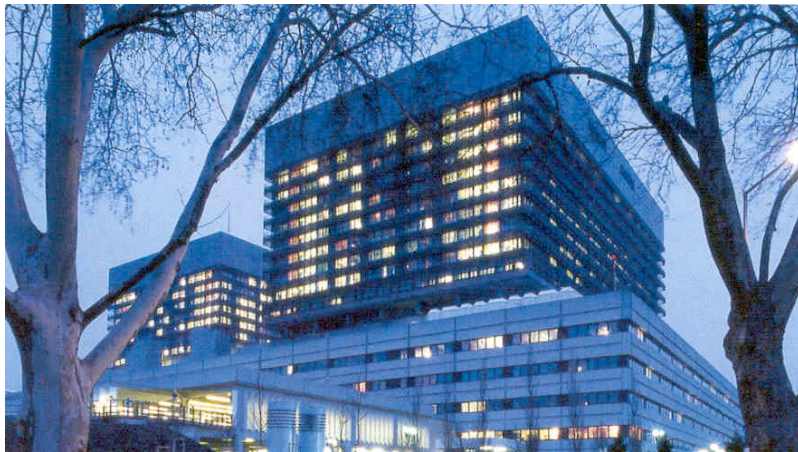




Pathologists meet Clinicians

# New techniques for the identification of HLA antibodies



**Georg Böhmig**  
**Division of Nephrology and Dialysis**  
**Department of Medicine III**



*Vienna, Austria*

*Krakow, Poland, September 2<sup>nd</sup>, 2010*



# Innovative techniques



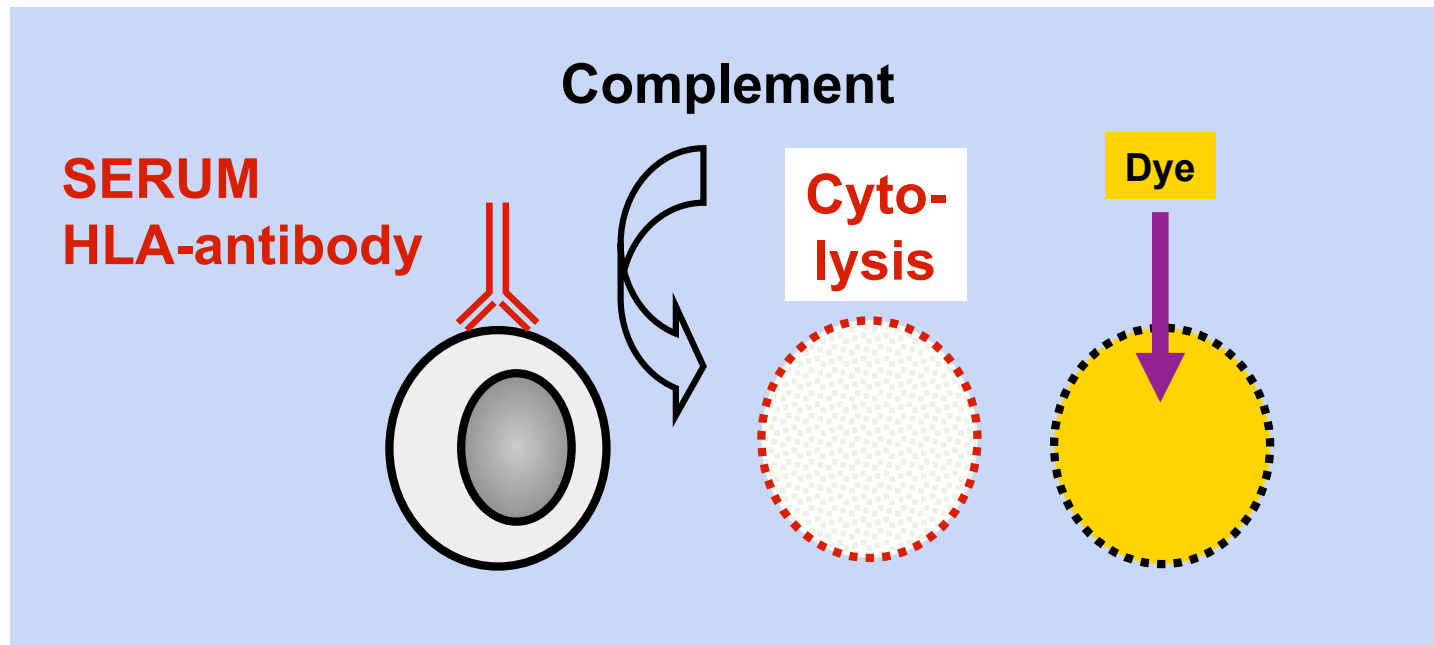
**Pre-Tx  
HLA Ab detection**

**(independent)  
predictive marker?**

**Antibody-mediated rejection**



# Complement-dependent cytotoxicity



Donor cells  
(PBMC, Spleen, Ln)

**Crossmatch  
(CDC-XM)**

PBMC from  
≈50 HLA-typed subjects

**Panel-reactive Ab  
(CDC-PRA)**



# Lymphocytotoxic crossmatch (CDC-XM)

## Clinical relevance

57 pre-sensitised KTX recipients

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Outcome	XM +ve (N=30)	XM -ve (N=27)
Immediate graft loss	80%	14.8%
Graft loss >3Mo	3.3%	25.9%

---

Patel&Terasaki, *N Engl J Med* 1969; 280(14):735



# Risk factors for C4d<sup>+</sup> AMR

388 biopsied kidney transplant (KTX) recipients (1999–2002)  
**17% C4d positive**

---

Parameter	Odds ratio	95% CI	p value
Donor age	0.9	0.7–1.2	0.5
Cold ischaemia time	1.0	0.8–1.4	0.8
HLA mismatch	1.8	0.9–3.5	0.008
<b>CDC-PRA reactivity &gt; 10%</b>	<b>3.1</b>	<b>1.5–6.1</b>	<b>0.002</b>
<b>Retransplantation</b>	<b>3.6</b>	<b>1.8–7.2</b>	<b>&lt;0.001</b>
2-hour CyA monitoring	1.2	0.4–3.6	0.7
CNI/MMF pretransplant	0.5	0.2–0.8	0.03

---

Lorenz et al. *Transplantation* 2004;78:447–52



# CDC testing



not HLA-specific



HLA Class II: inaccurate



Only indirect definition of HLA antigen specificities



%PRA reflects probability of positive XM

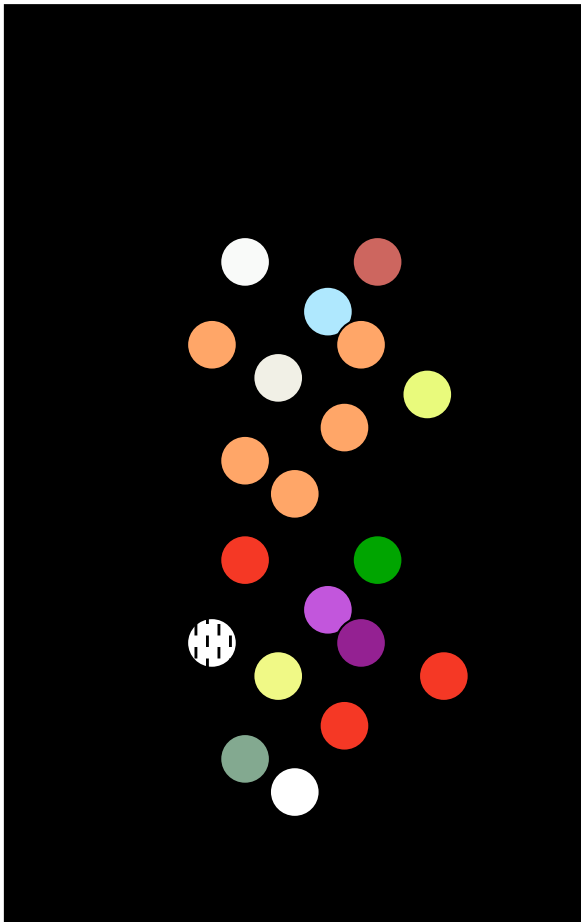
**≠ donor specificity**



# Solid phase HLA antibody detection

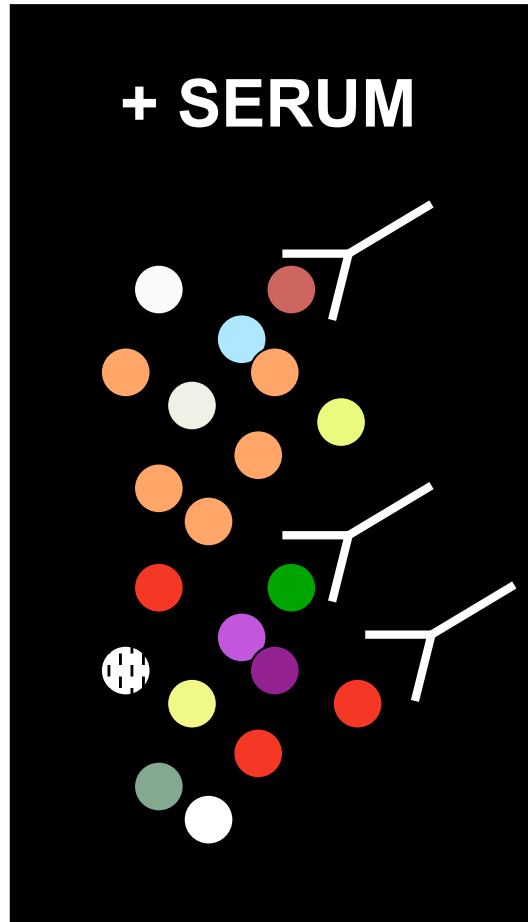
## Principle

HLA-coated microbeads

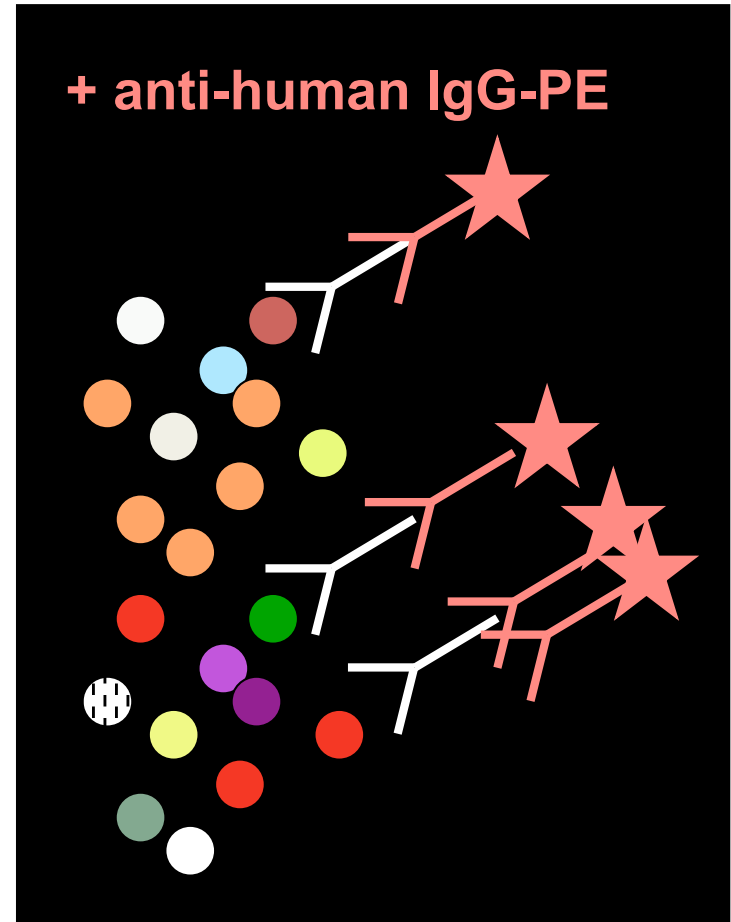


Indirect immunofluorescence (FACS, Luminex)

+ SERUM

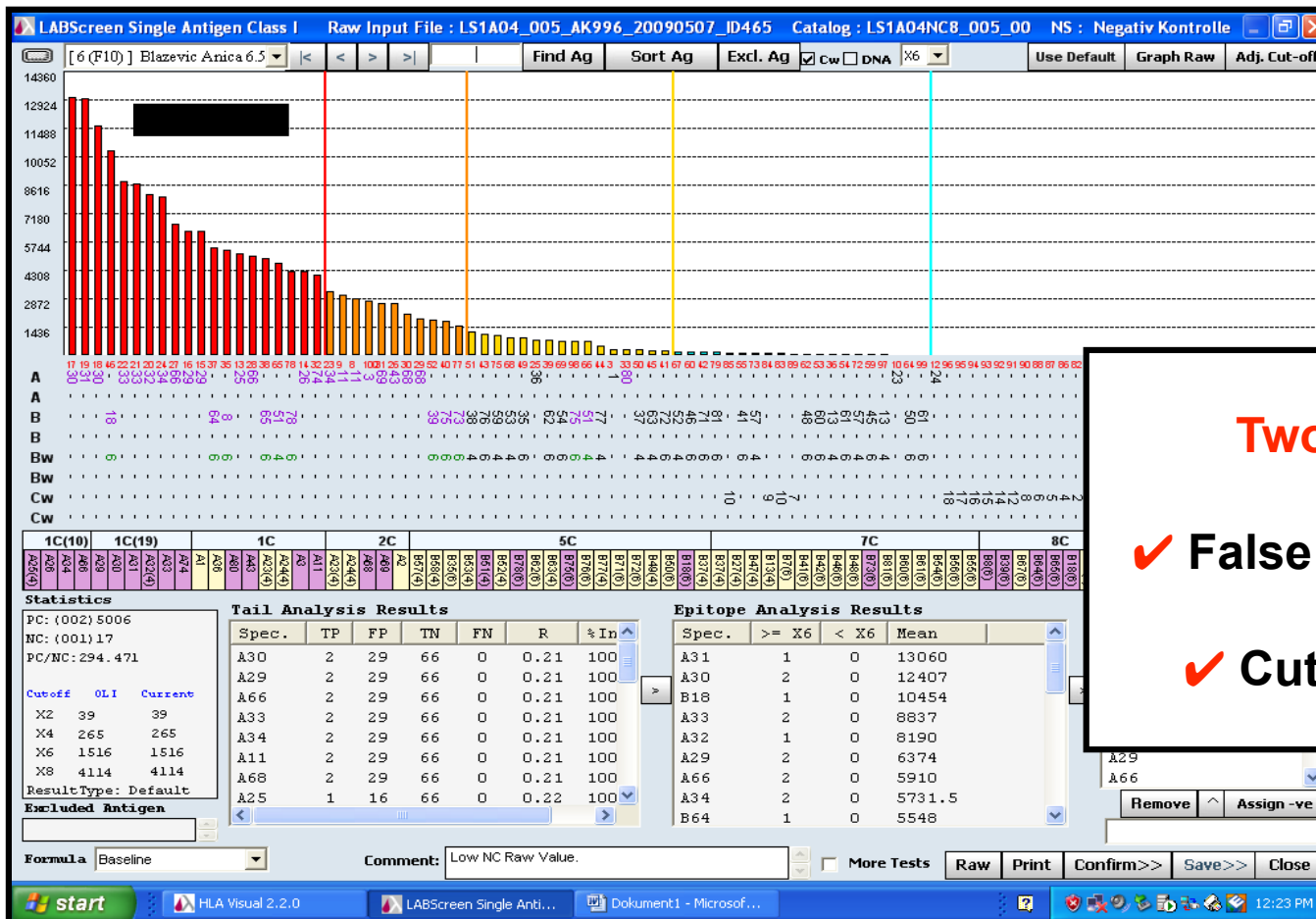


+ anti-human IgG-PE





# Identification of DSA w/o need for donor-derived cells



**Two drawbacks:**

- ✓ False positive results?
- ✓ Cut-off definition?



# Luminex - DSA and AMR

Retrospective analysis of pre-Tx sera  
338 recipients, Tx 2001-2002

	$\alpha$ -HLA class I and/or II		IgG neg
	DSA	no DSA	
Patient number	39	16	283
Retransplantation	77%	50%	10%
CDC-PRA>10%	80%	44%	17%
<b>AMR (C4d+)</b>	33%	0%	4%
Cellular rejection	21%	17%	21%

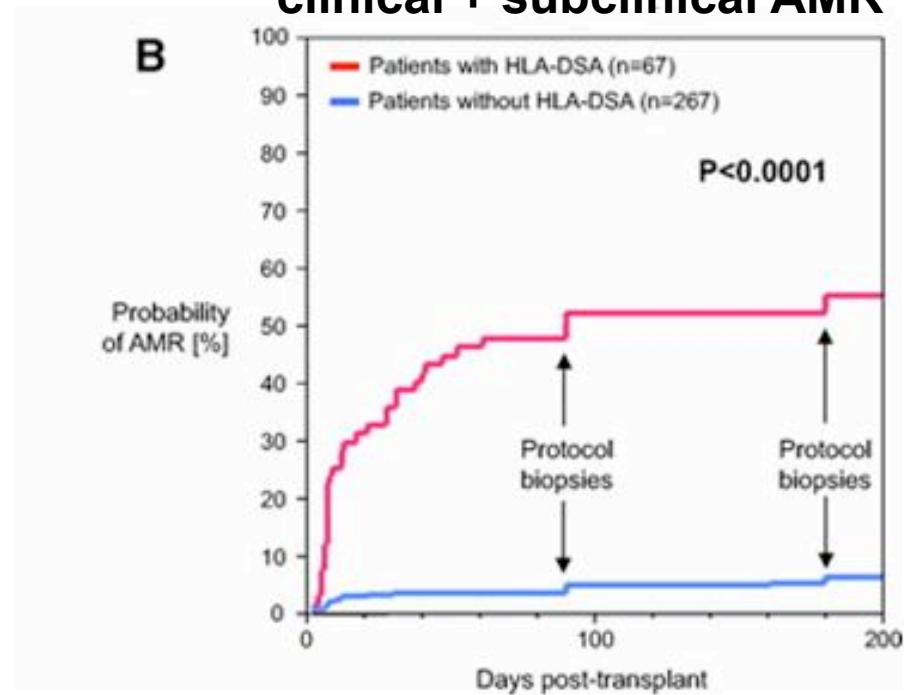
Wahrmann et al, *Transplant Int*, 2009;22(10):982-89



# Luminex - DSA and AMR

Retrospective analysis of pre-Tx sera  
*334 recipients*

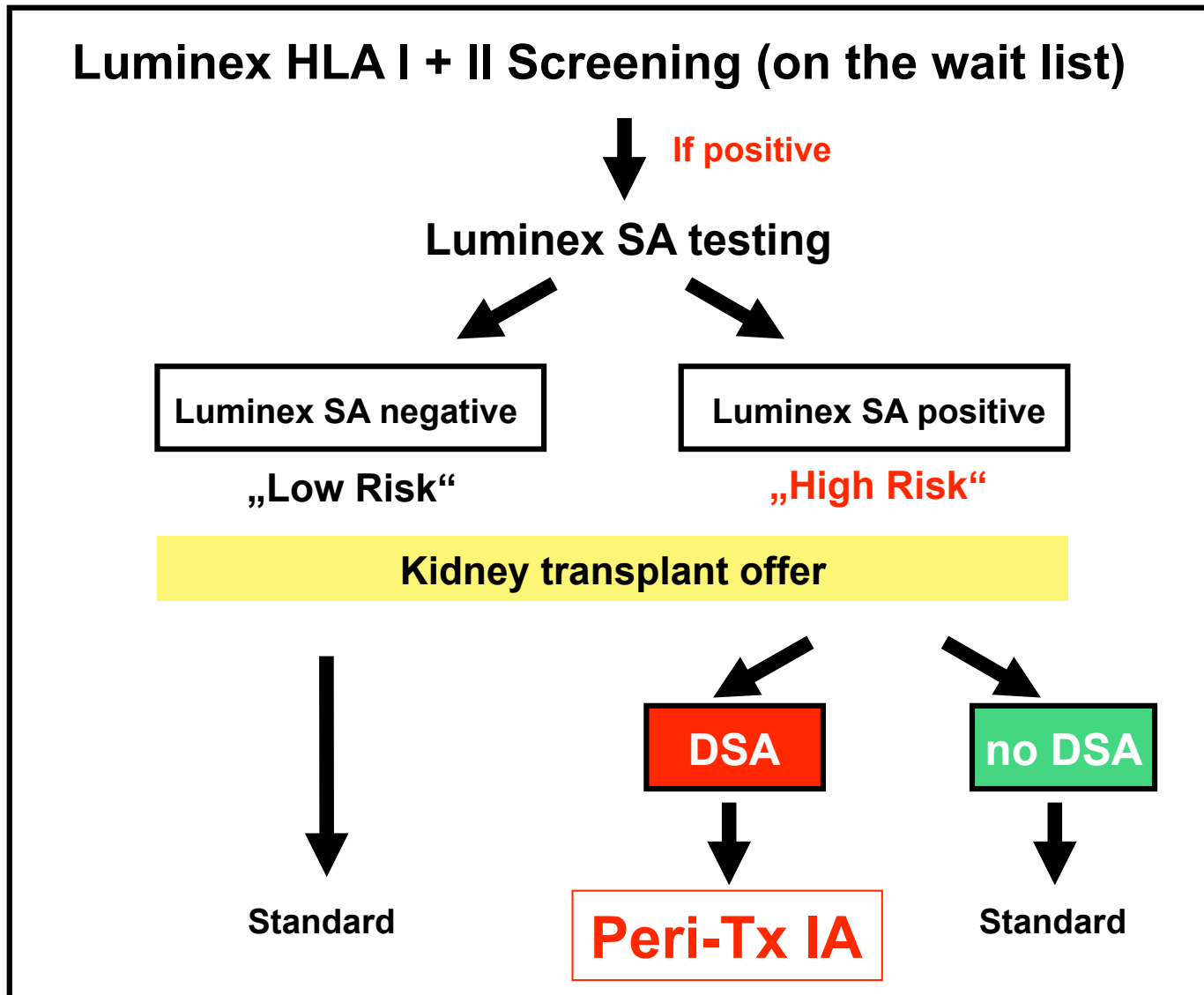
clinical + subclinical AMR



Amico et al, *Transplantation*, 2009; 87(11):1681-8

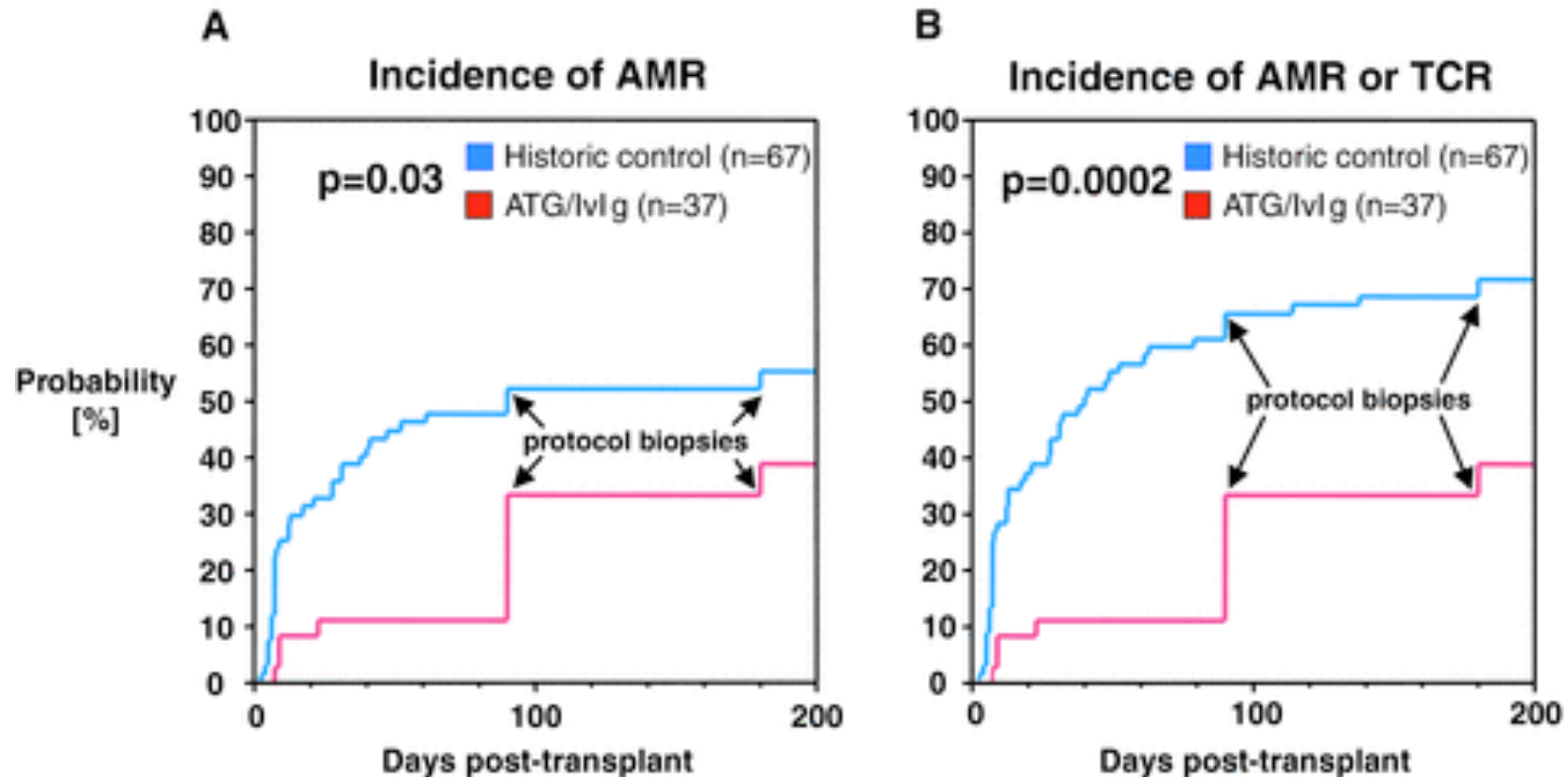


# Serological risk stratification + treatment allocation (since 2009)





# ATG+IVIg in kidney transplants with „low level“ DSA (CDCXM negative) (deceased & living donor Tx)



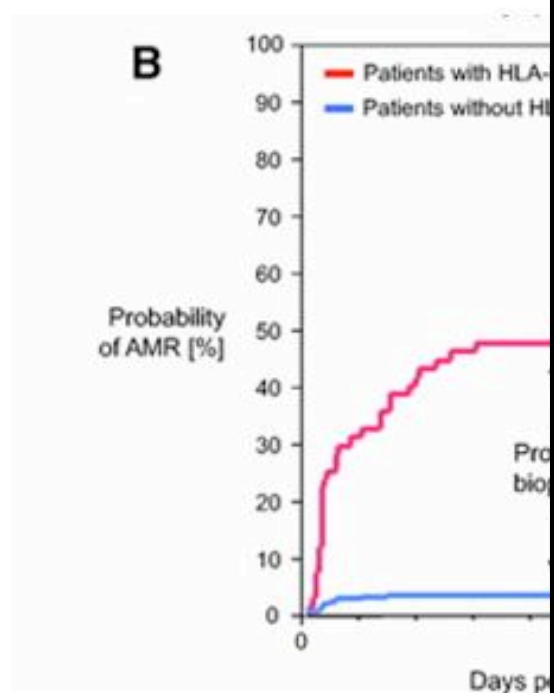
**Still high rate (30%) of subclinical AMR at months 3 and 6**

Bächler et al., *Am J Transplant*, 2010;10(5):1254-62



# Luminex – DSA

≥50% of DSA+ recipients do not develop rejection – **Why?**



**No difference:**

✓ **Specificity**

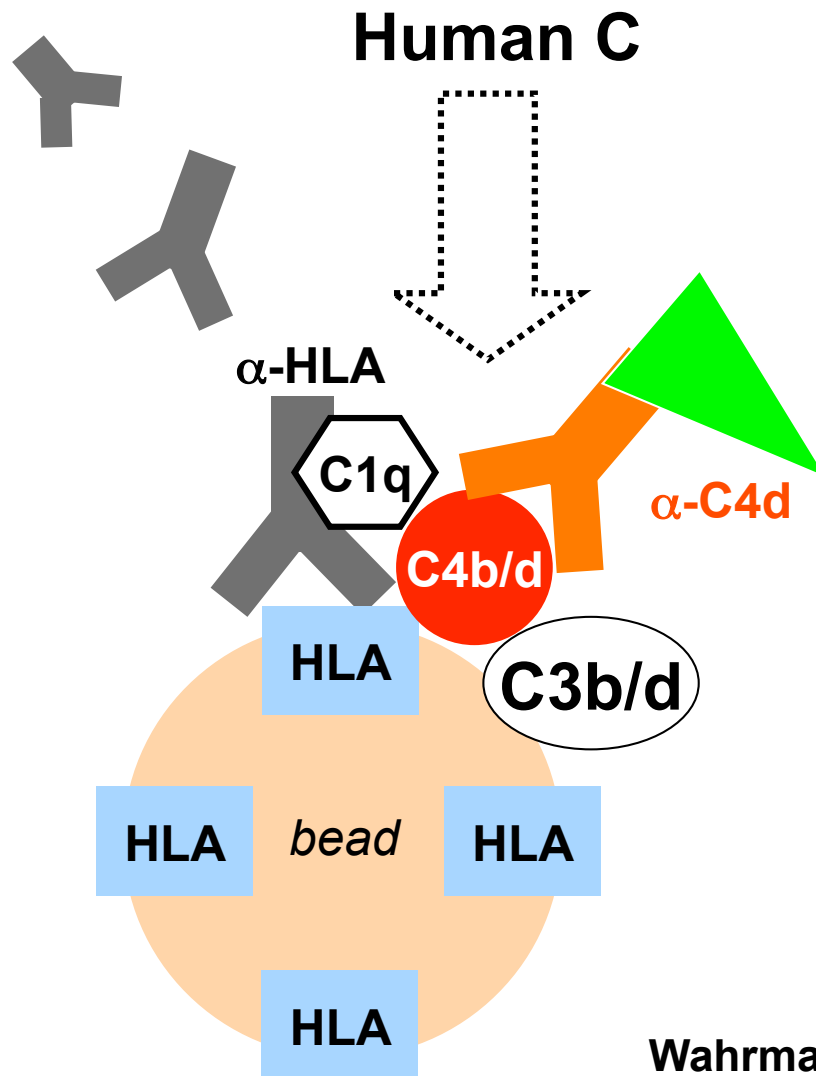
✓ **DSA strength**

**Other parameter?**

Amico et al, *Transplantation*, 2009; 87(11):1681-8



# *In vitro* C4d fixation to HLA-coated beads



**FlowPRA®**

## **Immunofluorescence**

For Screening/SA testing

✓ **Flow cytometry**  
(„FlowPRA“)

✓ **Luminex platform**  
(„Luminex testing“)

Wahrmann et al., *Immunological Methods*, 2003, 275:149

Wahrmann et al., *Human Immunol*, 2005, 66:526

Wahrmann et al., *Transplant Int*, 2009;22(10):982-89



# C4d-fixing DSA

No predictive value in CDC-XM-negative patients

	Patients with C4d-fixing DSA (n=11)	Patients without C4d-fixing DSA (n=53)	
AMR any type, n (%)	<b>6 (55%)</b>	<b>28 (53%)</b>	
AMR phenotype, n			
Clinical AMR C4d+	1	15	
Clinical AMR C4d-	-	6	<b>n.s.</b>
Clinical C4d+ only	2	4	
Subclinical AMR C4d+	1	1	
Subclinical AMR C4d-	-	1	
C4d in PTC, n (%)	<b>6 (55%)</b>	<b>21 (40%)</b>	

Hönger, Wahrmann et al, *Transplantation*, 2010, 89: 1471



# C4d-fixing (Luminex-) DSA before Tx?

## Three retrospective studies

Author, year	Organ	CDC-XM	Predictive
Wahrmann et al. 2009	Kidney	Some positive (conversion by IA)	no
Hönger et al. 2009	Kidney	All negative	no
Smith et al. 2007	Heart	Some positive (no conversion)	yes

**Useful for *virtual XM* - prediction of CDC-XM?**



**Innovative technologies**



**Post-Tx  
Allo-Ab detection**

**(independent)  
diagnostic marker?**

***ongoing or pending***

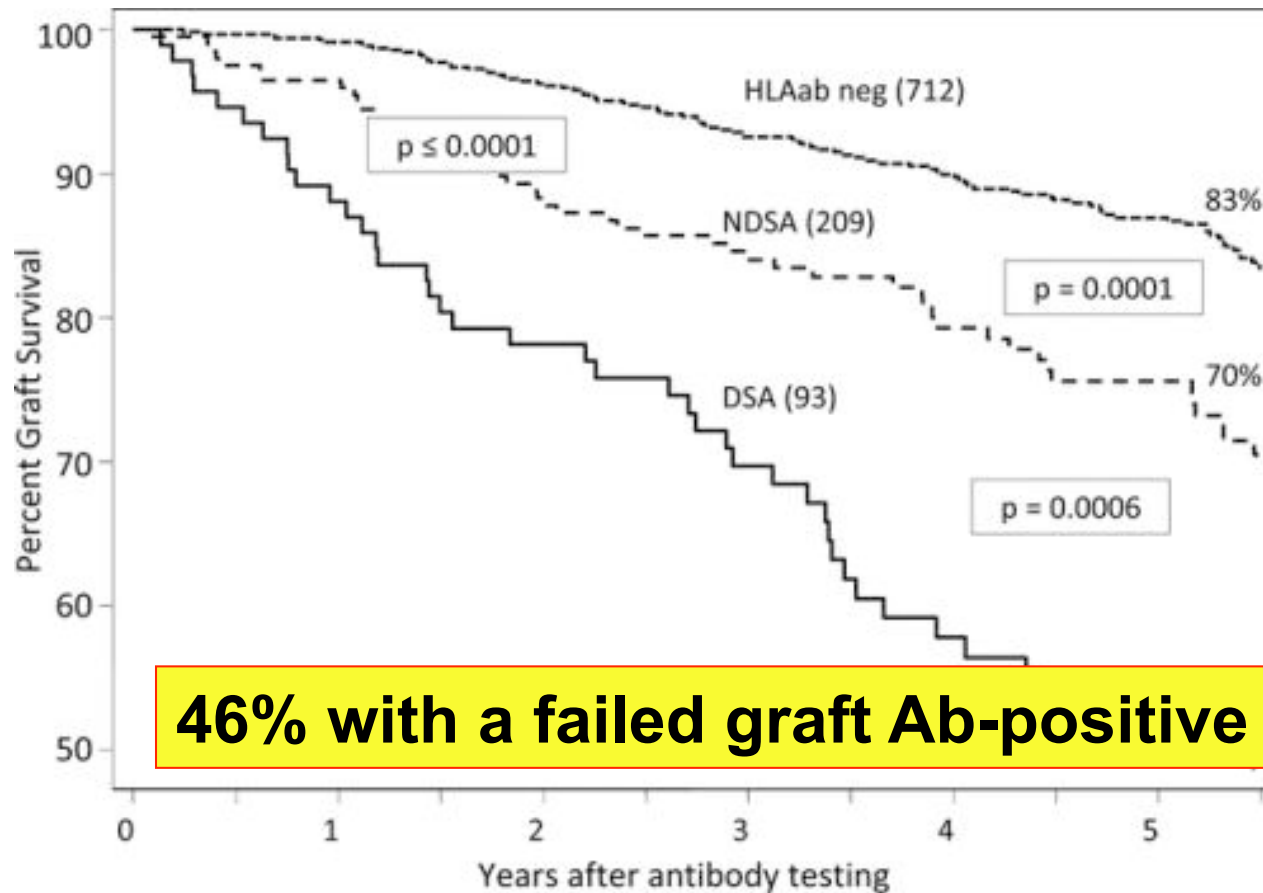
**Antibody-mediated rejection**



# Single center study (Berlin)

1014 deceased donor Tx recipients (Tx 1984 – 2004)

**Luminex SA** at  $\geq 6$  mo after Tx (median 5 yrs)



Lachmann et al, *Transplantation*, 2009, 87: 1505



# Antibody-mediated rejection criteria

An addition to the Banff'97 classification of renal allograft rejection

Racusen et al, *Am J Transplant*, 2003

## 1. Normal

## 2. Antibody-mediated rejection

Rejection due, at least in part, to **documented anti-donor antibody**  
(„suspicious for“ if antibody not demonstrated);

*may coincide with 3, 4, and 5*

### Type (Grade)

I. ATN-like - C4d+, minimal inflammation

II. Capillary - margination and/or thromboses, C4d+

III. Arterial - v3, C4d

## 3. Borderline changes

## 4. Acute/active cellular rejection

## 5. Chronic/sclerosing allograft nephropathy

## 6. Other

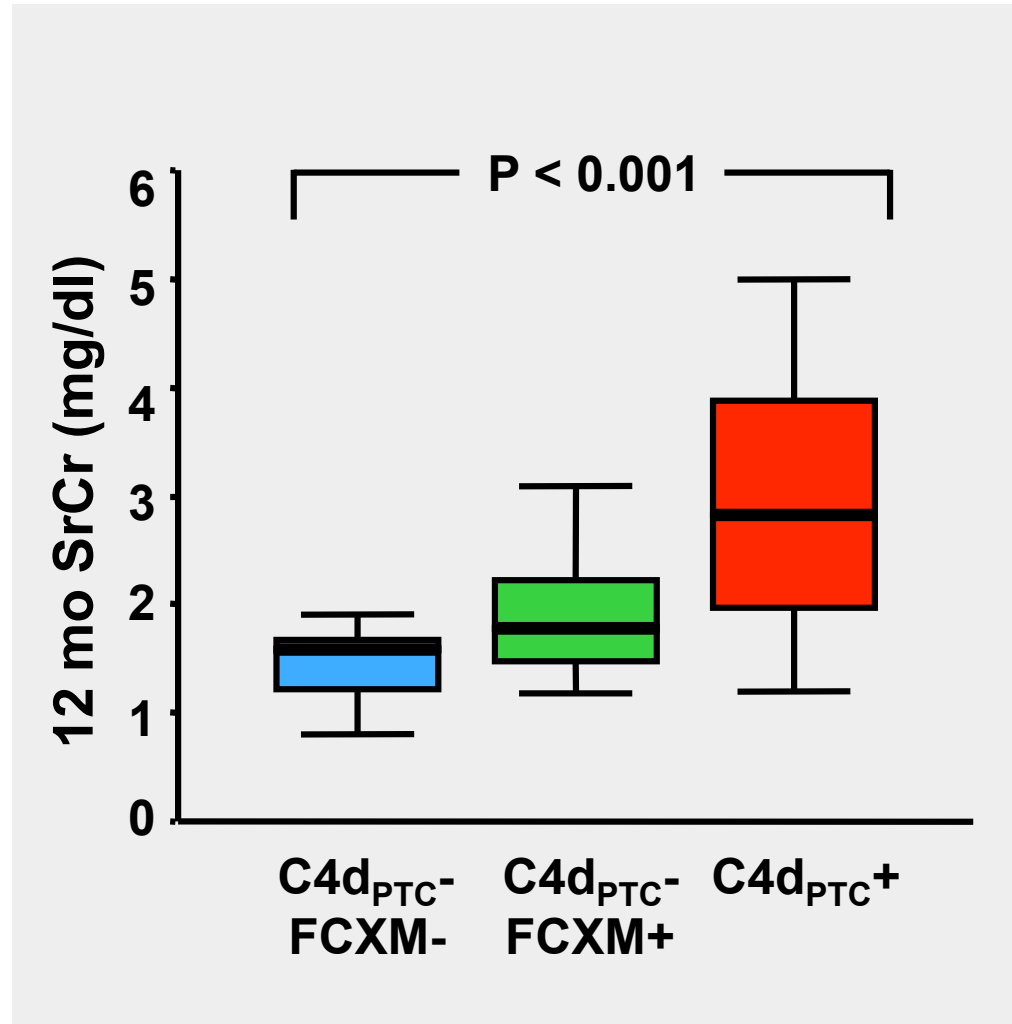
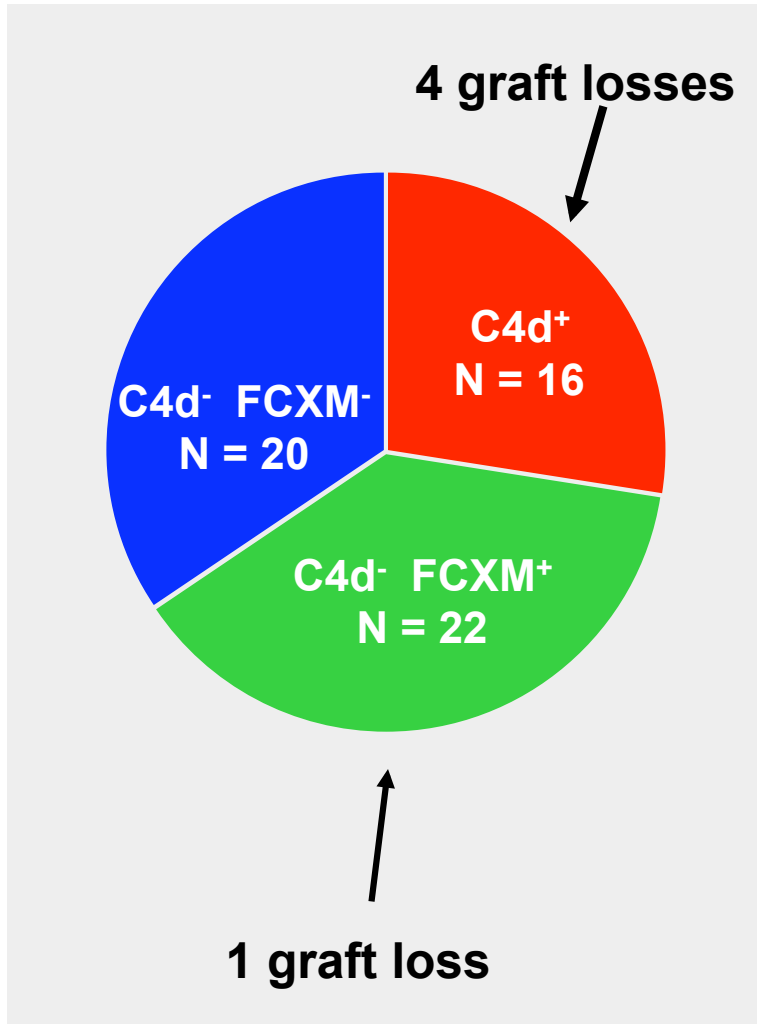


# Alloantibody detection ↔ Indication biopsy

Author	N	Assay	C4d-		C4d+	
			Ab-	Ab+	Ab+	Ab-
Böhmig (2002)	113	FCXM FlowPRA	53%	88%	38%	88%
			0%	22%	0%	14%
Mauyyedi (2002)	67	AHG-XM or FCXM	2%	90%	0%	94%
			0%	0%	31%	88%
Worthington (2007)	92	ELISA ELISA-DSA	0%	22%	0%	14%
			0%	0%	31%	88%
Bartel (2008)	105	FlowPRA FlowPRA-DSA	40%	94%	31%	88%
			33%	79%	0%	0%
Álvarez-Márquez (2009)	58	Luminex/ELISA (HLA, MICA, GSTT1)	33%	79%	0%	0%
			0%	0%	0%	0%



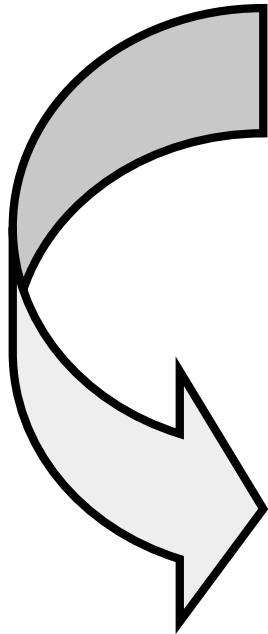
# Capillary C4d, FCXM & allograft outcomes



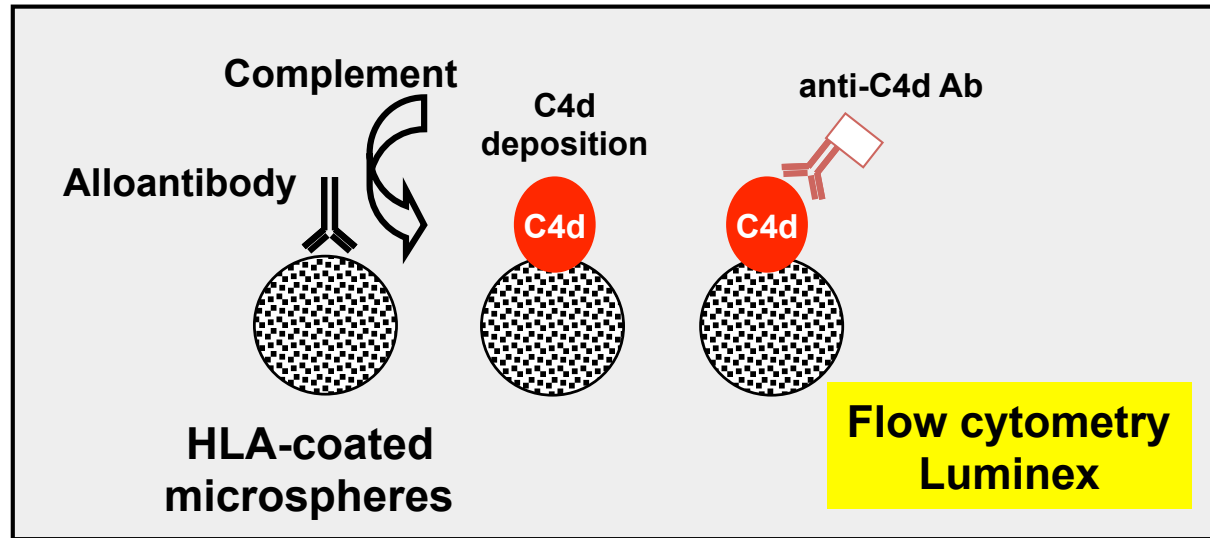
Böhmig et al., *J Am Soc Nephrol*, 2002, 13: 1091



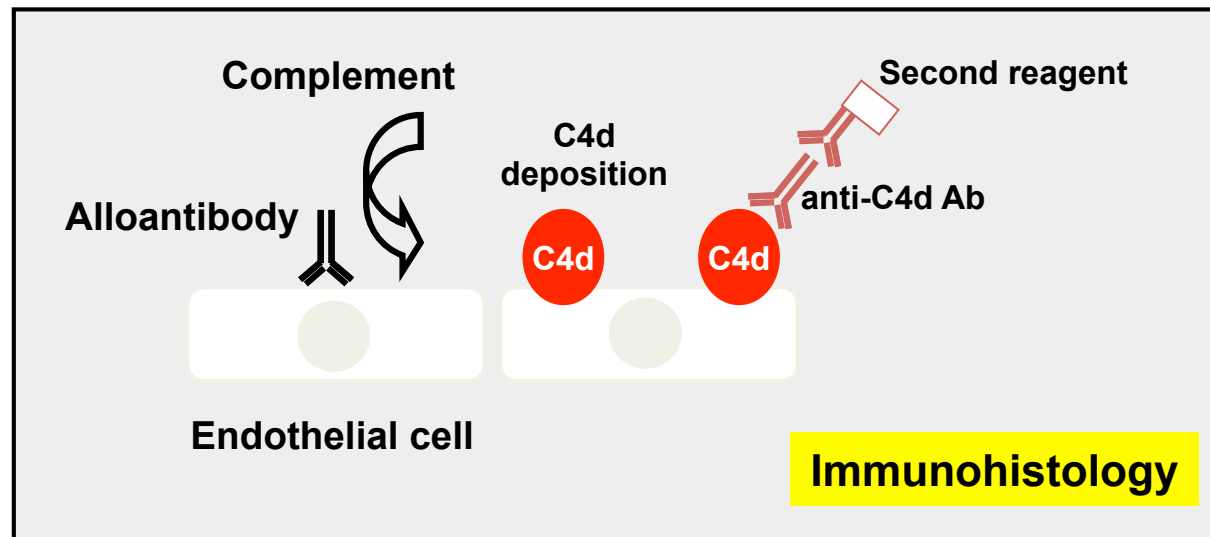
Surrogate of  
AMR?



## Solid-phase C4d staining in vitro



## Capillary C4d deposition in vivo



Böhmig et al., *Human Immunol*, 2009, 70:640



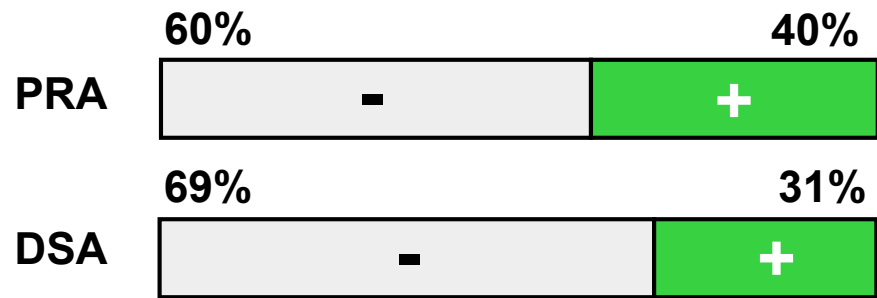
105 patients

Biopsy

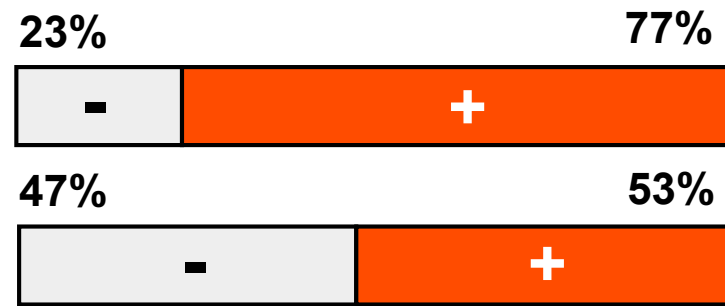
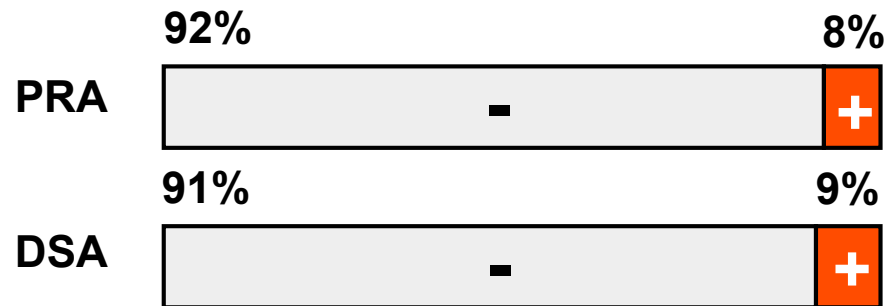
C4d negative, N=88

C4d positive, N=17

[IgG]FlowPRA



[C4d]FlowPRA



Bartel et al., *Am J Transplant*, 2008, 8:41

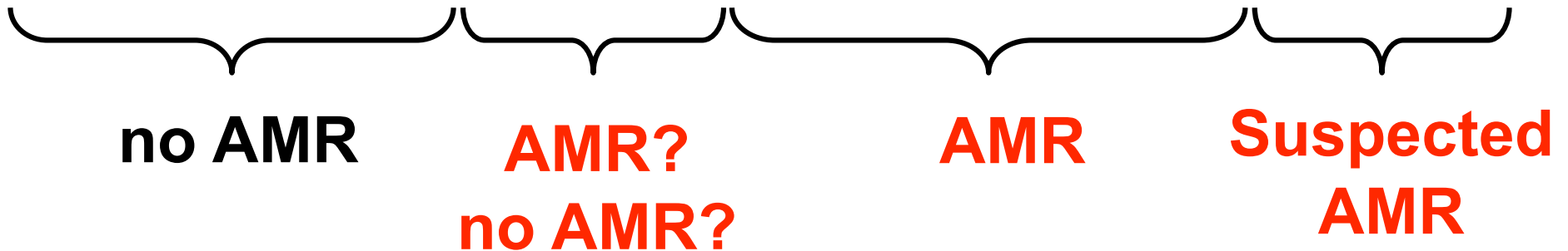
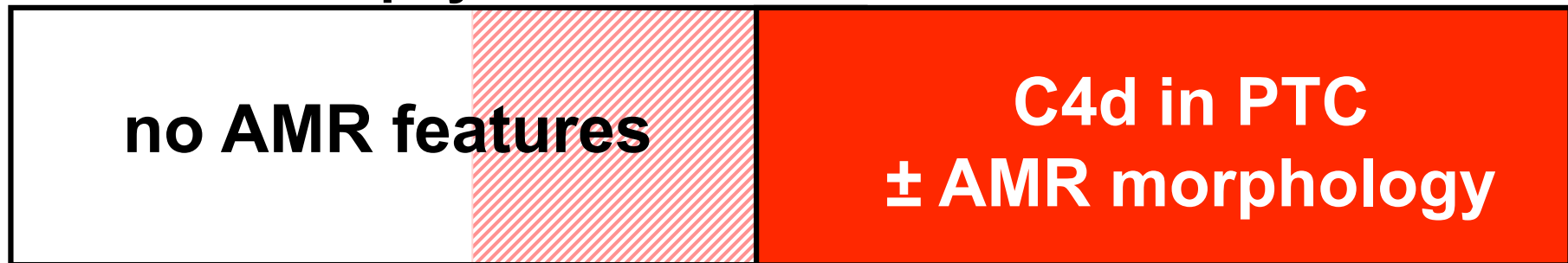


# Serology in acute/chronic dysfunction

## Circulating HLA-DSA



## Indication biopsy





# Serology and pending rejection

## Two major questions



**Does early Ab detection reliably predict chronic graft damage/loss?**



**Could early Ab detection guide targeted pre-emptive treatment?**



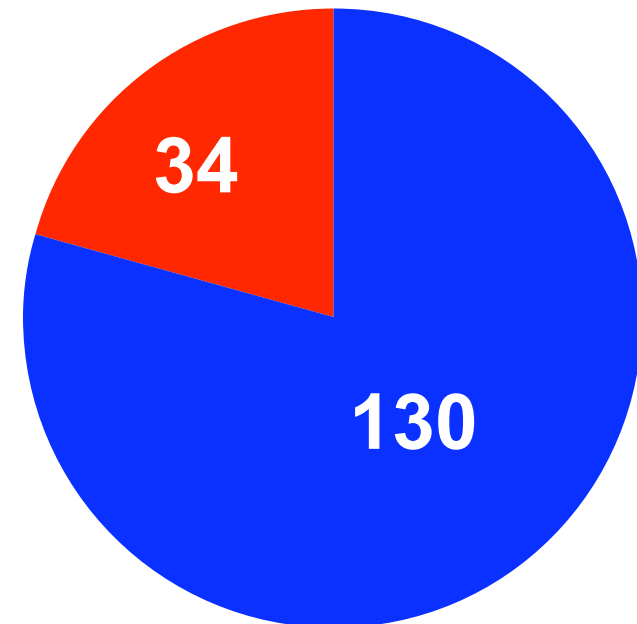
# Alloantibodies and excellent Tx function?

164 recipients with  $\geq 1$  a transplant function

## Separate analysis:

Patients with excellent 1-yr clinical course

1. GFR  $\geq 60$  ml/min (MC equation)
2. 24h protein excretion  $\leq 0.5$  g
3. no dysfunction/indication biopsy
4. no desensitization or rejection treatment

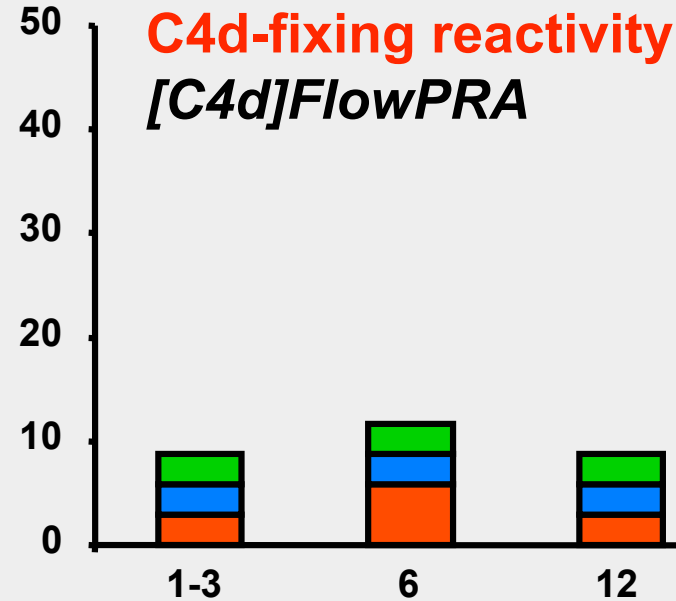
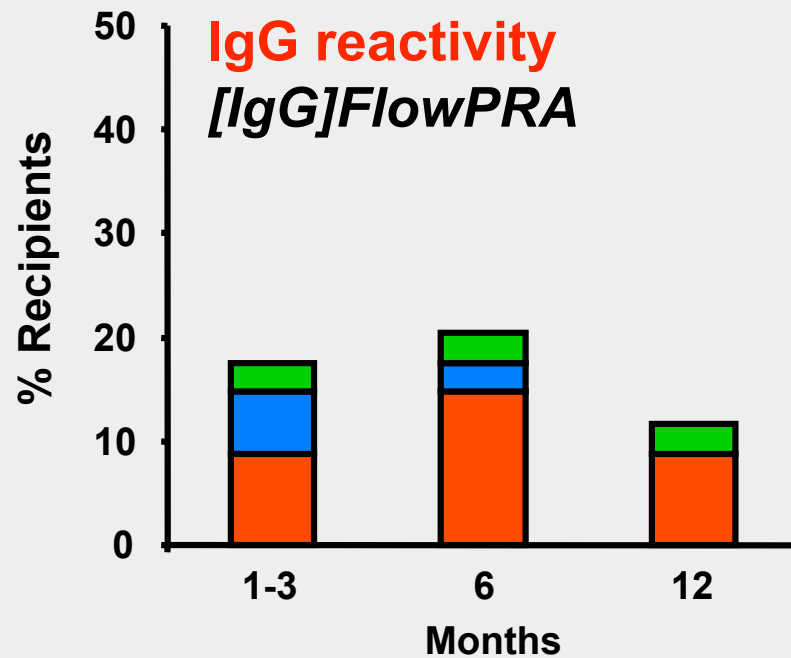


Bartel et al., *Am J Transplant*, 2008, 8:2652



# Post-Tx $\alpha$ HLA reactivities

- 34 Tx recipients with excellent 1-yr course -



- HLA class II
- HLA class I+II
- HLA class I

**No difference to „non-stable“ patients**

- ✓ Incidence
- ✓ Binding strength
- ✓ DSA frequency
- ✓ C4d-fixation in vitro

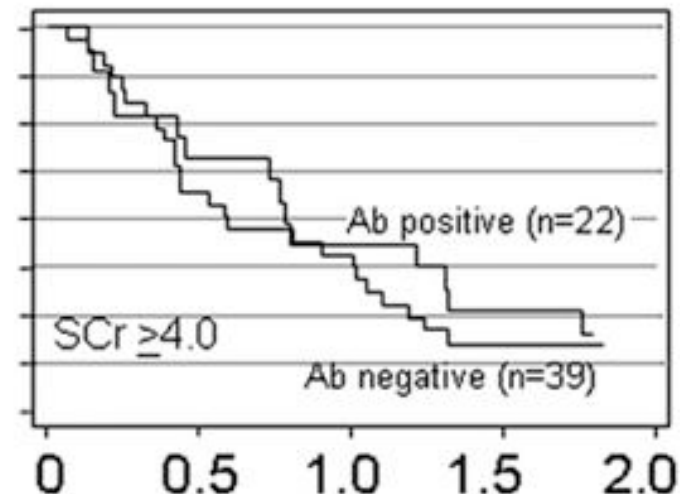
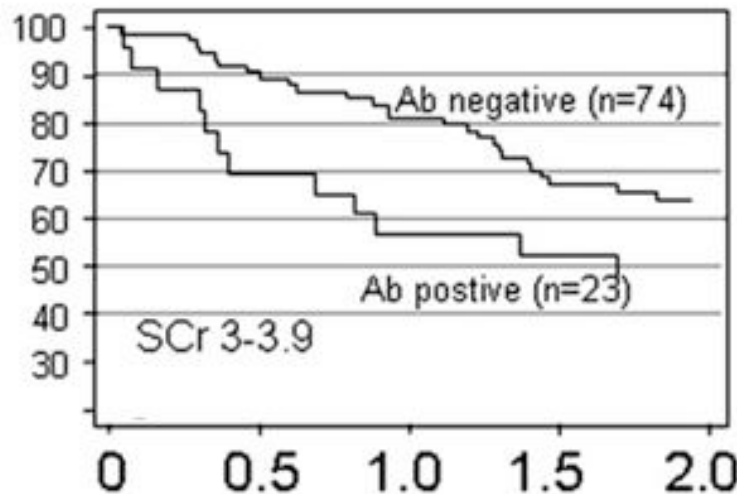
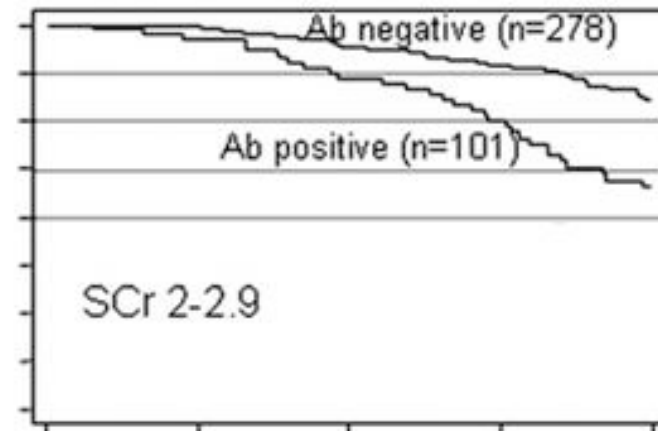
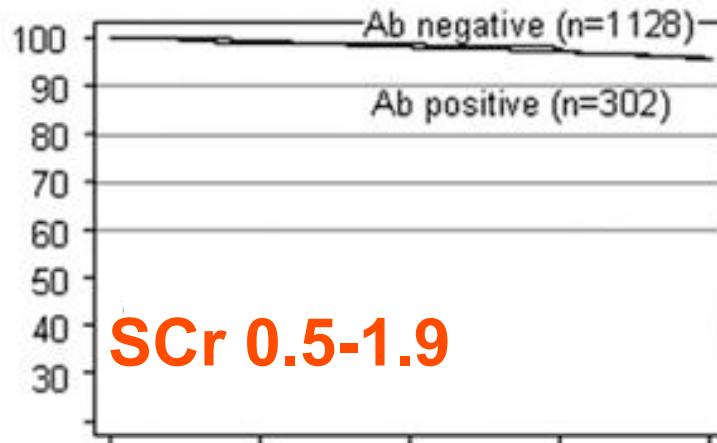
Bartel et al., *Am J Transplant*, 2008, 8:2652



# SrCr and predictive value of HLA Ab

(Single „snap shot“ after 6 months to >10 years)

Percent graft survival



Years after testing

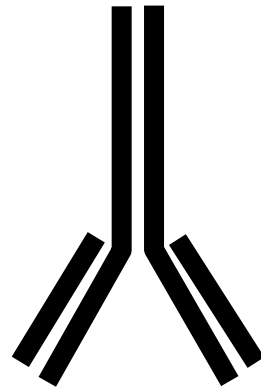
Terasaki&Ozawa, *Transplantation*, 2005, 80:1194



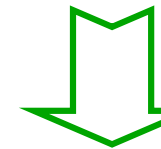
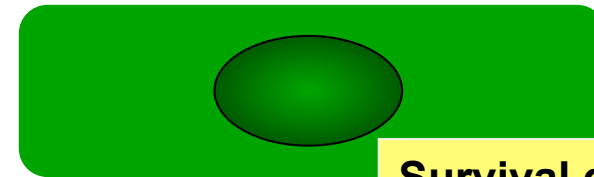
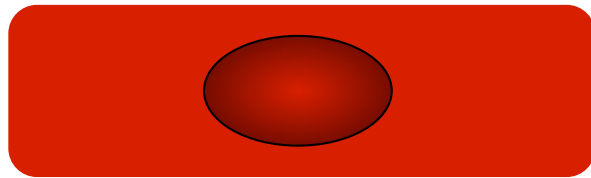
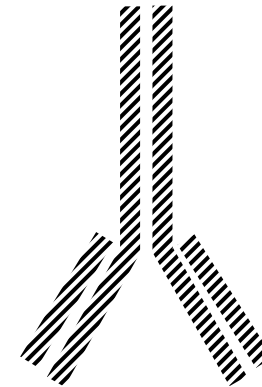
# Accommodation – Mechanisms?

„saturating“

„subsaturating“  
(e.g. following depletion)



BG/Xeno/HLA



**Activation/injury**

**Resistance**

Survival genes  
C-Regulation  
Reduced adhesion

Reviews: Tang & Platt, *Human Immunol*, 2007;68: 645  
Zhang and Reed, *Am J Transplant*, 2009;9:2459-65



**Circulating alloantibodies  
±C4d in PTC  
±AMR morphology**

**Graft dysfunction?**

**yes**

**no**



Böhmig et al, *Human Immunol*, 2009, 70:640



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