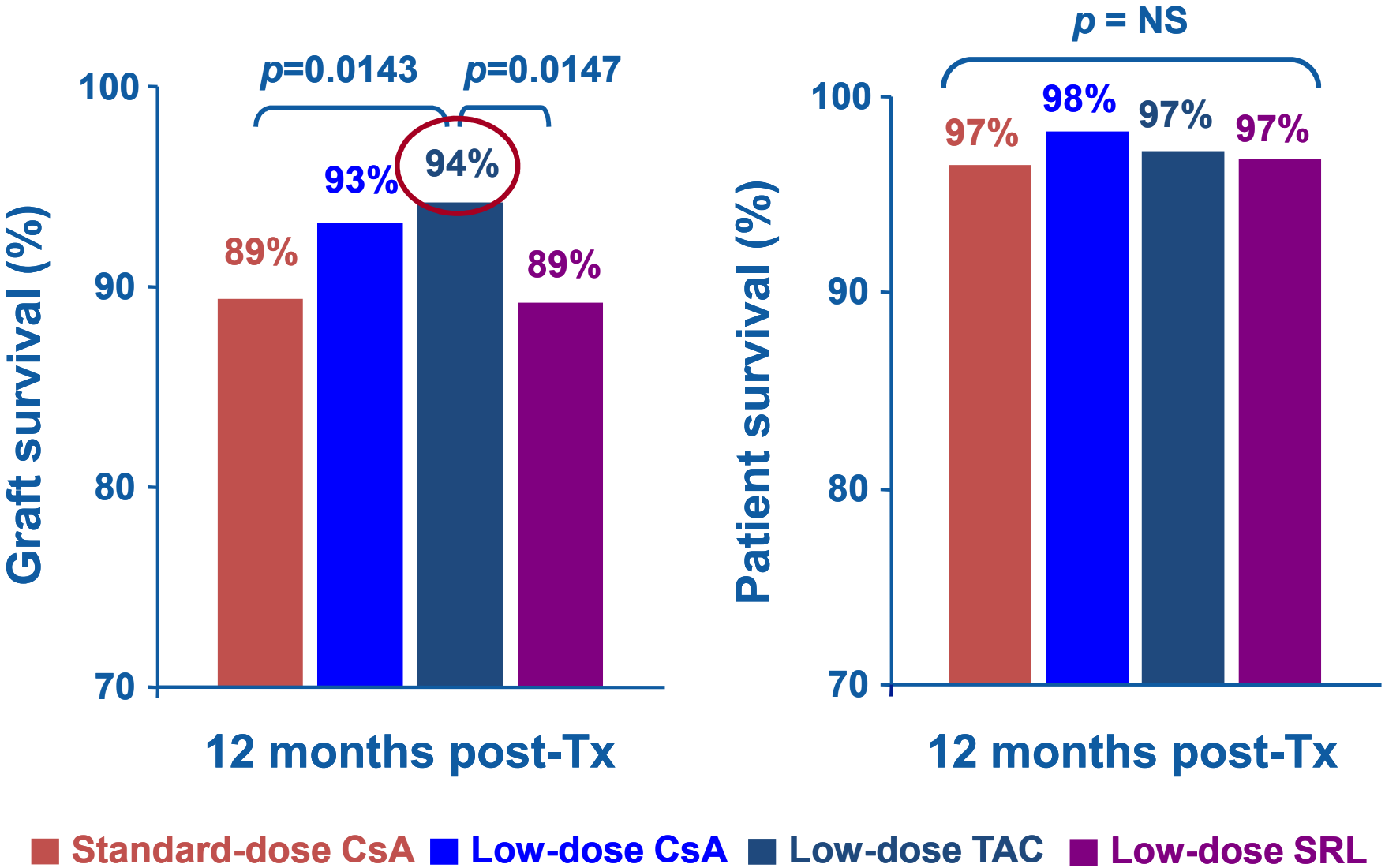
Ekberg H et al *NEJM* 2007; 357: 2562.

Less Biopsy Proven Acute Rejection with Low-dose Tac

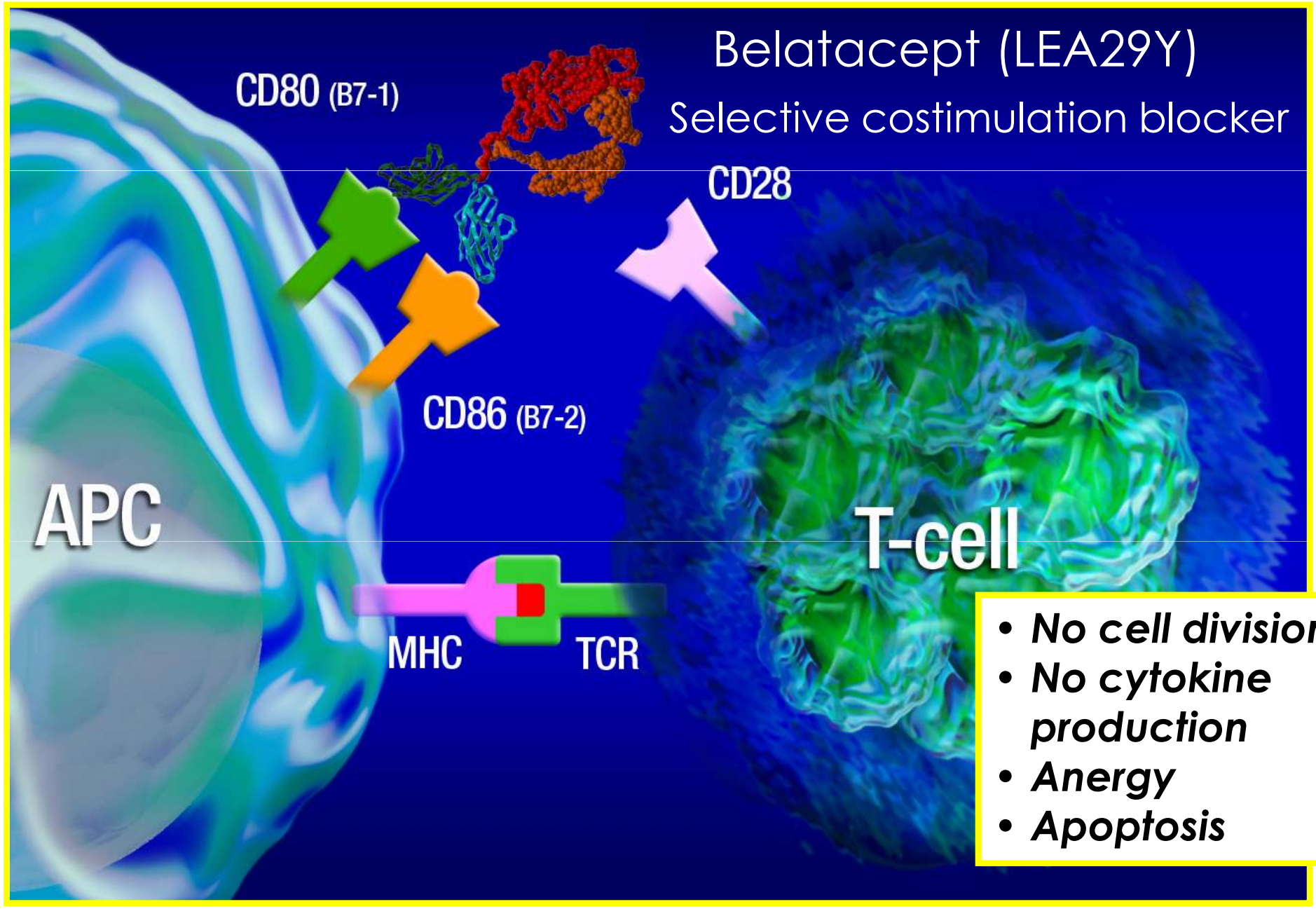


Ekberg H, et al. NEJM 2007; 357: 2562

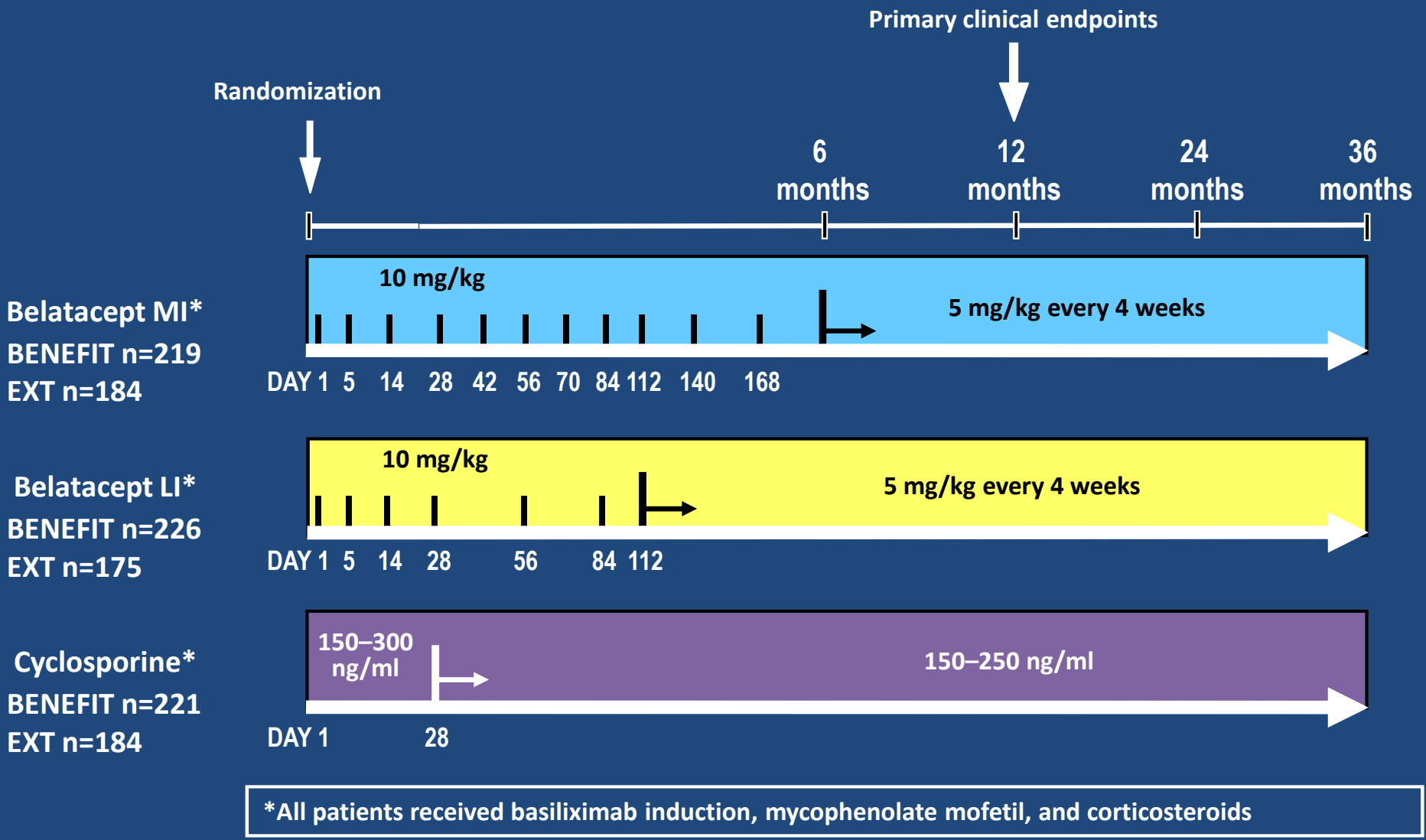
Graft Survival was superior with Low-dose Tac



Belatacept Potently and Selectively Blocks T-Cell Activation



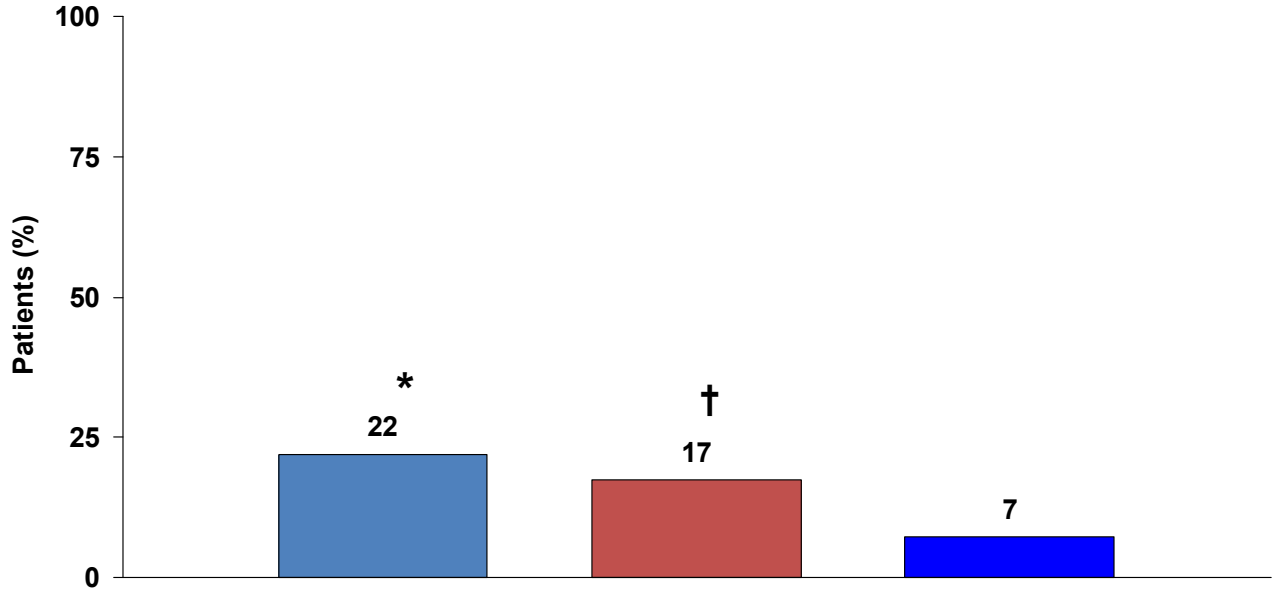
BENEFIT [Living and Standard Criteria Deceased Donors] and BENEFIT-EXT [Extended Criteria Donors] Phase 3 Clinical Trials of Belatacept in Kidney Transplantation



LI = less intensive; MI = more intensive.

Vincenti F, et al. *N Engl J Med*. 2005;353(8):770-781.

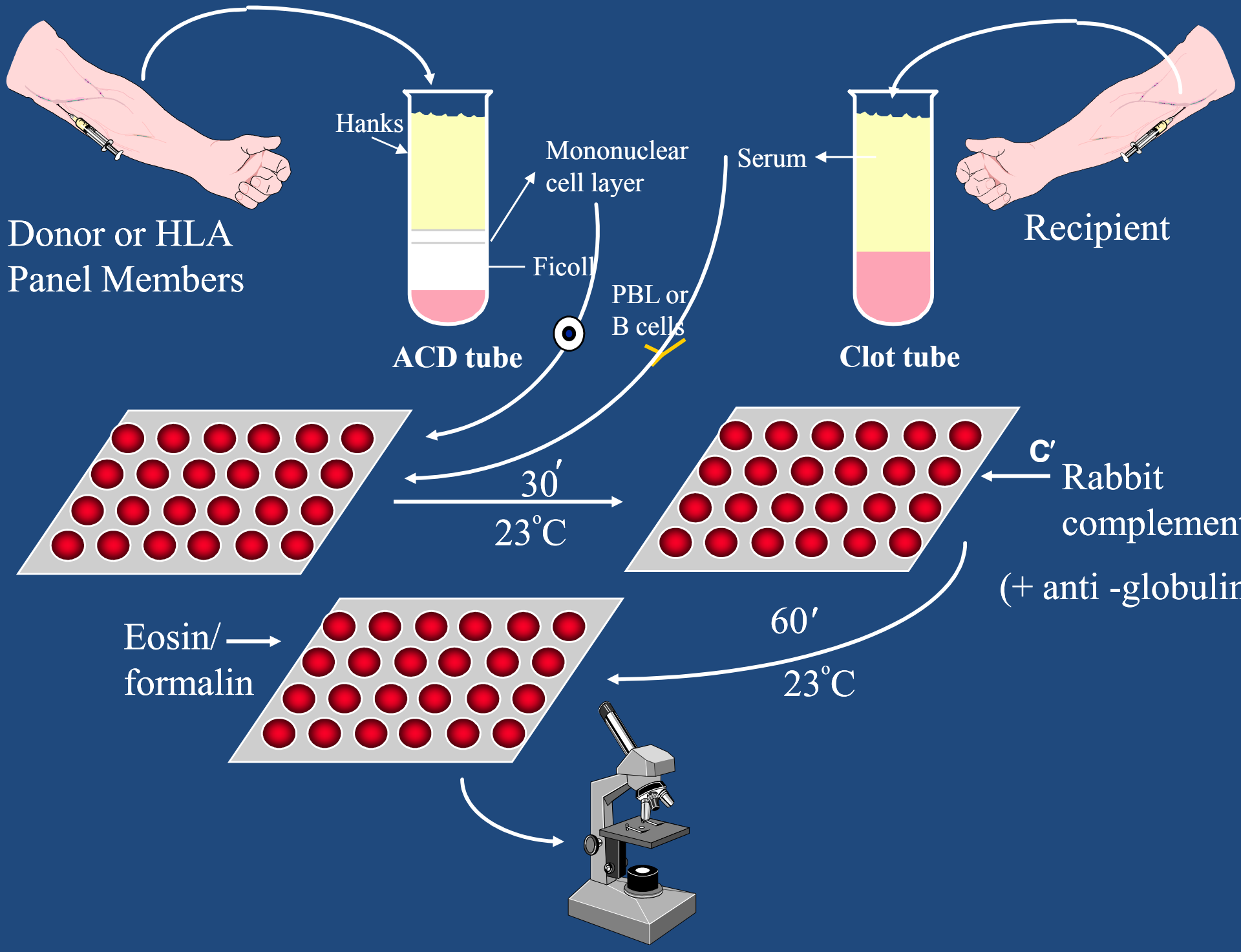
Incidence of Acute Rejection Episodes



Banff grade, n (%)	Belatacept MI	Belatacept LI	CsA
Mild acute (1A)	7 (3)	4 (2)	3 (1)
Mild acute (1B)	3 (1)	8 (4)	5 (2)
Moderate acute (IIA)	16 (7)	16 (7)	6 (3)
Moderate acute (IIB)	20 (9)	10 (4)	2 (1)
Severe acute (III)	2 (1)	1 (<1)	0

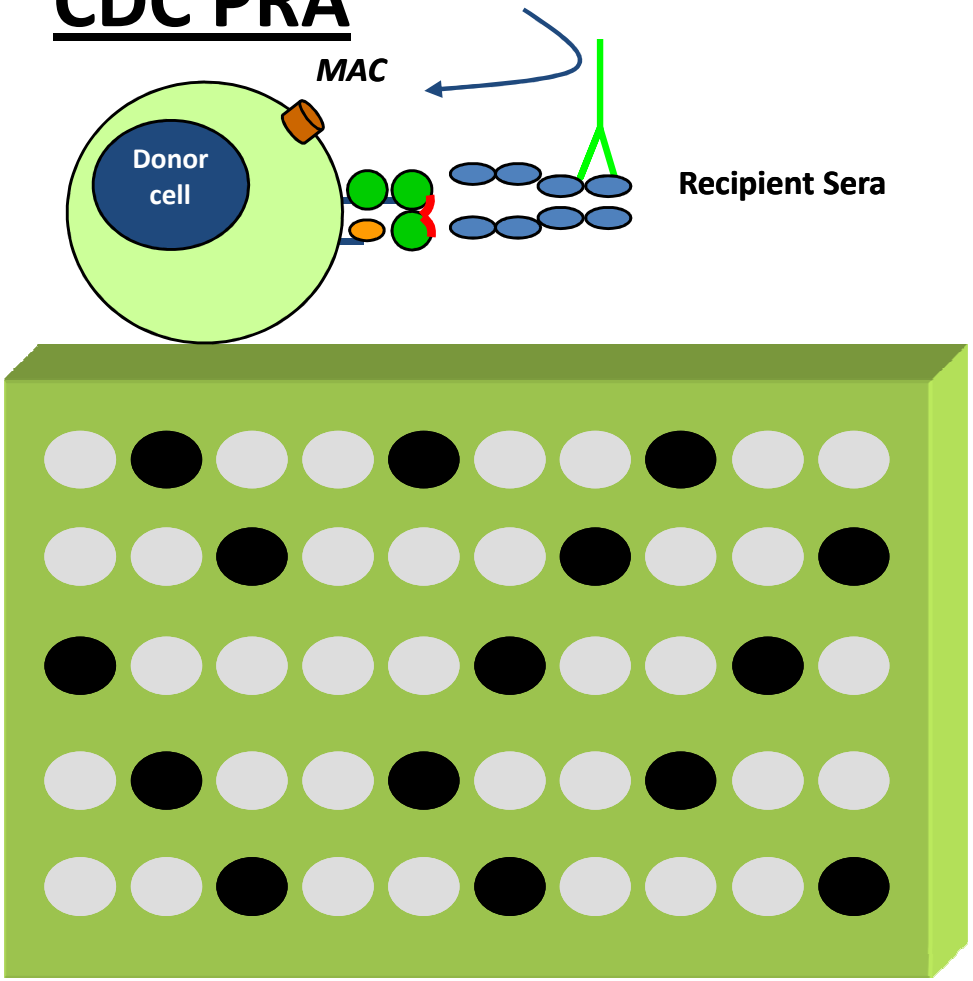
Pre-transplant Immunologic Risk Assessment (Humoral Immune Response)

	Assays
Detection of anti-HLA antibodies **PANEL REACTIVE ANTIBODY (PRA)**	-Cell based assays: Complement-dependent-cytotoxicity (CDC) (CDC PRA) -Solid phase assays: -ELISA -Luminex beads (Luminex PRA)
Cross-match	- CDC-Anti-Human Globulin (AHG-CDC) -CDC T cell CXM -CDC B cell CXM -Flow cytometry (FC) cross-match -FC T cell CXM -FC B cell CXM
Quantitative antibody measurement	- Antibody titer (CDC or FC) - FCXM – Semiquantitative (channel shift) - Luminex – Semiquantitative (Median Fluoresence Intensity; MFI)

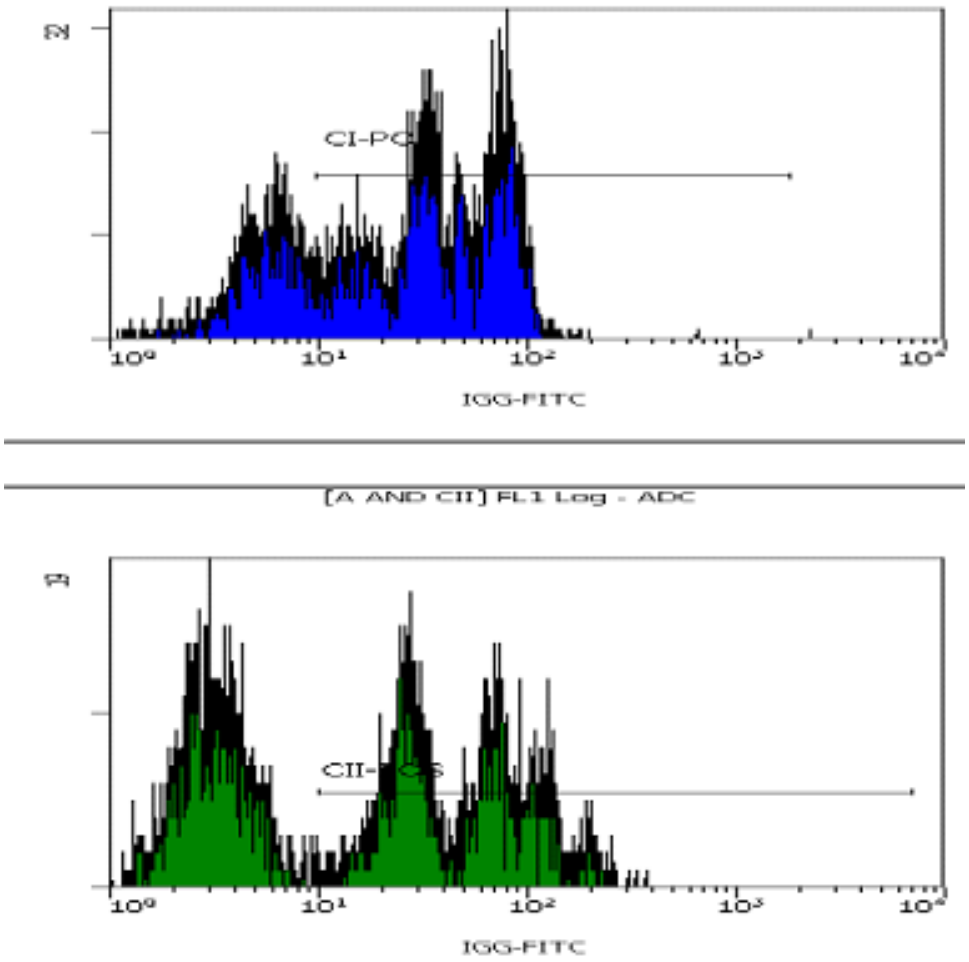

Donor or HLA
Panel Members
Hanks
Mononuclear
cell layer
Ficoll
ACD tube
PBL or
B cells
Serum
Clot tube
Recipient
30′
23°C
C′
Rabbit
complement
(+ anti -globulin)
60′
23°C
Eosin/
formalin

Panel Reactive Antibody (PRA)

CDC PRA



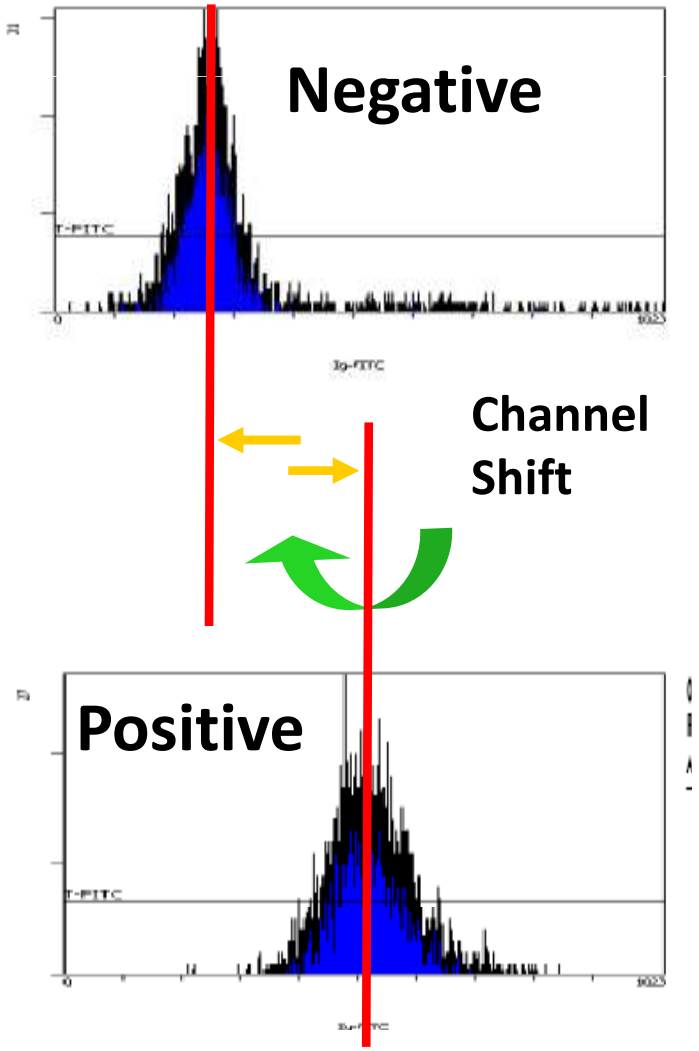
LUMINEX PRA



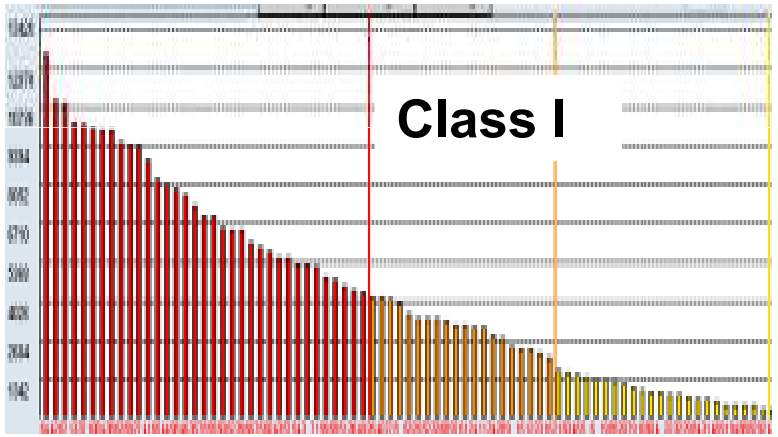
Quantitating Antibody: Flow Cytometry and Luminex Single Antigen Bead Assays

Flow Cytometry Positive Readout:
Median Channel Shift

T cell cross match >50 / B cell cross match >150



Luminex Single Antigen Bead Assay
Readout: Mean Fluorescence Intensity

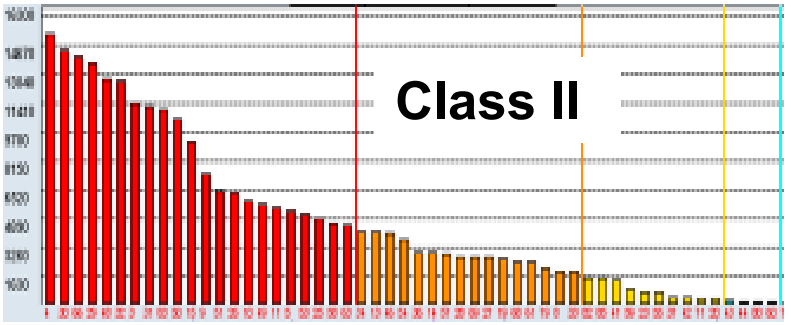


Epitope Analysis Results

Spec.	>= X6	< X6	Mean
B50	1	0	12238.4
B52	1	0	1064...
B71	1	0	1064...
A25	1	0	1000...
B64	1	0	9909.77
A3	1	0	9744.72
A26	1	0	9711.84

MFI

Spec.	>= X6	< X6	Mean
DR1	2	0	1310...
	5	0	1293...
	1	0	1088...
	1	0	9065.36
DP4	2	0	8383.75
DP2	2	0	7538.98
DP3	1	0	5773.52



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Perception Versus Reality?: Virtual Crossmatch—How to Overcome Some of the Technical and Logistic Limitations

A. R. Tambur[a,b,*], D. S. Ramon[a], D. B. Kaufman[b], J. Friedewald[c], X. Luo[c], B. Ho[c], A. Skaro[b], J. Caicedo[b], D. Ladner[b], T. Baker[b], J. Fryer[b], L. Gallon[c], J. Miller[b], M. M. Abecassis[b] and J. Leventhal[b]

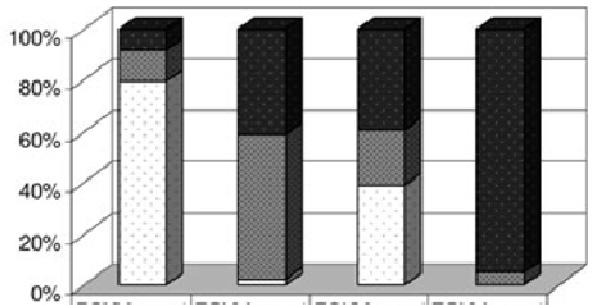
[a] *Transplant Immunology Laboratory,* [b] *Division of Organ Transplantation, Department of Surgery,* [c] *Department of Internal Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL*
* *Corresponding author: Anat R. Tambur, a-tambur@northwestern.edu*

Introduction

The introduction of solid-phase-based methods for detecting anti-HLA antibodies has been a significant technical advance that has increased the specificity and sensitivity of detecting antibodies directed against HLA class I and class II antigens (1,2). Some concerns, however, have been expressed regarding the utility of applying these tests as a method to predict a 'true positive' actual crossmatch (XM) in the clinical scenario. Several studies, including abstracts from scientific meetings, suggest that disparate sets of guiding principles have been applied by different laboratories to define the presence of HLA

	Virtual/DSA	
	Positive	Negative
Actual/FCXM		
Positive	515	28
Negative	83	854

Sensitivity = 86.1%; specificity = 96.8%; positive predictive value; = 94.8%; negative predictive value = 91.1%.



	FCXM neg / vXM neg	FCXM neg / vXM pos	FCXM pos / vXM neg	FCXM pos / vXM pos
PRA >80%	71	34	11	488
PRA 20-80%	104	47	6	27
PRA <20%	679	2	11	0

	Total	%			
FCXM positive/ DSA positive	515	100			
Strong DSA	147	28.5			
Moderate DSA	279	54.2			
Weak DSA	87	16.9	Single weak	33	6.41%
FCXM negative/ DSA positive	83	100			
Strong DSA	5	4.8			
Moderate DSA	36	42.2			
Weak DSA	44	53.0	Single weak	35	42.17%

Table 6: The likelihood of having a positive actual FCXM based on DSA strength

Antibody strength	Likelihood of positive FCXM
Strong DSA	97%
Moderate DSA	86%
Weak DSA	66%
Multiple weak DSA	86%
Single weak DSA	49%

Variation in Results by Choice of Anti-HLA Antibody Detection Technique

Method	Positive	Negative
CDC	102	162
AHG-CDC	116 (+13%)	148
ELISA	127 (+10%)	137
Flow-PRA	139 (+10%)	125

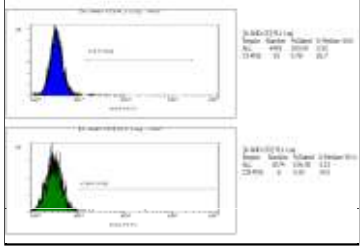
Gebel, HM & Bray, RA. Transplantation. 2000;69: 1370

Strategies to Identify Anti-HLA Antibodies

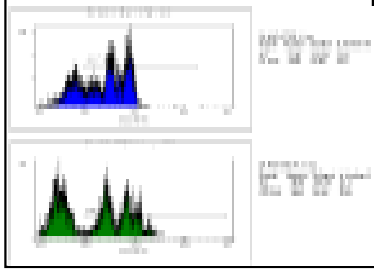
Patient's sample

Flow PRA screening

Negative



Positive



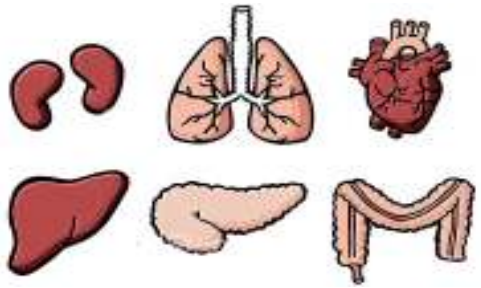
Luminex Single Ag

Interpretation:

Match with Patient and Donor HLA typing

Unacceptable HLA Ag to UNOS for DD match

DSA Pre- and Post-Tx



Basic Concepts in Desensitization

Removal of existing antibodies:

- Plasmapheresis
- Immunoadsorption

Depletion of antibody producing cells:

- Naïve and memory B cells – Rituximab (anti-CD20)
- Plasma cells – Bortezomib (proteosomal inhibitor)

Inhibition of residual antibody and complement system cascade:

- Intravenous Immunoglobulin (IVIg)
- Eculizumab (C5 inhibitor)

Suppression of the T cell response

- Induction agents
- Triple immunosupression with CNI, mycophenolate, and steroids

Outcomes in Kidney Recipients Receiving Desensitization Treatment 2000-2010

Author/Year PP/ Low-Dose IVIG	N	Follow up (Months)	Patient Survival	Graft Survival	Acute Rejection	AMR
Schweitzer 2000	11	13	100%	100%	36%	27%
Montgomery 2000	4	14	100%	100%	100%	100%
Gloor 2003	14	15	86%	78%	43%	43%
Magee 2008	28	22	93%	89%	42%	39%
Thielke 2009	51	23	95%	93%	43%	33%
High Dose IVIG						
Jordan 2003	43	24	98%	89%	31%	31%
Akalin 2003; 2005	17	15	100%	88%	18%	18%
Vo 2006	58 39	24 24	96% 100%	84% 90%	36% 31%	22% 21%
Vo 2008	54 16	14 12	98% 100%	96% 94%	35% 50%	20% 31%
Mai 2009	20	36	94%	89%	50%	30%
Bachler 2010	37	24	95%	87%	38%	38%
Vo 2010	76	24	95%	84%	37%	29%

Outcomes in Kidney Recipients Receiving Desensitization Treatment 2000-2010

- 21 published studies
- All single center and retrospective studies
- Total 725 patients
- Mean follow-up 23 months
- Patient survival 95%
- Graft survival 86%
- Acute rejection 36%
- Acute antibody-mediated rejection 28%

SINAI MEDICAL CENTER

Akalin et al. Transplantation 2003; 76:1444 and 2005; 79: 742

Akalin et al. CJASN 2008; 3: 1160

Median Age	51 (24-76)
Sex (Female %)	74%
Race (African-American %)	39%
Transplant type (living %)	63%
Previous transplant	33%
Median peak PRA	60% (10-100)
Pre-transplant cross-match	
CDC T cell	0%
CDC B cell	37%
Flow not done (DSA+)	27%
Flow T cell	59% (Flow Chd 110 ± 65)
Flow B cell	86% (Flow Chd 262 ± 92)
DSA	
Class I only	33%
Class II only	27%
Class I and II	40%
Mean # of DSAs	2.5 ± 0.9
Desensitization protocol	
Low-dose IVIG(n=10)	14%
High-dose IVIG (n=40)	57%
High-dose IVIG and PP (n=20)	29%

Clinical Outcomes per Luminex MFI Values

	IVIG only DSA MFI < 6,000 (n=33)	IVIG only DSA MFI > 6,000 (n=17)	IVIG/PP DSA MFI>6,000 (n=20)
Median F/U (mos)	30 (4-80)	40 (14-53)	16 (12-28)
Patient survival	100%	100%	90%
Graft survival	97%	65%	75%
Living	100%	67%	88%
Deceased-donor	88%	64%	67%
Acute rejection	0%	59%	20%
AMR	0%	47%	15%
ACR	0%	12%	5%
Biopsy proven CAN	6%	36%	20%
Transplant glomerulopathy	6%	12%	10%
Median Cr (mg/dl)	1.1 (0.6-3.1)	1.2 (1.0-3.1)	1.4 (0.8-1.9)
Patients with Cr < 1.4	81%	73%	87%
DSA loss during F/U	77%	31%	36%

Akalin et al. Transplantation 2003; 76:1444 and 2005; 79: 742
Akalin et al. CJASN 2008; 3: 1160

IVIG+Rituximab Article.pdf - Adobe Reader
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Use of Intravenous Immune Globulin and Rituximab for Desensitization of Highly HLA-Sensitized Patients Awaiting Kidney Transplantation

Ashley A. Vo,[1] Alice Peng,[1] Mieko Toyoda,[2] Joseph Kahwaji,[1] Kai Cao,[3] Chih-Hung Lai,[3] Nancy L. Reinsmoen,[3] Rafael Villicana,[1] and Stanley C. Jordan[1]

Background. We have shown that high-dose intravenous immune globulin (IVIG; 2 g/kg ×2 doses)+rituximab (1 g ×2 doses) was effective in lowering anti-human leukocyte antigen (HLA) antibodies and improving rates of transplantation. The aim of this report was to evaluate the efficacy of IVIG+rituximab on reduction of anti-HLA antibodies to a level that was permissive for living donor (LD) or deceased donor (DD) transplantation without incurring the risk of antibody-mediated rejection and immediate graft loss.

Methods. From July 2006 to February 2009, 76 HLA-sensitized (HS) patients who met strict sensitization criteria received kidney transplants after desensitization using IVIG 2 g/kg (days 1 and 30)+rituximab (1 g, day 15). Parameters evaluated included rates of transplantation, previous transplants, panel reactive antibodies, donor specific antibody, crossmatches (CMXs), patient and graft survival, acute rejection, serum creatinines, and infections.

Results. Seventy-six HS CMX^+ treated patients (31 LD/45 DD) were transplanted. For LD and DD recipients, significant reductions were seen in T-cell flow cytometry CMXs from pretreatment (T cell 183.5±98.4 mean channel shifts (MCS) for LD and 162.8±41 MCS for DD) to time of transplant (T cell 68.2±58 MCS for LD [$P<0.00006$] and 125±49 for DD [$P=0.05$]), respectively. Time on wait list for DD recipients was reduced from 95±46 months to 4.2±4.5 months after treatment. Twenty-eight patients (37%) experienced acute rejection (29% $C4d^+$/8% $C4d^-$). Patient and graft survival up to 24 months was 95% and 84%, respectively. The mean serum creatinines, at 12 and 24 months were 1.5±1.1 and 1.3±0.3 mg/dL, respectively. Viral infections were seen in six patients.

Conclusions. IVIG and rituximab seems to offer significant benefits in reduction of anti-HLA antibodies allowing improved rates of transplantation for HS patients, especially those awaiting DD, with acceptable antibody-mediated rejection and survival rates at 24 months.

Keywords: Rituximab, IVIG, Highly sensitized, Alemtuzumab, Antithymocyte globulin, Daclizumab, Acute cell–mediated rejection, Acute antibody-mediated rejection, Kidney transplant.

(*Transplantation* 2010;X: 000–000)

TABLE 3. AR episodes and graft losses in the patients receiving transplants after desensitization

Patient DSA characteristics	Outcomes	Significance
Total AR episodes	28/76 (37%)	
Total CMR episodes (3DD/3LD)	6/76 (8%)	
Total graft loss to CMR	0/6 (0%)	
Total AMR episodes (11DD/11LD)	22/76 (29%)	
AMR+ (DSA <100,000 SFI units)[a]	5/22 (23%)	
AMR+ (DSA >100,000 SFI units)[a]	17/22 (77%)	[b]$P<0.0000004$
AMR− (DSA <100,000 SFI units)	42	
AMR− (DSA >100,000 SFI units)	12	
Graft loss to AMR by DSA	7/22 (32%)	
Graft loss to AMR by DSA <100,000 SFI units	2/5[c] (40%)	
Graft loss to AMR by DSA >100,000 SFI units	5/17[c] (29%)	P=NS

Relationship of AMR to DSA levels at time of transplant is also shown.

[a] DSA values obtained at time of transplant.

[b] Statistics is by χ^2 analysis.

[c] This data include two patients with late AMR because of noncompliance 12 mo posttransplant.

AR, acute rejection; AMR, antibody-mediated rejection; DSA, donor-specific antibody; SFI, standard fluorescence intensity; CMR, cell mediated

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Blackwell Munksgaard

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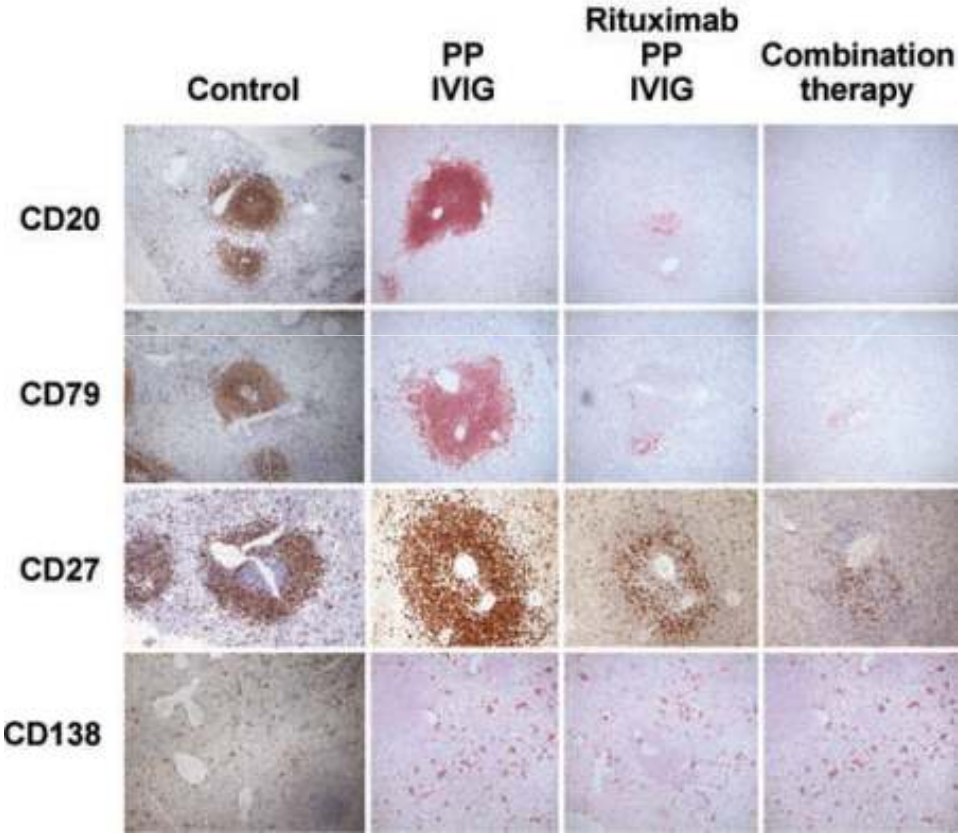
The Effect of Desensitization Protocols on Human Splenic B-Cell Populations *In Vivo*

E. J. Ramos[a], H. S. Pollinger[a], M. D. Stegall[a,*], J. M. Gloor[b], A. Dogan[c] and J. P. Grande[c]

[a]*Division of Transplantation Surgery,* [b]*Division of Nephrology and Hypertension and* [c]*Department of Pathology, von Liebig Transplant Center, Mayo Clinic College of Medicine, Rochester, MN*
**Corresponding a*
stegall.mark@may

or donor HLA (1–10). Some of these protocols have included agents such as intravenous immunoglobulin (IVIG), rituximab and rabbit antithymocyte globulin (rATG) because of their perceived effect on B cells. The aim of the current study was to assess the impact of desensitization protocols involving these agents on splenic B-cell subsets *in vivo*.

Immunostain Intensity of Splenic Follicles



Perry DK et al. AJT 2008; 8 : 133

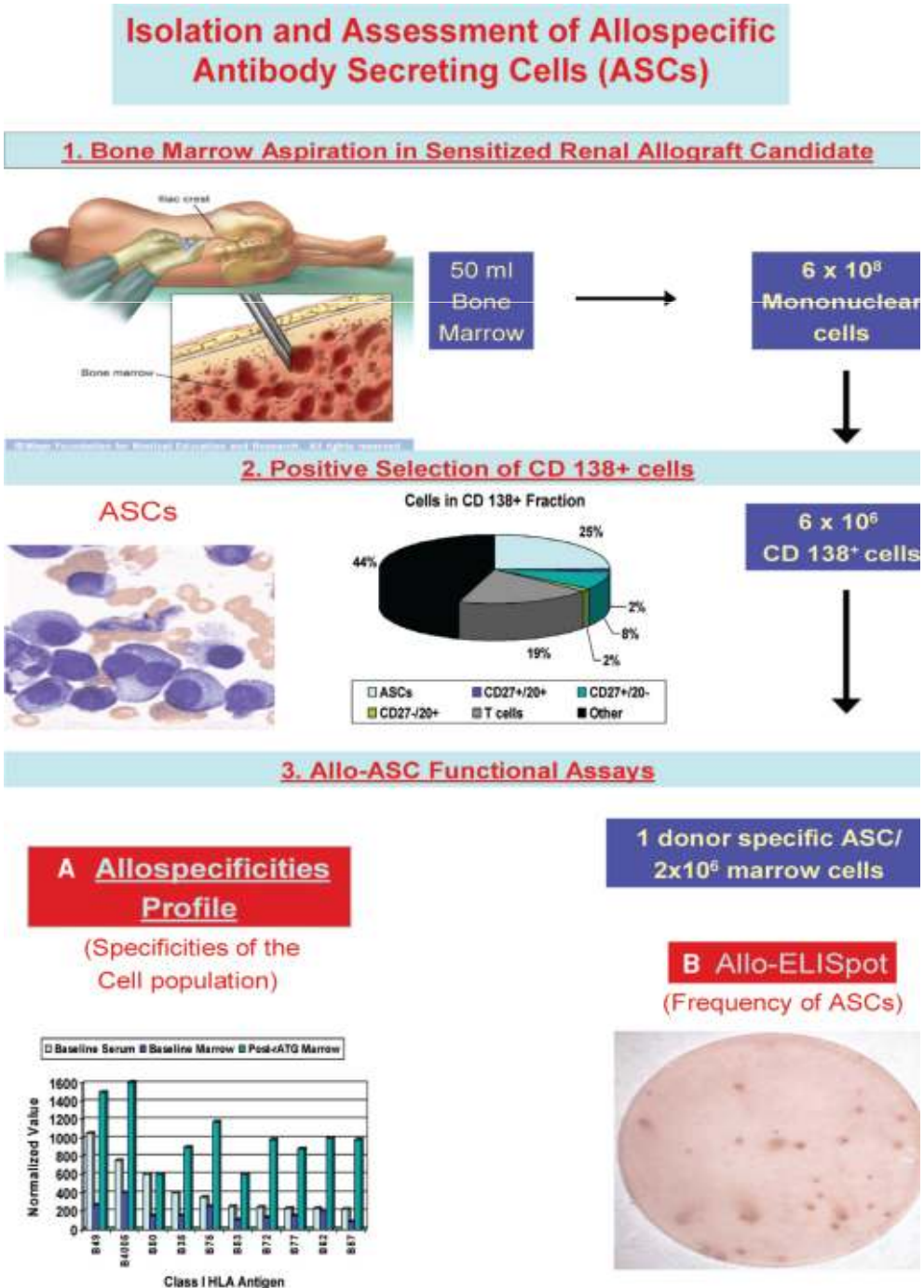


Table 2: Effect of desensitization therapy on bone marrow derived ASCs

Paired samples (n = 6)	Baseline	Post rATG	p-Value
PC/mL	17789.8 ± 12086.2	19374.8 ± 7961.6	NS
Tetanus and alloantibody ELISpots/1 × 10^6 cells	T: 74.0 ± 30.8 A: 49.3 ± 23.5	T: 86.0 ± 37.0 A: 50.0 ± 24.6	NS
Alloantibody specificity profile	69 of 107 (65%)	87 of 107 (81%)	NS
Loss serum DSA	0	0	1.00

Values in mean ± standard deviation.
Paired marrow samples pre and posttreatment (desensitization with multiple PP, low-dose IVIG and rATG prior to transplantation) had no effect on the percentage of CD138+ cells in the marrow and their function as determined by the number of tetanus and allospecific cells in the ELISpot assays and the ASC alloantibody specificity assay. All ASC HLA specificities measured pretreatment were present posttreatment in all six patients studied.

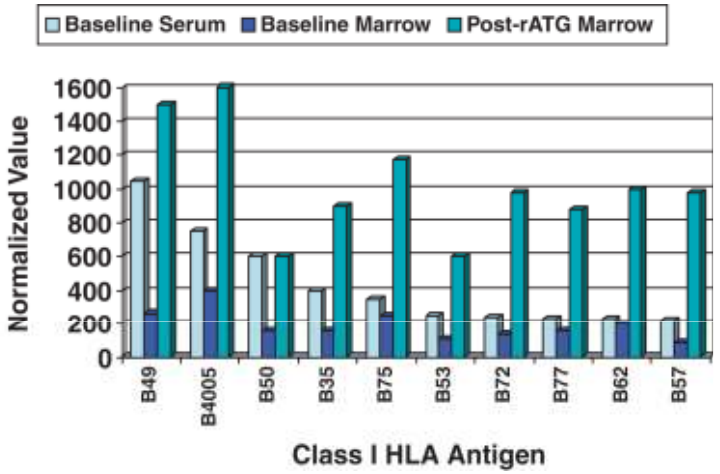


Figure 7: This represents a typical example of a paired marrow sample in a patient that received pretransplant PP, IVIG and rATG. There is no loss of alloantibody after desensitization when comparing the allospecificities profile before and after therapy. There is a high correlation with the serum antibody detected.

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Proteasome Inhibition Causes Apoptosis of Normal Human Plasma Cells Preventing Alloantibody Production

D. K. Perry[a], J. M. Burns[a], H. S. Pollinger[a], B. P. Amiot[d], J. M. Gloor[b], G. J. Gores[c] and M. D. Stegall[a,*]

[a]*Division of Transplantation Surgery, Department of Surgery,* [b]*Division of Nephrology and Hypertension and* [c]*Division of Gastroenterology and Hepatology, Department of Internal Medicine, von Liebig Transplant Center, Mayo Clinic College of Medicine, Rochester, MN*
[d]*Brami Biomedical, Incorporated, Minneapolis, MN*

Introduction

Antibodies are an integral component of the body's immune system (1). However, antibody production by normal plasma cells (PCs) is an important component of many human diseases (2,3), and antibody against human leukocyte antigens (HLA) may present a major barrier to the successful transplantation of kidneys and hearts. Novel protocols have been developed to allow for the successful transplantation of sensitized renal transplant candidates, however,

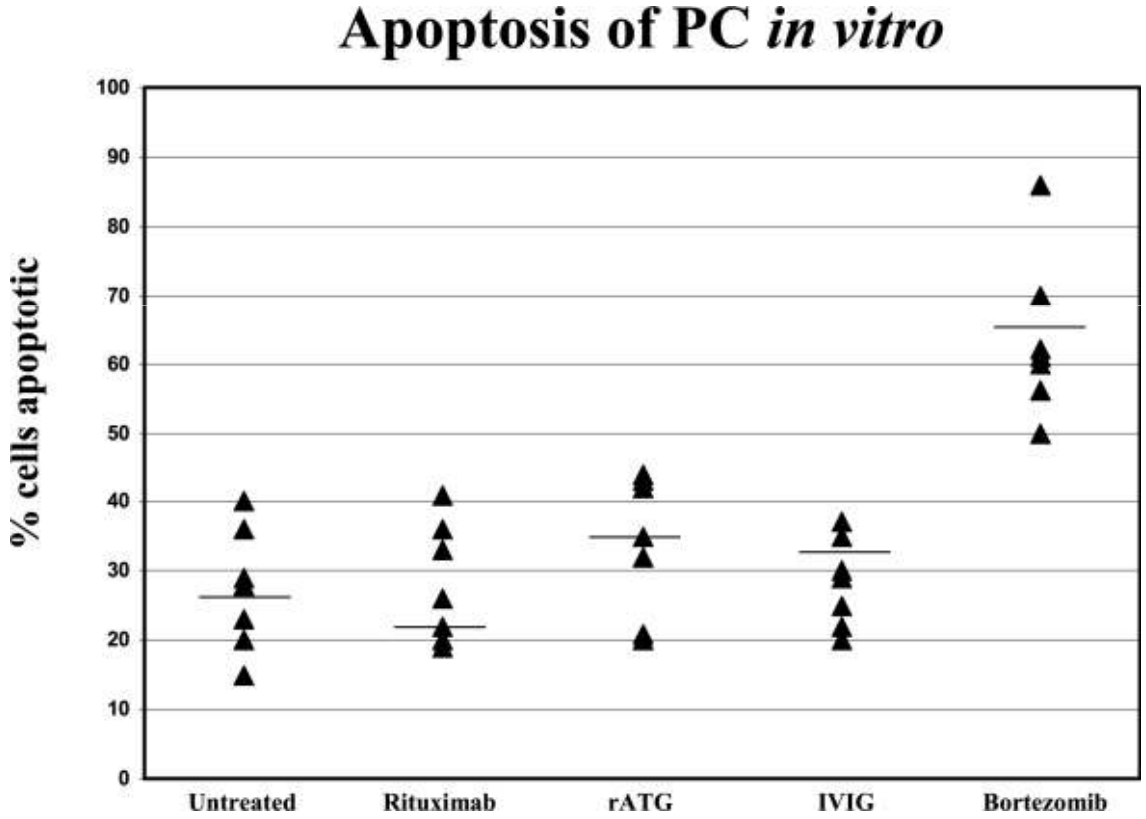


Table 1: Proteasome inhibition (bortezomib) blocks production of antibody by normal plasma cells

	Mean # ELISpots	p-Value	Mean # of allo-specificities detected	p-Value	Mean highest normalized value	p-Value
Untreated	69.33 ± 16.11	0.88	8.67 ± 10.29	0.85	3078.18 ± 6180.34	0.923
rATG-treated	66.66 ± 16.76		9.83 ± 9.19		3456.50 ± 6671.30	
Untreated	69.33 ± 16.11	0.45	8.67 ± 10.29	0.53	3078.18 ± 6180.34	0.812
Rituximab-treated	56.66 ± 14.27		14.50 ± 15.36		4148.33 ± 7596.54	
Untreated	76.00 ± 8.18	**0.03**	24.92 ± 18.01	**0.006**	5385.75 ± 5491.43	0.007
Bortezomib-treated	18.33 ± 2.36		6.33 ± 6.51		421.75 ± 906.75	

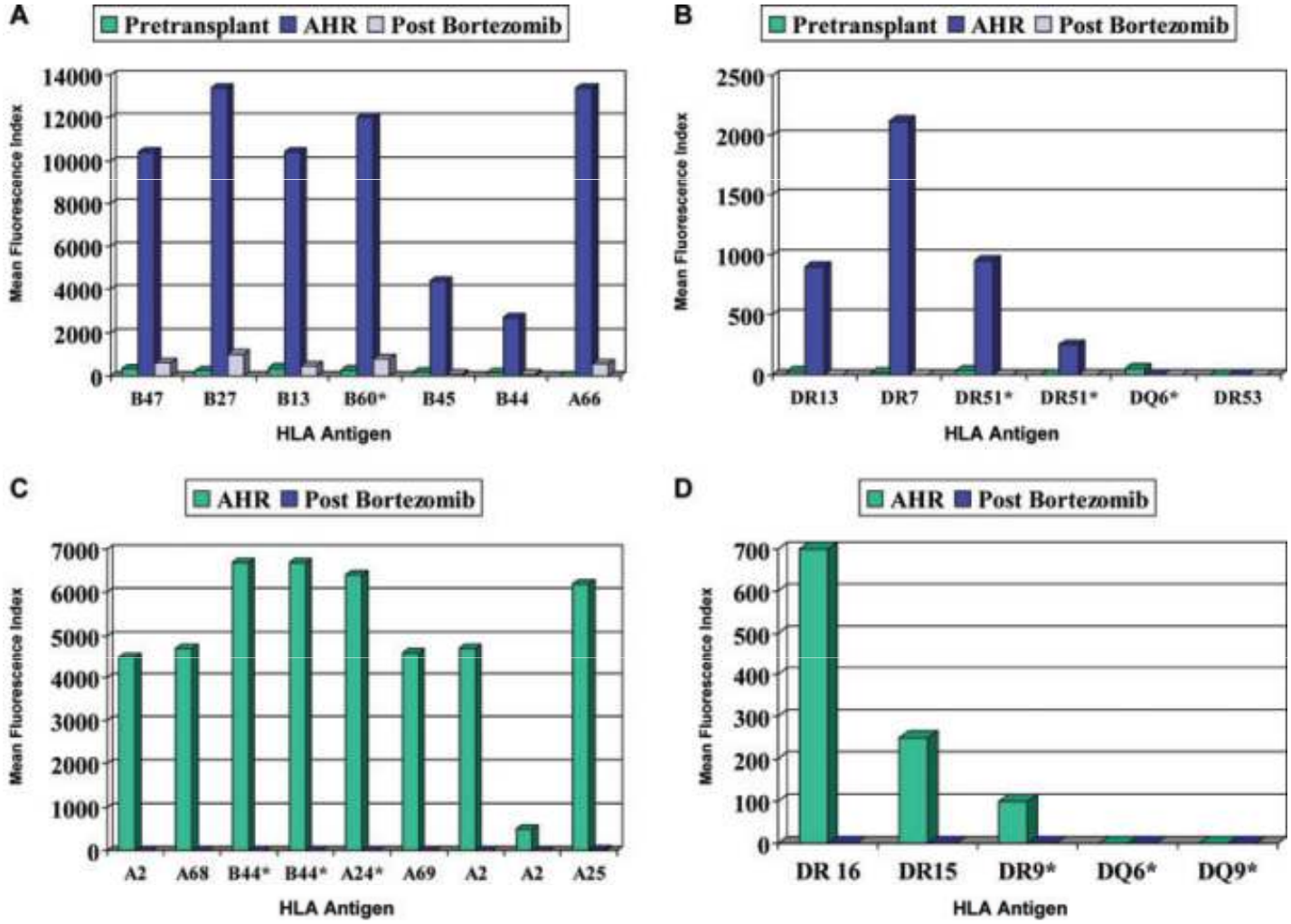


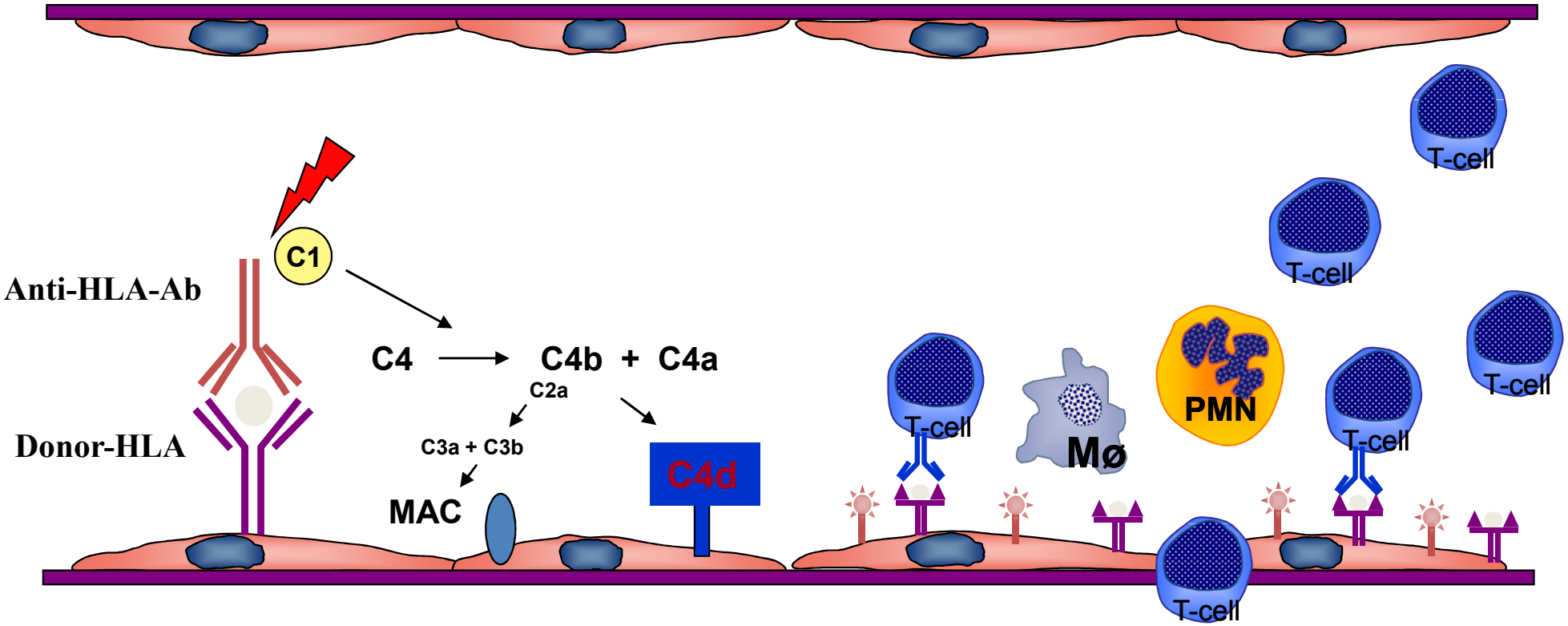
Figure 6: Bortezomib *in vivo* decreases overall levels of alloantibody and allospecificities produced by bone marrow-derived PCs. Patient 1 underwent bone marrow aspirates at the time of transplant, during AHR and 1 week after bortezomib treatment. There was an increase in antibody production at the time of AHR. Bortezomib therapy resulted in a decrease in the number of allospecificities produced and levels of anti-HLA antibody. (A) HLA class I allospecificities profile. (B) HLA class II allospecificities profile. Patient 2 underwent bone marrow aspirates at the time of AHR and 1 week after completion of bortezomib treatment with the same pattern of response. (C) HLA class I allospecificities profile. (D) HLA class II allospecificities profile. *Represents DSA.

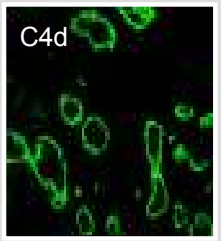
Pre-transplant Immunologic Risk Assessment (Humoral Immune Response)

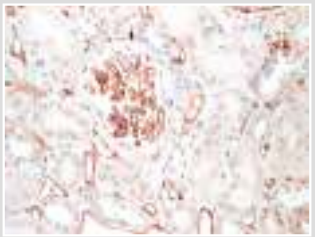
	Assays	Immunologic Risk
Pre-transplant cross-match	- CDC T cell CXM + - CDC B cell CXM + - FC T and/or B cell CXM + - CXM negative, DSA+	- Contraindication to transplantation if positive - High risk if DSA+ - High risk if DSA+ - High risk
Donor-specific anti-HLA antibodies (DSA)	- CDC - Luminex single-antigen beads - ELISA	- High risk - High risk - High risk
Quantitative antibody measurement	- Antibody titer – CDC or Flow - FCXM – Semiquantitative - Luminex – Semiquantitative MFI	- Increased risk per titer - Increased risk per channel shift - Increased risk per MFI

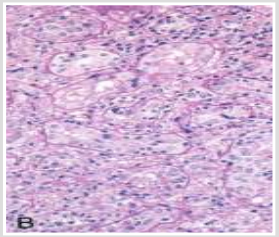
Antibody-mediated Rejection

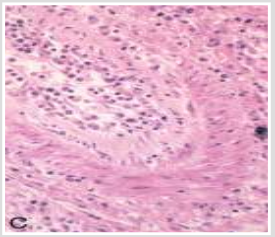
Cellular Rejection











Clinical Outcomes in AMR: The Mount Sinai Experience

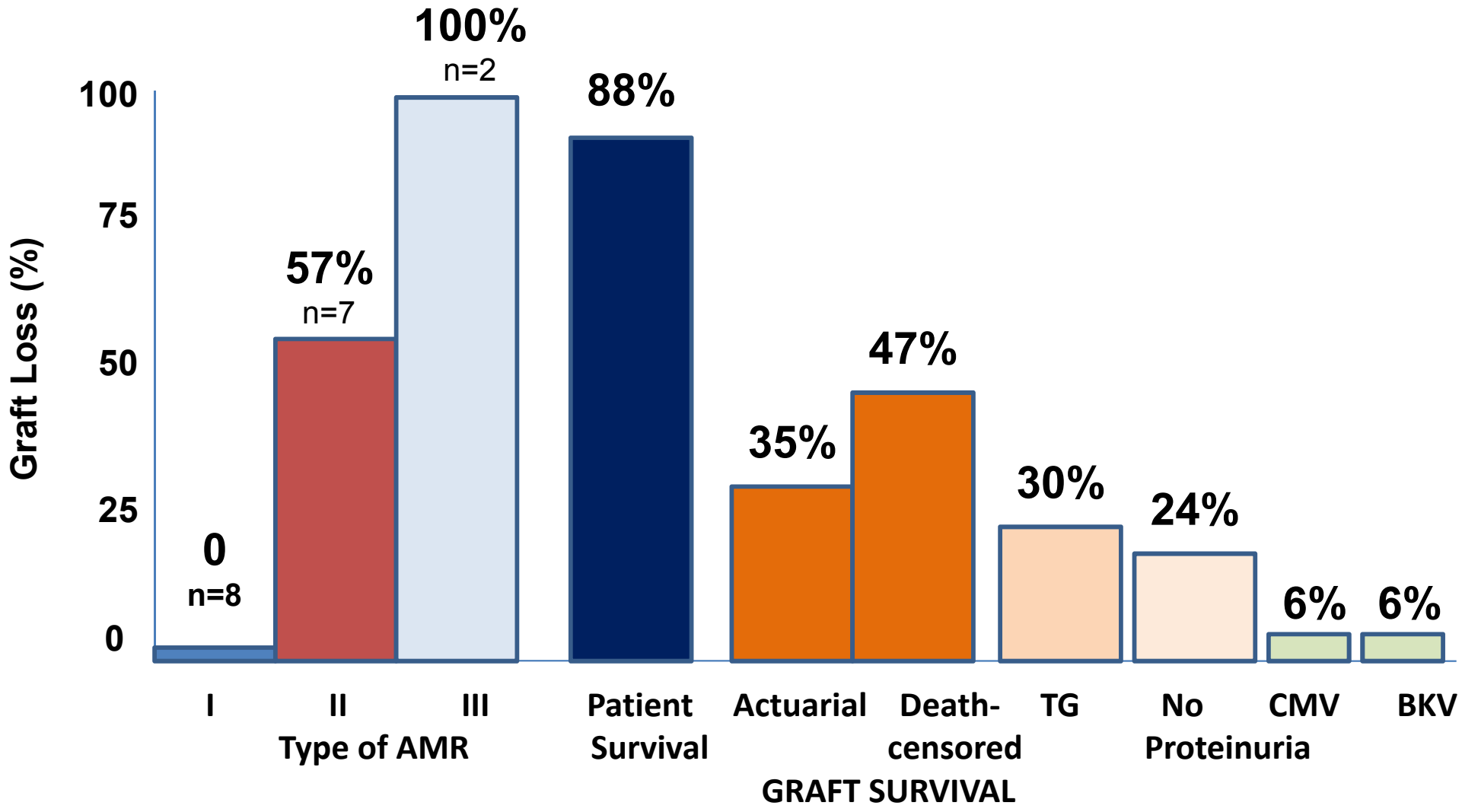
Retrospective analysis of 833 adult kidney recipients transplanted 2001-07
Acute cellular rejection 8.2% (n=68)
Acute antibody-mediated rejection 2.0% (n=17)

DEMOGRAPHICS OF PATIENTS WITH AMR

Median age **M:F** **AA race**	53 (34-68) 30:70 47%	**Median PRA**	51 (10-88)
Living donor **Previous tx**	59% 12%	**Cross-match**	CDC-TCXM 3% CDC-BCXM 9% FC-TCXM 11% FC-BCXM 10%
Median F/U **Median time to develop AMR**	28 months (12-38) 8 days (1-21)	**Pre-tx DSA**	Class I only 3 Class II only 2 Class I + Class II 10 Not studied 2

Rafiq MA et al Clin Transpl 2009

Differential Outcome in Three Types of AMR: The Mount Sinai Experience



Median SCr = 1.6 mg/dL (0.8-2.7 mg/dL)
TG – transplant glomerulopathy

Rafiq MA et al Clin Transpl 2009

Identifying DSA-negative Patients at High Risk for Cellular Rejection

- PRA >10%
- African American recipients
- Re-transplant recipients
- Deceased donor organ recipients with delayed graft function (DGF)

Do we need induction therapy in these patients?

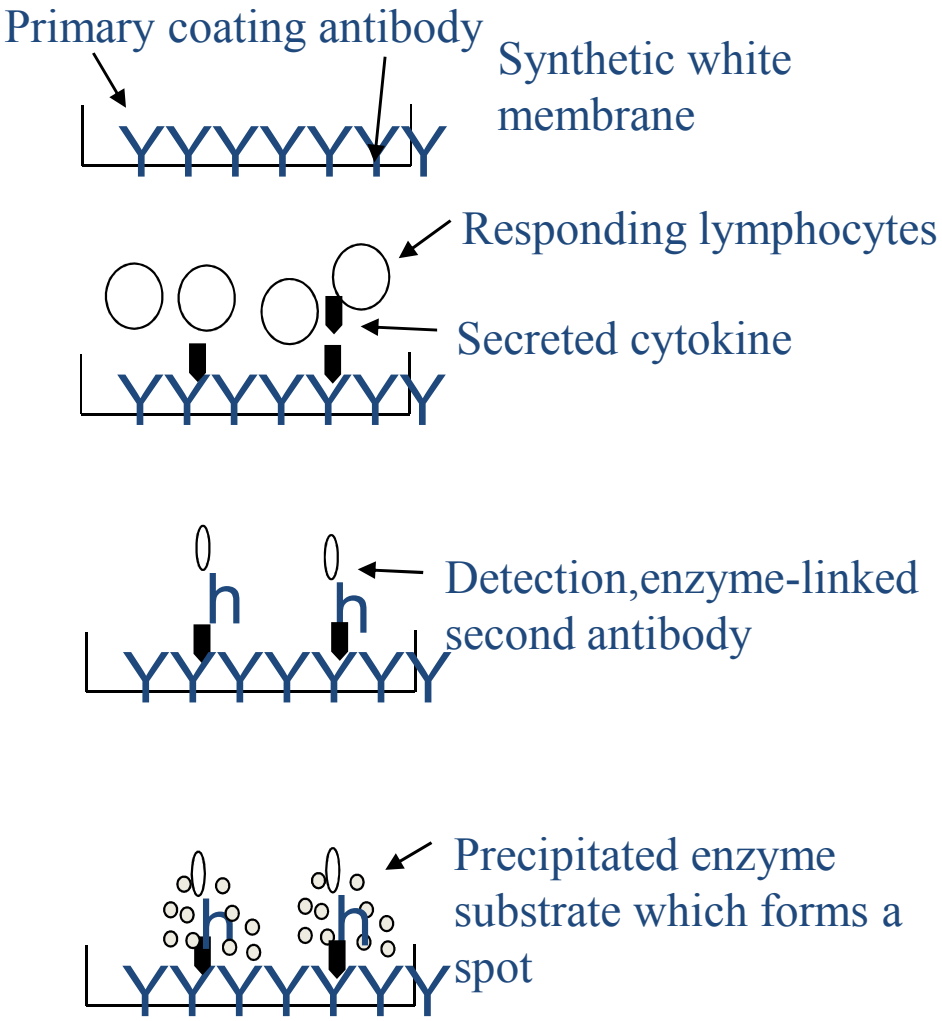
If so: Thymoglobulin, Alemtuzumab or Anti-IL-2R antibodies (Basiliximab or Daclizumab)?

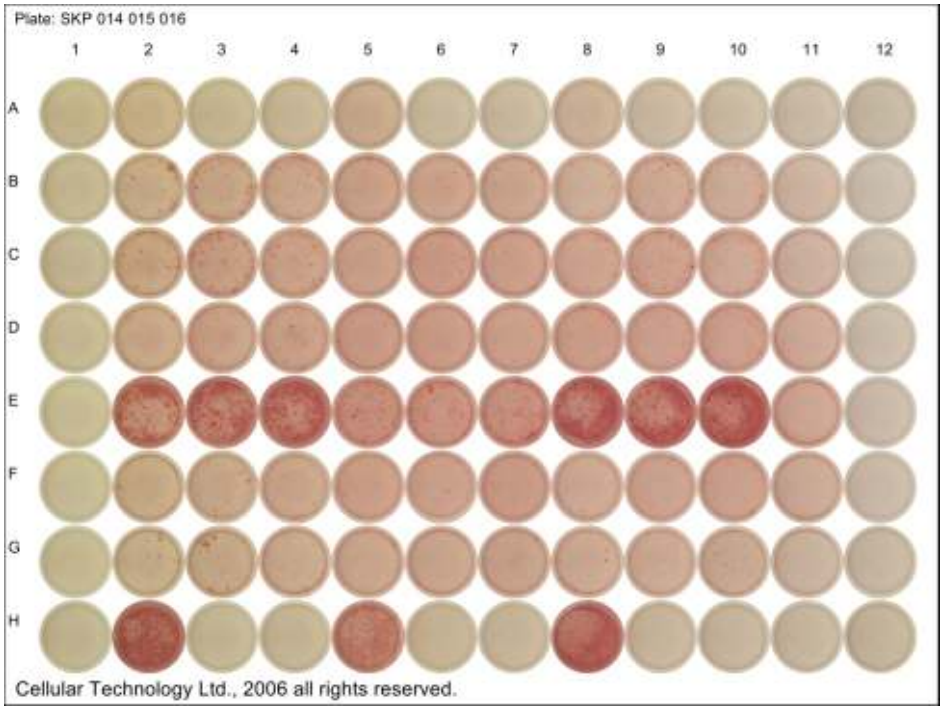
Pre-transplant Immunologic Risk Assessment (Cellular Immune Response)

Assay	Measurement	Clinical Relevance
Mixed lymphocyte reaction (MLR)[1]	CD4+ T cell activity	Highly variable
Cytotoxic T lymphocyte (CTL) assay[1]	CD8+ T cell activity	Measures direct, but not indirect alloreactivity
Enzyme-linked immunosorbent spot (ELISPOT) assay combined with Luminex technology[2]	*Ex vivo* frequency of cytokine-producing T cells	To be further studied
Panel reactive T cell (PRT) assay – ELISPOT-based[3,4]	PRT-75+ identifies patients with >25 spots/300,000 PBL, against > 75% of stimulator cells	To be validated and further studied

[1]Reviewed in Iacomini J, Sayegh MH J Am Soc Nephrol 2006; 17:328-330
[2]Gebauer BS et al Am J Transplant 2002; 2:857-866
[3]Andree H et al J Am Soc Nephrol 2006; 17:573-580
[4]Poggio ED et al. J Am Soc Nephrol 2006; 17:564-572

Measuring T Cell Activation – The ELISPOT Assay and the Panel Reactive T Cell Assay





The ELISPOT and the Panel Reactive T Cell Assay: Cleveland Clinic Experience

- **PRA and PRT are not correlated** (Poggio et al JASN 2006; 17:564)
- 41 HD pts
  -54% AA, 37% female, 22% PRA>50%
- 8 stimulators. PRT >25 spots/well is positive
- PRT>75% and PRA > 50%
  - 34% -/-, 12% +/+, 20% -/+, 34% +/-
- PRT > 40% and PRA > 10%
  - 66% -/-, 5% +/+, 17% -/+, 12% +/-

- **Increased PRT with longer HD vintage** (Augustine et al. JASN 2007; 18: 1602)
- 100 patients. AR 38% in ELISPOT+ patients versus 14% in ELISPOT- patients
- Median HD vintage: 46 months for ELISPOT+ patients and 24 months for ELISPOT- patients
- Odds ratio for 12-mo incidence of AR:
  - ELISPORT+ 4.6
  - HLA mismatch 1.48

- **ELISPOT correlates with acute rejection** (Poggio et al. Transplantation 2007; 83:847)
- 30 patients. 11/30 (37%) PRT+
- 7/30 had acute rejection (23%)
- 6/7 AR patients were PRT+ (86%)
- 1/7 patients with PRA > 15% had AR (14%)
- Mean pre-tx PRT 40% for no AR versus 81% for AR

- **Benefit of induction therapy for ELISPOT+ patients** (Augustine et al. Transplantation 2008; 86:529)
- Retrospective analysis of 130 patients enrolled in immune monitoring study
- 32 ELISPOT+ patients. No AR in 8 patients who received induction versus 46% AR in no induction
- 86% ELISPOT+ patients receiving induction became neg comparing to 35% who did not

Choice of Induction Therapy May Influence Acute Rejection with Increasing HLA Mismatch

Scientific Registry of Transplant Recipients (SRTR) Database Analysis 1998-2003

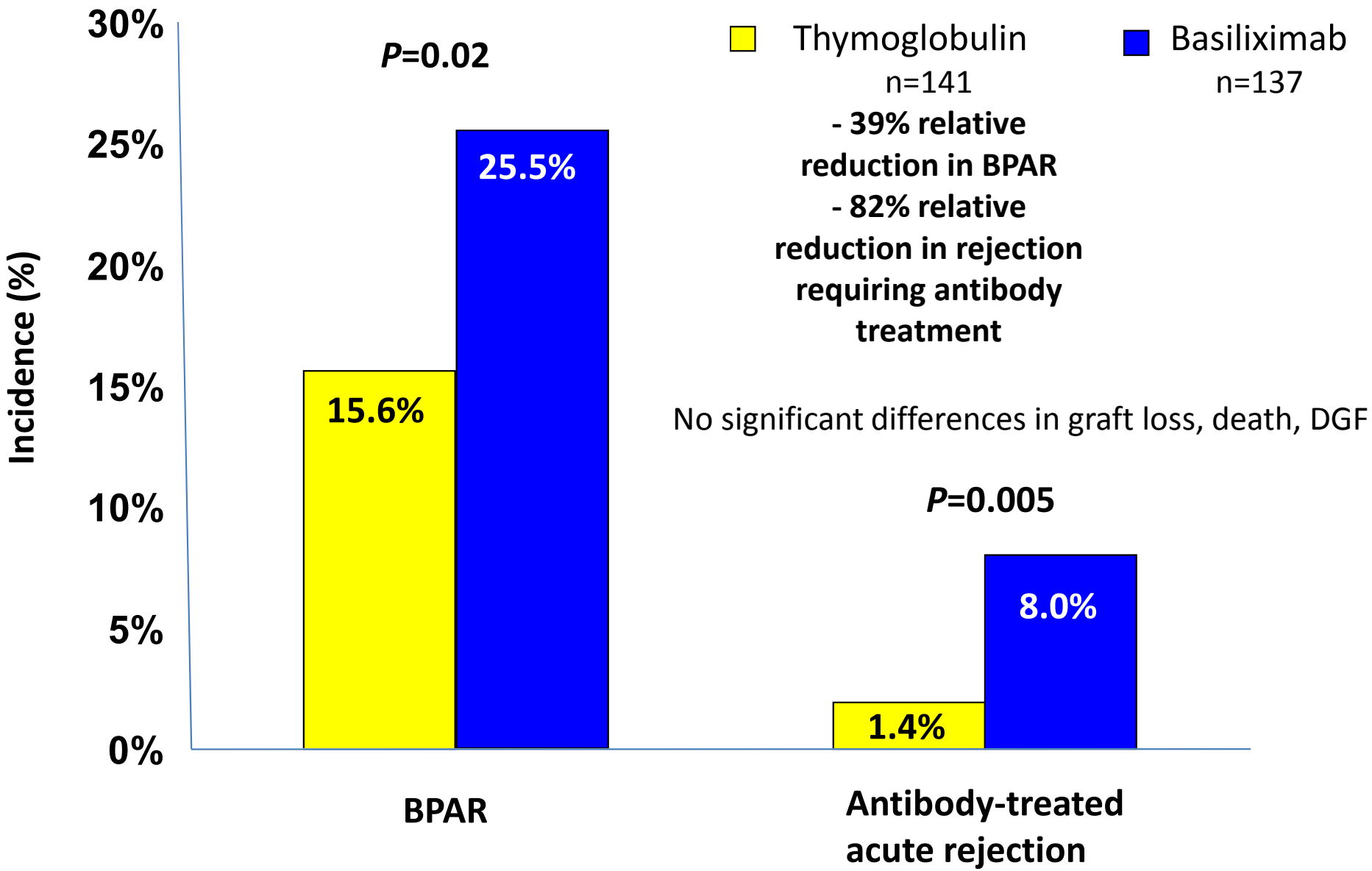
	Acute rejection at 1 year Odds ratio (95% CI)	Death-censored graft failure Hazard ratio (95% CI)
IL-2R antibodies Reference: No induction - 0 HLA antigen mismatch - 6 HLA antigen mismatch	**0.85 (0.79-0.91)** **0.99 (6 mos)** **0.69* (6 mos)**	**0.91 (0.84-0.99)**
ATG Reference: IL-2R antibodies	**0.90 (0.83-0.99)**	**1.11** (0.99–1.23)**

N=49,948 recipients of first kidney transplants
Acute rejection at 1 year: No induction 12.5%,IL-2R Ab 10.4%, ATG 10.2%
ATG – antithymocyte globulin
*P=0.007; **P=0.07

Patlolla V et al Am J Transplant 2007; 7:1832

Induction Antibody Treatment Differentially Affects Incidence and Severity of Acute Rejection

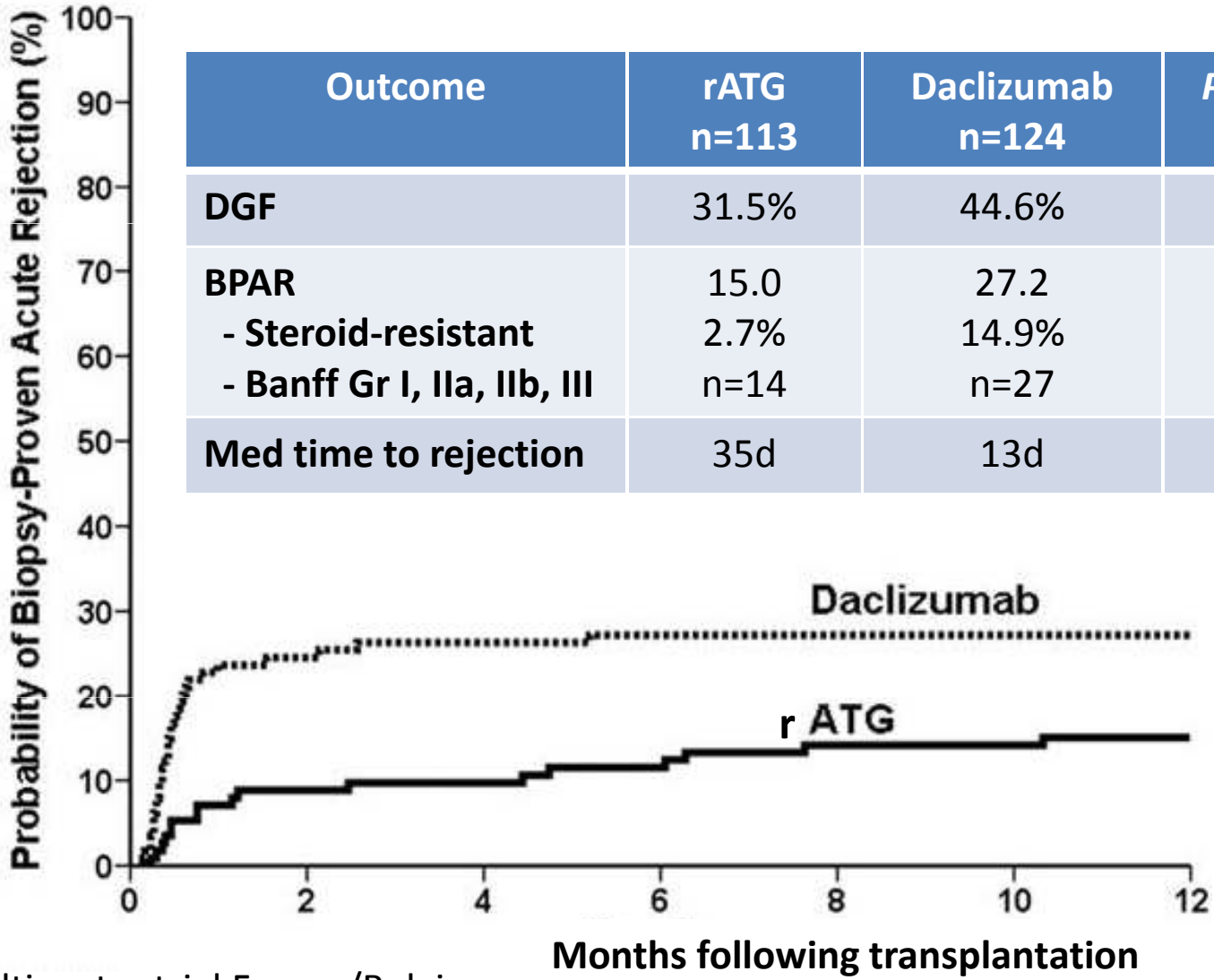
(Deceased-donor recipients high-risk for acute rejection or delayed graft function)



Brennan D et al N Engl J Med 2006; 355:1967

Thymoglobulin Induction Associated with Improved Outcomes in High Risk Kidney Recipients

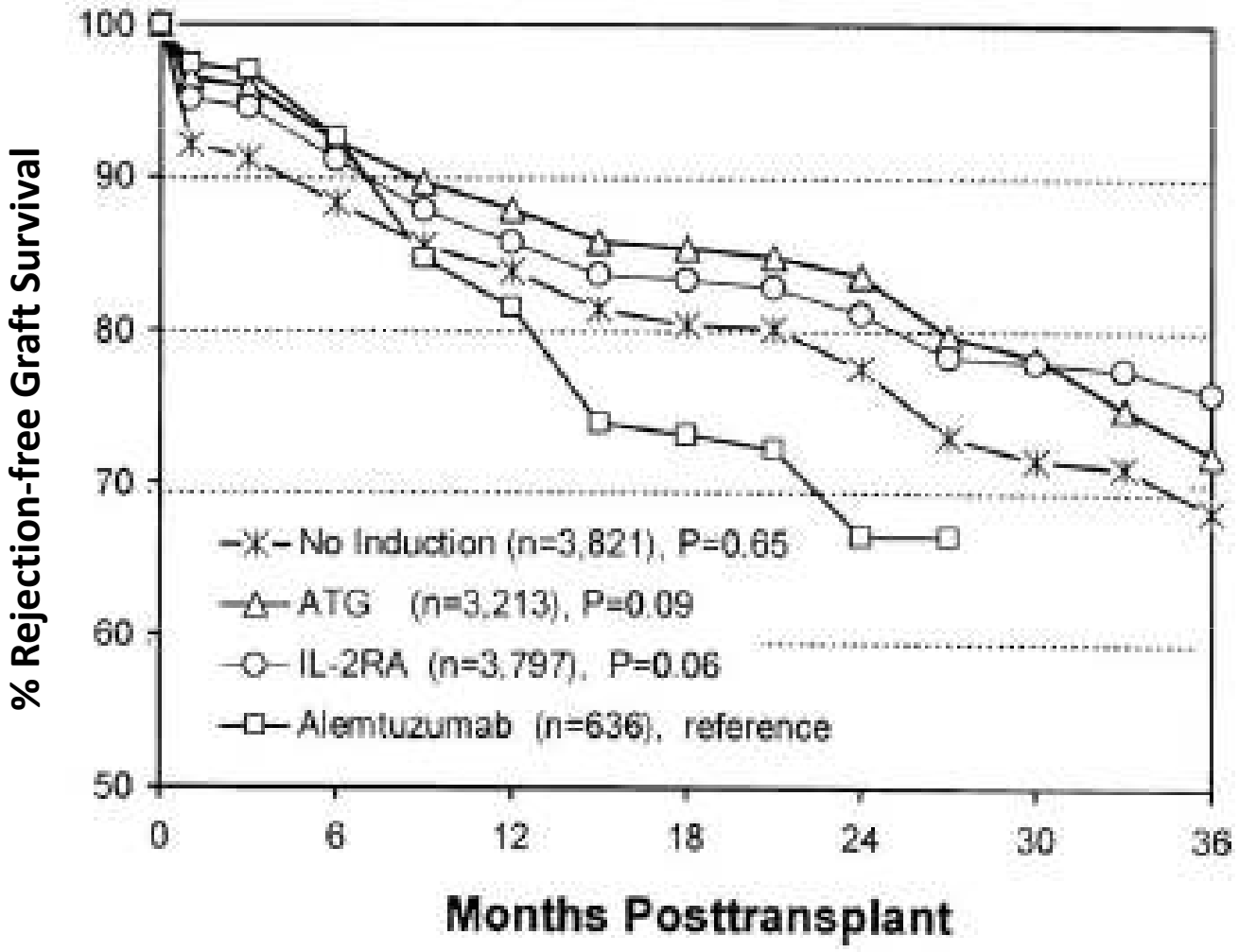
Outcome	rATG n=113	Daclizumab n=124	*P*-value
DGF	31.5%	44.6%	0.044
BPAR **- Steroid-resistant** **- Banff Gr I, IIa, IIb, III**	15.0 2.7% n=14	27.2 14.9% n=27	0.016 0.002 0.10
Med time to rejection	35d	13d	0.007



Randomized, multicenter trial France/Belgium
Recipients: current PRA >30% / peak PRA >50 / retransplant
Donors: mean CIT >23h;>50% CVA

Noel, C. et al. J Am Soc Nephrol 2009;20:1385

Potential of Alemtuzumab as Induction Therapy in Recipients of Deceased-Donor Kidney Transplants: OPTN Analysis 2003-2004



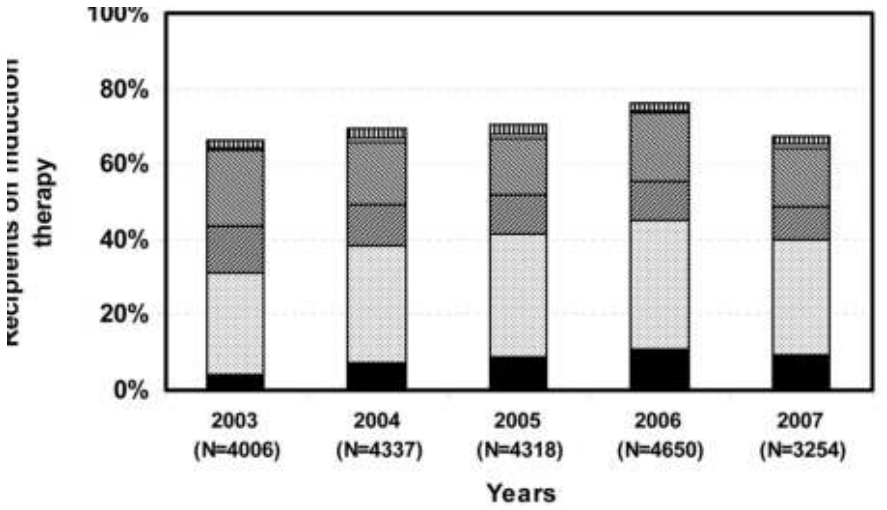
- No multicenter, randomized and control study with Alemtuzumab
-Retrospective or randomized single center studies with small number of patients
-Alemtuzumab has been used in conjunction with low-dose CNI, CNI-free, and steroid sparing regimens

N=14,362 recipients of deceased donor transplants

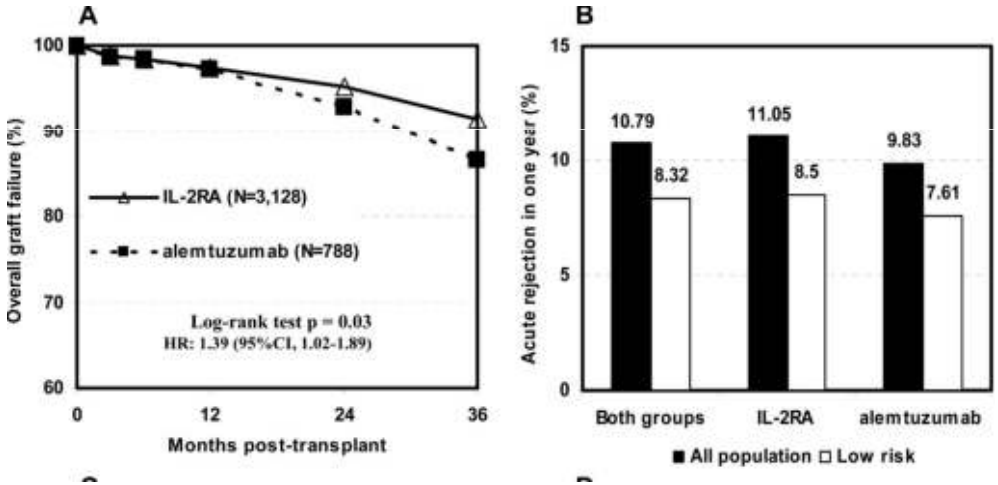
Huang E et al Transplantation 2007; 84:821

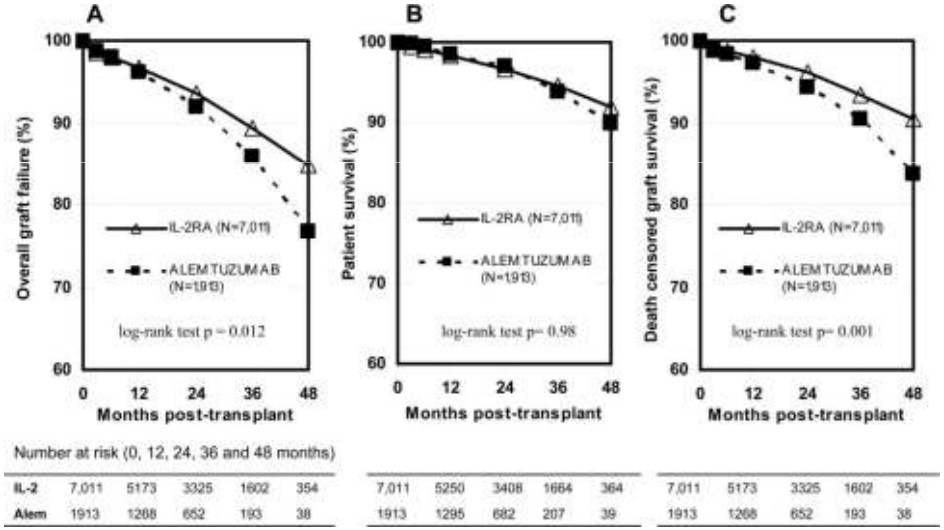
Alemtuzumab as Induction Therapy in Living-donor Kidney Transplant Recipients OPTN/UNOS database

Sampaio et al. Transplantation 2009; 88:904



IL-2RA (%)	49.28	39.54	36.61	37.44	36.75
Thymo (%)	40.64	45.05	46.02	45.27	45.08
Alem (%)	6.24	10.58	12.64	14.15	14.04





Anti-HLA Antibody Detection Techniques for Solid Organ Transplantation

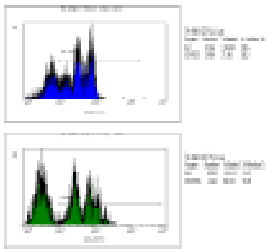
Anti-HLA Antibody

Solid Phase Assay

ELISA-PRA

Flow Cytometer

Flow Screening-PRA



Luminex Single Antigen-CPRA/DSA

Strength of antibodies

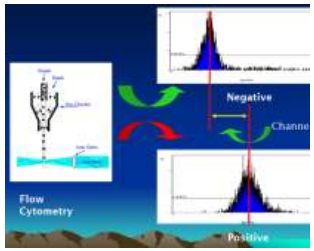
MFI; SFI; MESF

CDC Techniques PRA

HLA Cross-match

CDC Cross-match
T: AHG
B: Amos

Flow Cross-match T and B cell (IgG) (With or without Pronase)



Strength of T and B cell Flow Cross-match:
T cutoff: <60 MCS
B cutoff: < 100 MCS are negative cross-match.

Abbreviations: HLA:human luekocyte antigen; CDC: complement-dependent-cytotoxicity; DSA: donor specific antibody; CPRA: calculated panel reactive antibody; MFI: mean fluorescence intensity; SFI: standardized fluorescence Intensity; MESF: molecular equivalent soluble fluorescence; MCS:median channel shift.

Summary – Clinical Implications of Risk Assessment

Test Result	Transplant	Treatment
CDC-TCXM- and BCXM- Luminex-negative No history of sensitization	Proceed	Standard post-transplant immunosuppression -DDKTx (Thymo vs anti-IL2R) -Living tx HLA-ID (no induc) -Living Tx non-HLA-ID (Anti-IL2R)
CDC-TCXM- and BCXM- DSA negative Previous history of sensitization PRA > 10% (non-DSA) Rapid steroid withdrawal	Proceed	Standard post-transplant immunosuppression with Thymoglobulin or Alemtuzumab induction
CDC-TCXM– CDC-BCXM+ and/or FCXM+ Low MCS values Low titer/strength/MFI DSA+	Proceed	- No pre-tx desensitization - Peri-transplant IVIg + Thymoglobulin or Alemtuzumab induction
CDC-TCXM+ High FCXM channel shift Luminex DSA+ with high MFI values	Do not transplant	Pre-tx desensitization with PP + IVIg ± rituximab

Einstein-Montefiore Transplant Center Desensitization Protocol

Patients evaluated for kidney transplant

Patients with a living donor

Patients have no DSA and cross-match negative

Transplant

Patients have DSA and cross-match positive

- Internal and NKR kidney paired exchange programs

- CDC cross-match T or B-Pos or Flow T/B Pos MCS >300 and DSA MFI>5,000: Desensitization with PP, IVIG and rituximab. If DSA MFI<5,000 and MCS <300, after desensitization: Transplant with anti-thymocyte globulin and IVIG.
- CDC cross-match T-Neg/ B-Pos or Flow T/B Pos MCS <300 and DSA MFI<5,000: No pretransplant desensitization. Transplant with anti-thymocyte globulin and IVIG.
- CDC cross-match T and B-pos with more than 3 DSAs or more than 1 strong DSA with MFI > 5,000: Do not desensitize and transplant

Patients without a living donor

UNOS DONATE LIFE

List to UNOS with any antibody >5000 MFI as unacceptable HLA antigens

- Patients with CPRA >50% and on top of the waiting list: 2 g/kg IVIG at day 0 and day 30; 375mg/m2 rituximab at day 15. If any antibody strength after treatment decreases to <5000, remove from UNOS as unacceptable antigen

Post-TX monitoring : monthly DSA, BKV up to 6 months; and at 9th and 12th months; biopsy if creatinine level or DSA MFI increases

Abbreviations: CDC: complement-dependent-cytotoxicity; NKR: National Kidney Registration; DSA: donor specific antibody; HLA:human luekocyte antigen; CPRA: calculated panel reactive antibody; UNOS: United Network for Organ Sharing; MFI: mean fluorescence intensity; MCS:median channel shift.

NON-INVASIVE IMMUNE MONITORING

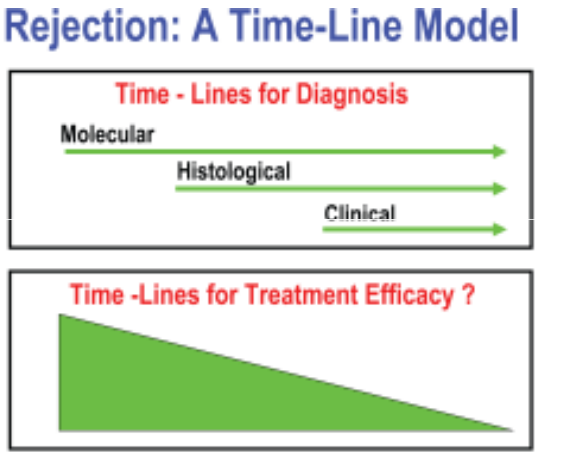


FIGURE 1. A time-line model for allograft rejection. In this formulation, rejection defined by molecular markers precedes histologically defined rejection and this precedes clinically defined rejection. The hypothesis that early intervention is efficacious is an important rationale for the development of molecular surveillance strategies to anticipate histologic and clinical rejection.

TABLE 1. Objectives of messenger RNA profiling of organ graft recipients

1. Diagnose rejection by noninvasive means and obviate the need for the invasive procedure of allograft biopsy
2. Anticipate the subsequent development of rejection before the development of tissue injury
3. Prognosticate the outcome of an episode of rejection, and responsiveness to antirejection therapy
4. Predict subsequent allograft function
5. Help develop mechanism-based therapy
6. Facilitate individualization or optimization of immunosuppressive drug therapy including weaning or reintroduction of therapy

Anglicheau and Suthanthiran Transplantation 2008; 86: 192

Clinical Trials in Organ Transplantation (CTOT)

- Clinical Goal: Development of noninvasive tests to facilitate safe minimization of immunosuppression.
- Funding Period: 2004-2009 NIAID
- Three consortia performing five studies: CTOT-1 thru CTOT-5
  - Cleveland/NYC-based consortium (Case Western Reserve, Cleveland Clinic, Mt Sinai NYC, Yale, Emory, U Manitoba, U Cincinnati (pediatrics) CTOT-1 and CTOT-5
    - PI: Peter Heeger
  - Brigham & Women's Hosp./UCSF-based consortium CTOT-2 and CTOT-5
    - PI: Mohamed Sayegh
  - U Penn/Cornell-based consortium CTOT-3 and CTOT-4
    - PI: Avi Shaked

CTOT-1 Assay Schedule – First 6 mo

Test	D-1	D3	D7	D14	D28	M2	M3	M4	M5	M6
Biopsy	X									X
ELISPOTs and Flow	X			X	X	X	X	X	X	X
Anti-HLA Ab	X						X			X
Blood-mRNA Profiling		X	X	X	X	X	X	X	X	X
Urine-Proteomics		X	X	X	X	X	X	X	X	X
Urine- mRNA Profiling		X	X	X	X	X	X	X	X	X
Urine-Luminex Cytokine/ Chemokines		X	X	X	X	X	X	X	X	X
Cylex® *	X		X	X	X	X	X	X	X	X