Anti-HLA alloantibodies : PRA, cPRRA and DSA

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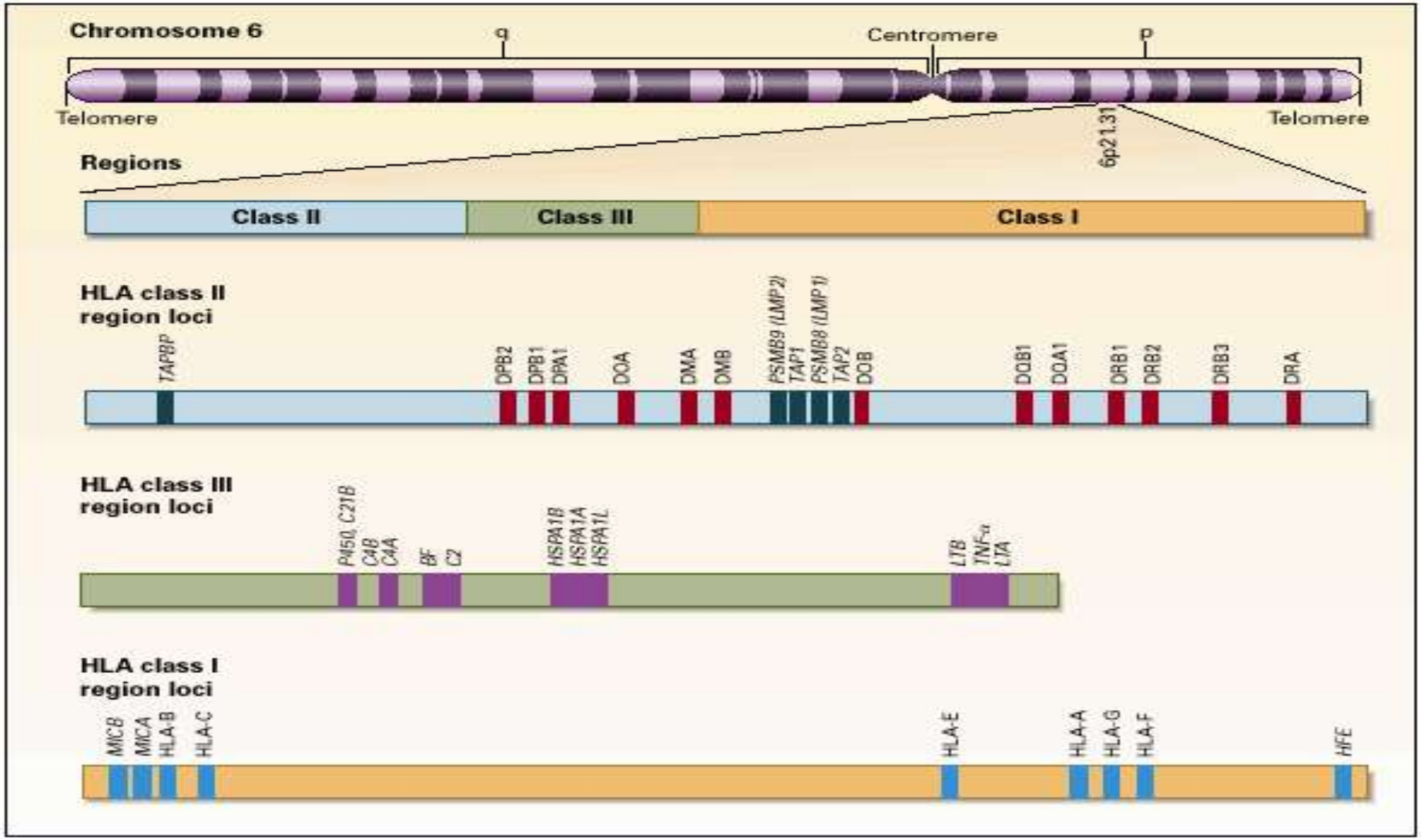
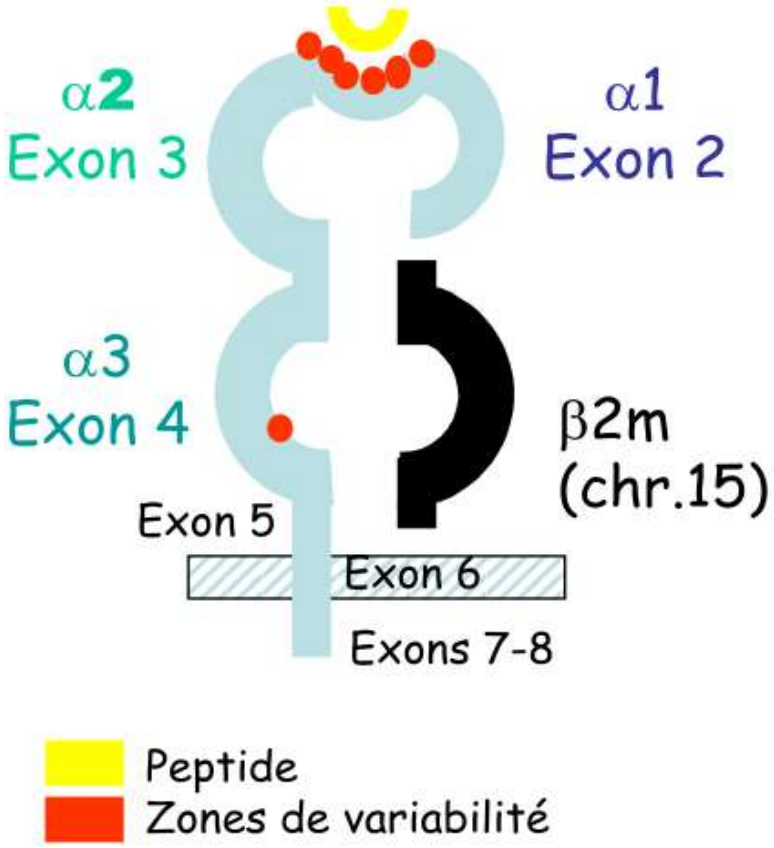
Tbilisi, September 2023

Genetic structure of HLA system on chromosome 6



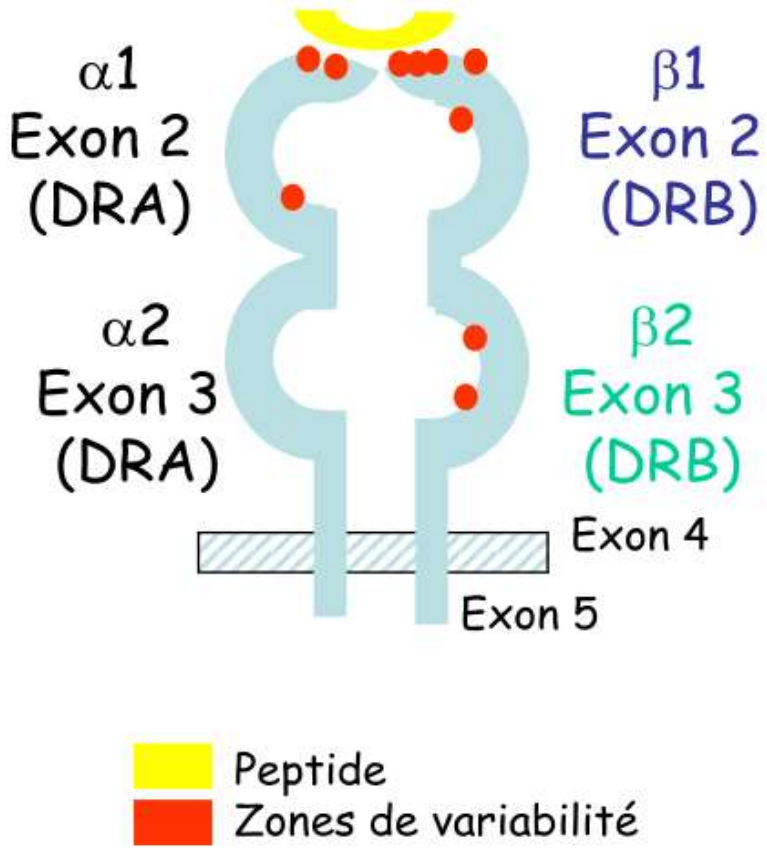
Structure of HLA class I and class II molecules

Class I



P. Bjorkman, Nature, 1987

Class II



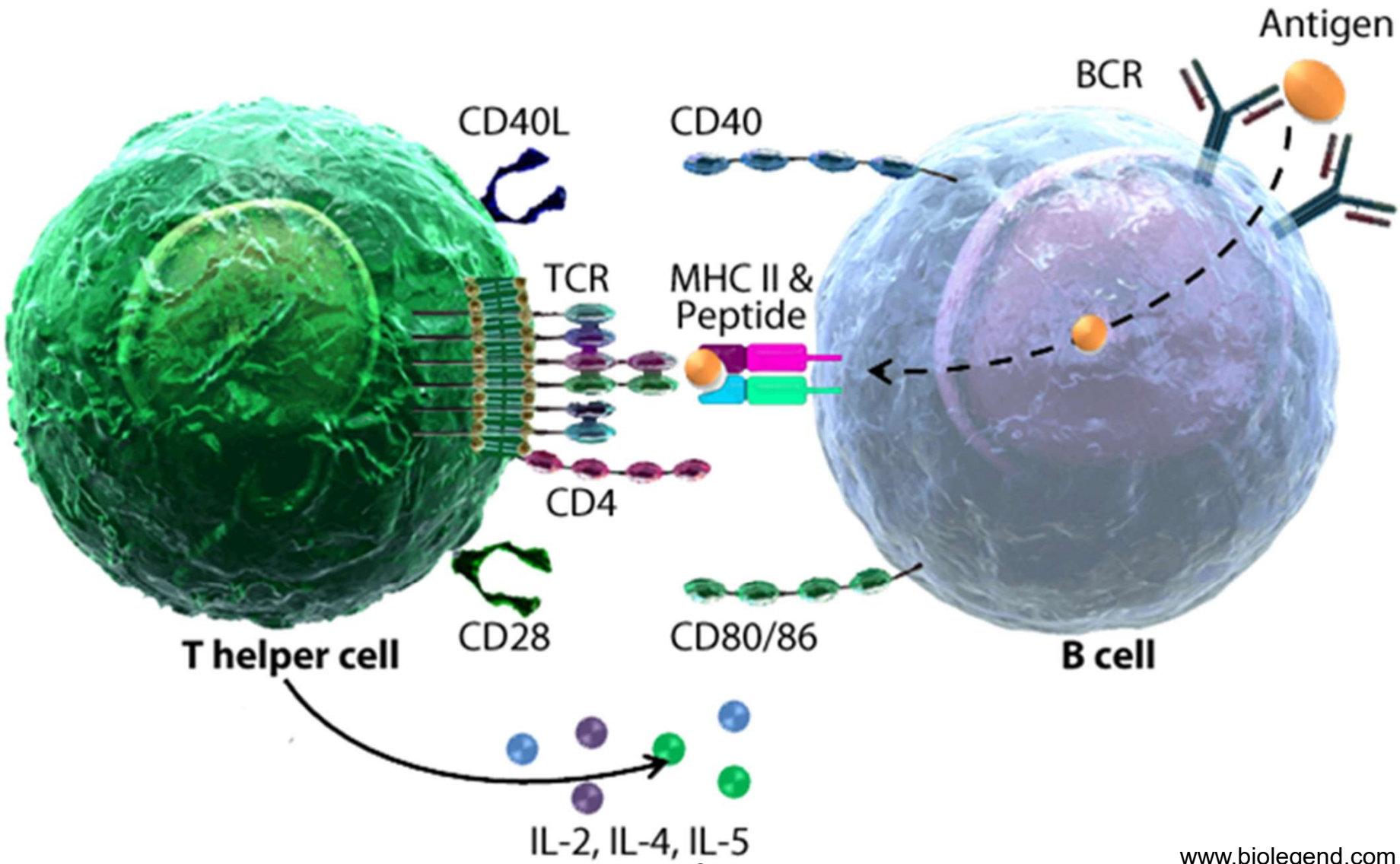
JC. Gorga, Crit. Rev. Immunol., 1992

What are the factors determining long-term allograft survival?

A good control of alloimmune responses mediated by T-cells and B-cells

- T-cells induce organ damage through cytotoxicity (CD8 + cells)
- B-cells induce organ damage through inducing synthesis of donor-specific alloantibodies (anti-HLA)
- NK-cells induce organ damage through ADCC

T-helper cells will activate B-cells

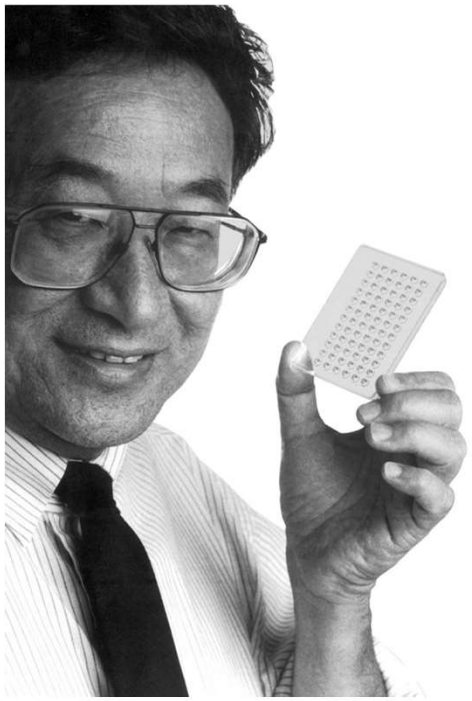


www.biolegend.com

Can we predict whether an organ will be (immediately) rejected?

YES thanks to the cross-match

Paul Terasaki- Inventor of the Crossmatch



The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.

The New England
Journal of Medicine

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ıme 280 APRIL 3, 1969 Number 14

SIGNIFICANCE OF THE POSITIVE CROSSMATCH TEST IN KIDNEY TRANSPLANTATION*

RAMON PATEL, M.R.C.P., AND PAUL I. TERASAKI, PH.D.

Abstract Crossmatch tests of the prospective kidney-transplant donor's lymphocytes with the serum of the prospective recipient in 225 transplants showed that eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch (p less than 0.001). Immediate failure occurred in significantly higher numbers among patients with a higher risk of having antibodies, such as multiparous females and patients receiving secondary transplants. The effect was not a nonspecific one, for more immediate failures occurred among transplants from unrelated than among those from related donors. The corresponding frequency of positive crossmatch was also lower among related donors. The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.

HLA sytem and organ transplantation

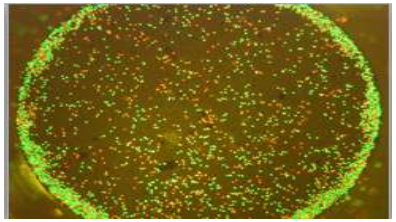
Very last test before transplantation :
the CROSS-MATCH

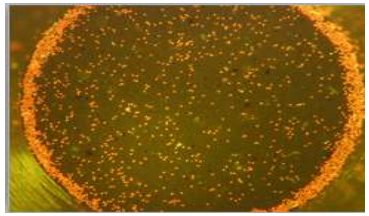
Performed 24 hours a day, 7 days a week

Rationale : to check within the recipient the ABSENCE of circulating complement-binding donor-specific alloantibody (ies)

Donor serums
+ donor cells (lymphocytes or splenocytes)
+ Complement
+ Dyes

Transplant: OK





Transplant: forbidden

Anti-HLA alloantibodies

* Are ONLY present after a sensitization event:
    * pregnancies/miscarriages
    * blood transfusions (platelets; red blood cells)
    * previous transplantation
* They have to be detected by a very sensitive technic
    * currently it should be assessed by Luminex technology (OneLambda or Immucor). Results are expressed in mean fluoresence intensity (MFI)
    * CDC tests only detect complement-binding anti-HLA antibodies
    * Elisa tests are not sensitive enough
* When they are positive they might be directed against a potential donor = donor-specific alloantibody (DSA)

- When they are positive for a given donor the cross-match has to be perform by CDC but also by flow cytometry (more sesitive)
- Should flow cross-match be positive it implies pretransplant desensitization

HLA and organ transplantation

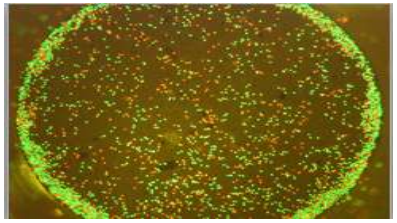
The cross-match : an example

Waitlisted women kidney transplant candidate,
HLA type-A1, 3; B7, ..; DR3, 4
She has had 3 pregnancies but no previous blood transfusions
Pretransplant anti-HLA alloantibodies were assessed every 6 months: she was sensitized against -HLA-A2, A24 and DR11 (i.e. cPRA is at 65%)

How was the patient sensitized?

Deceased kidney transplant proposal: HLA-A1, 28; B7, 8; DR4,..

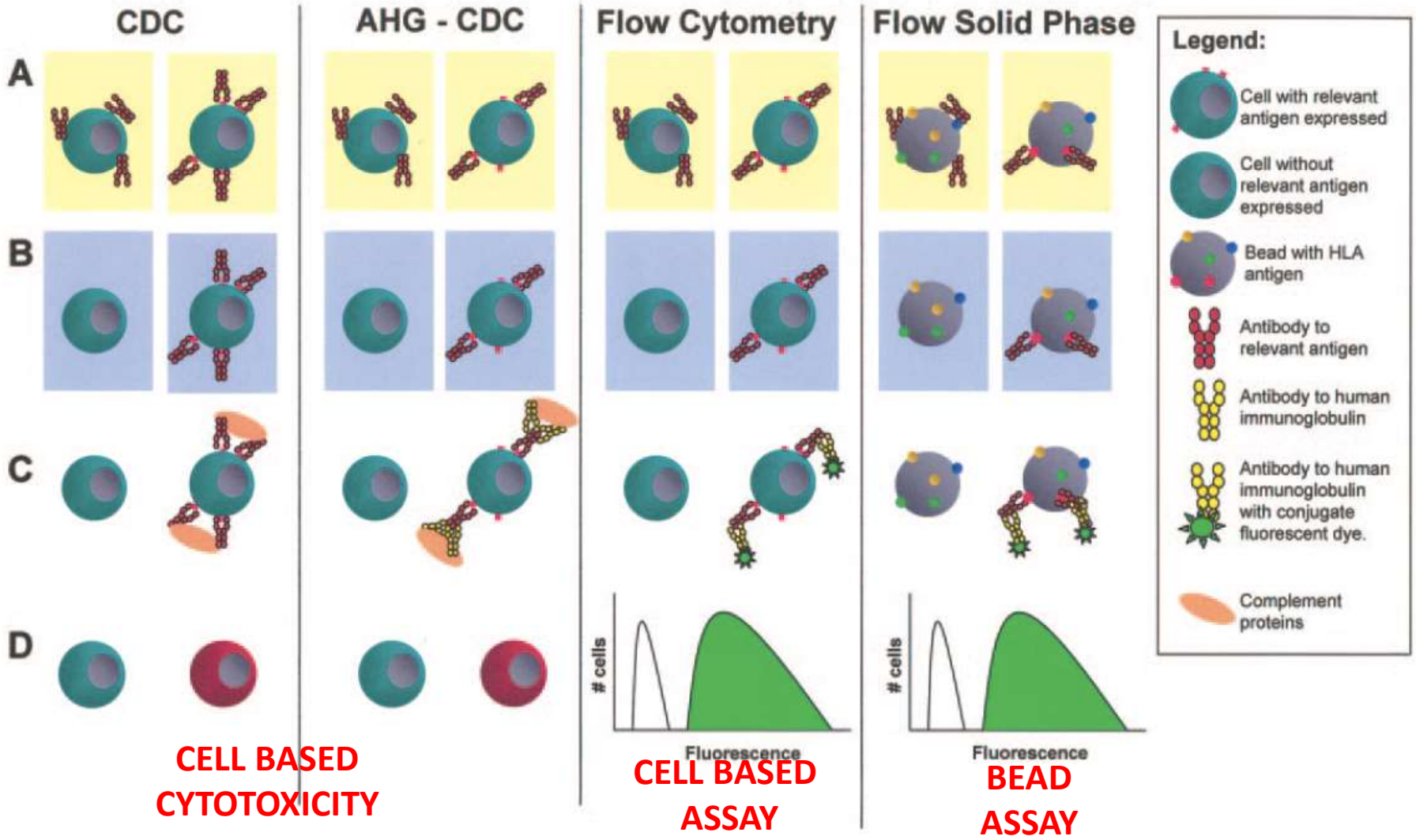
What do we decide



Conclusion : the cross match is negative;
kidney transplant is possible

Crossmatch, panel reactive alloantibodies (PRA) and anti-HLA antibodies

Complement dependent cytotoxicity (CDC) FACS Luminex/ELISA



Tinckam KJ and Chandraker A. CJASN 1: 404, 2006

cPRA in 2023

PRA = panel reactive alloantobodies, i.e. anti-HLA alloantibodies against a given panel of HLA antigens (which varies according to world areas)

* these antigens represent the MOST frequent HLA antigens in a given population, i.e. the antigens contributing to PRA panel are different in Western Europe vs. Middle East vs. China vs. America etc…
* PRA was derived from Elisa test

In 2023 we use calculated PRA (cPRA), i.e. the result is given based on the Luminex single bead test

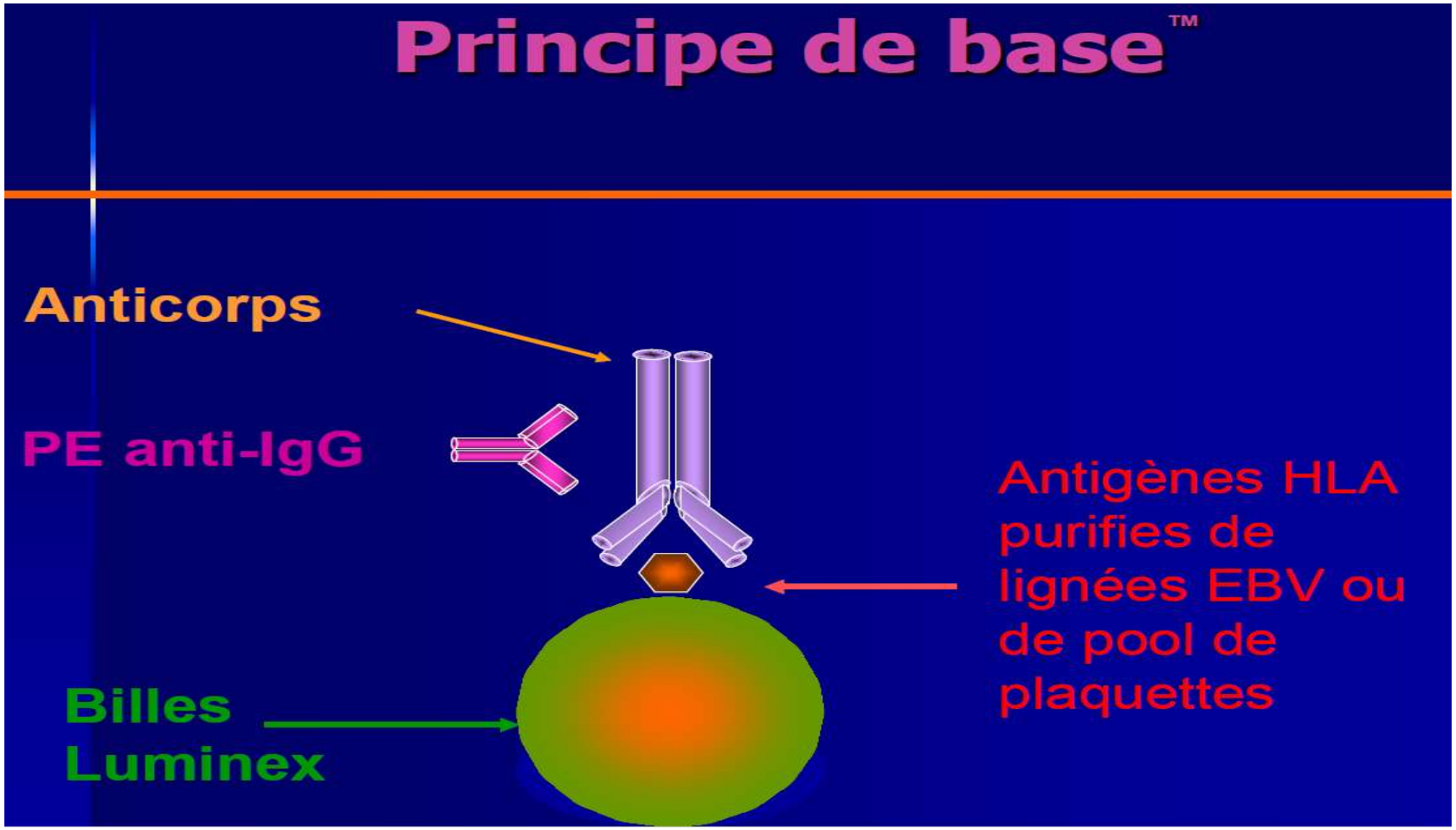
In 2023 in the setting of living transplantation we do NOT care about cPRA!!! What ONLY counts is the presence OR absence of donor-specific alloantibody (ies) = DSA

cPRA only gives you an idea of the magnitude of HLA sensitization

The LUMINEX technology

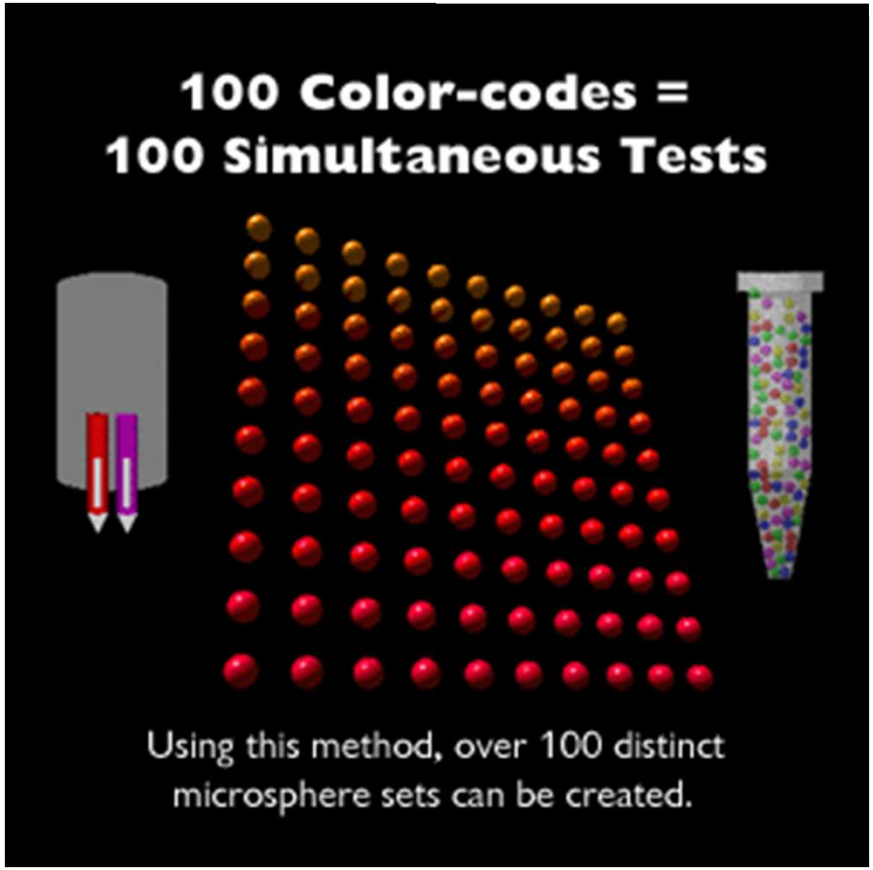
Multianalytical system including a flow cytometer and a constellation of microspheres

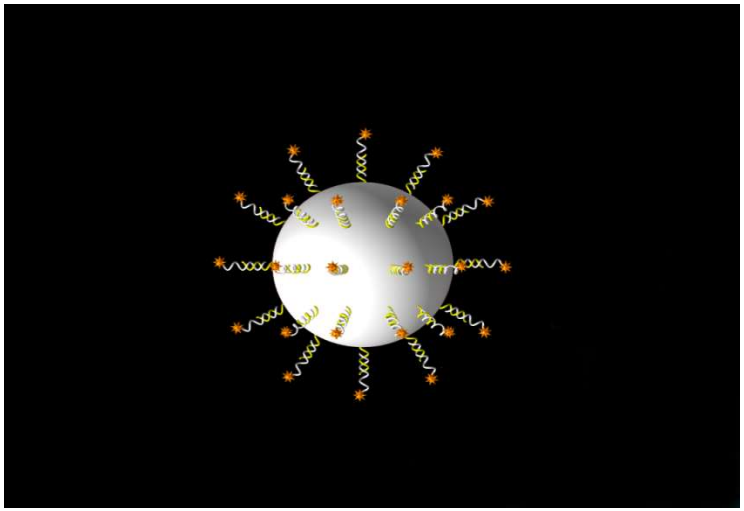
Luminex technology

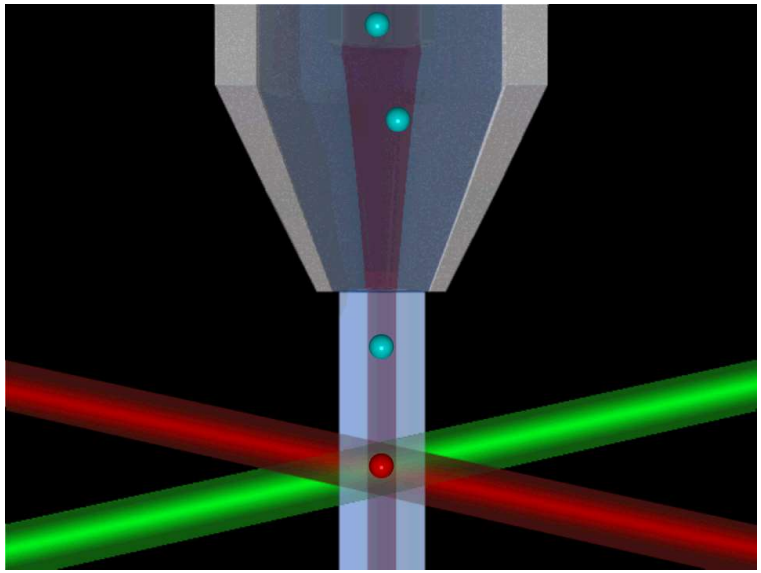


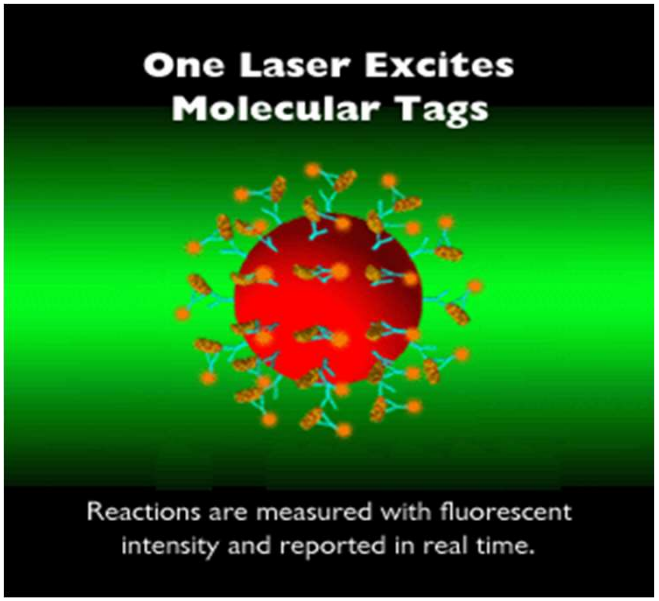
Luminex beads

Every single bead is covered by a SINGLE HLA antigen



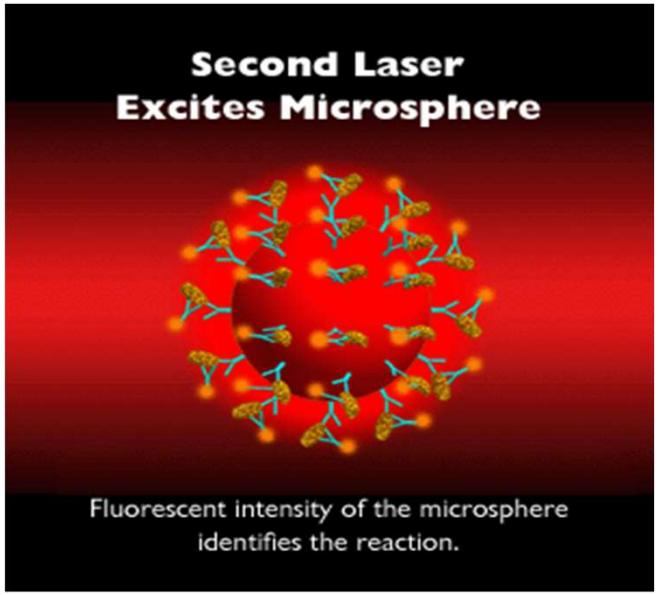






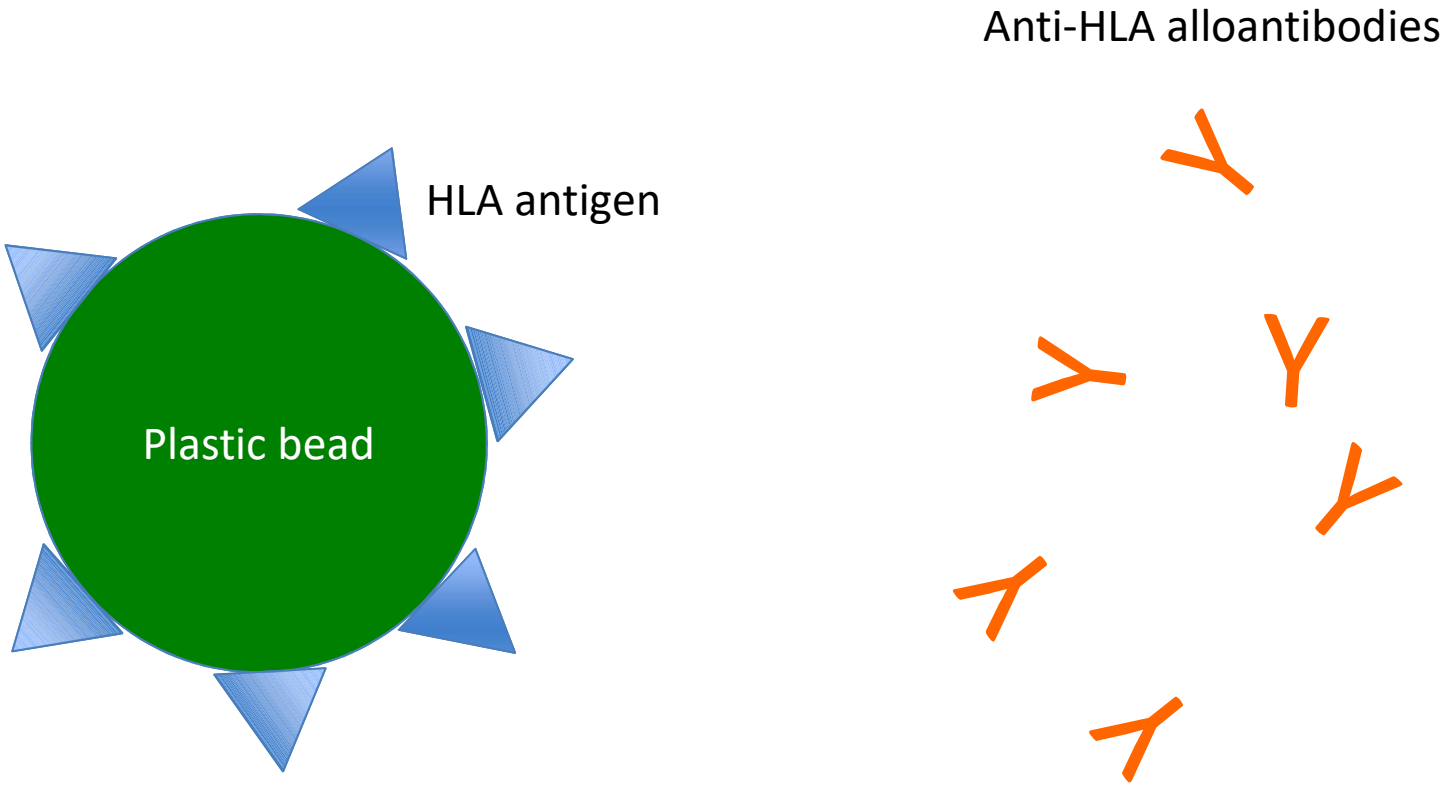
Détection par deux lasers différents:

- Le laser rouge discrimine les différents jeux de billes.
- Le laser vert quantifie la fluorescence émise par la molécule

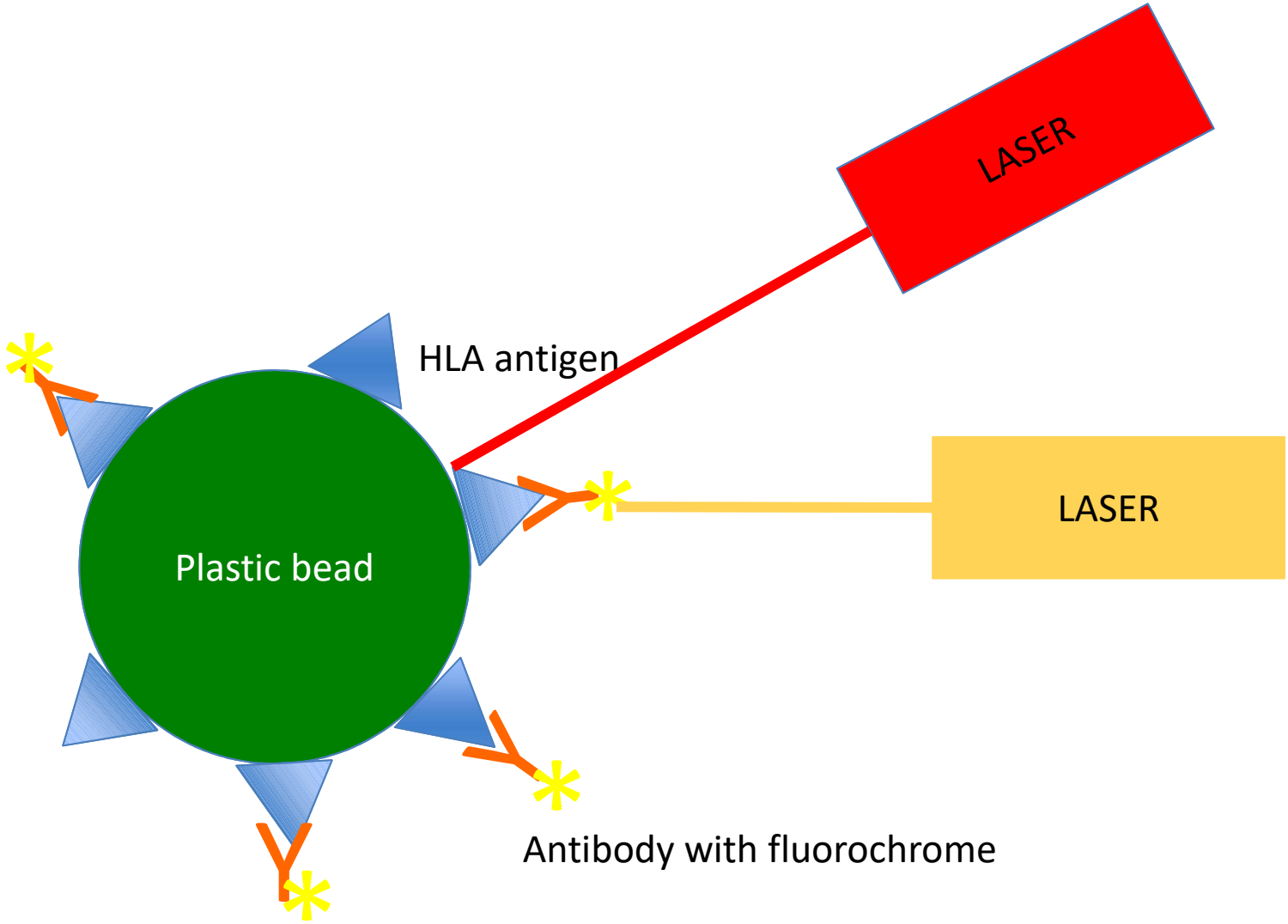


Red laser discriminates the beads; green laser quantifies fluorescence emitted by the molecule

Luminex- Single Antigen Technology



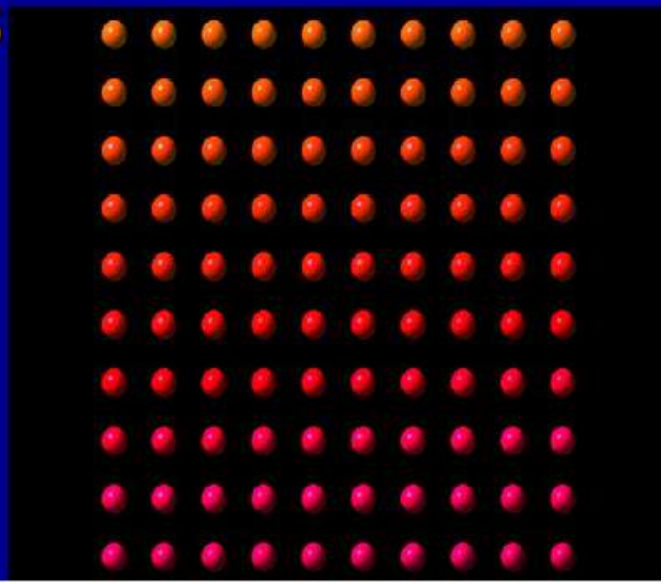
Luminex- Single Antigen Technology



Identification of anti-HLA alloantibodies

Principe du dépistage et d'identification des Ac anti HLA:

- utilise un panel de microbilles colorées recouvertes d'antigènes HLA classe I ou II, permet de détecter la présence d'AC anti HLA spécifique IgG et d'identifier les spécificités.



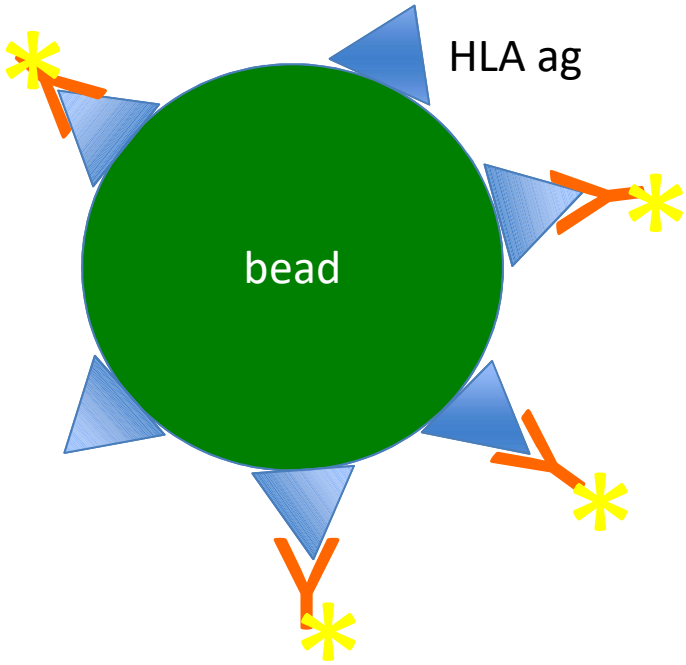
Screening and anti-HLA alloantibody identification: based on a panel of colored microspheres coated with HLA class I or class II antigens. This allows to detect the presence of anti-HLA specific Igs and to identify the specificities.

Limits and confusion around Luminex technology

- Luminex is an *in vitro* technology: it does not give information on the pathogenic nature of the antibody
- Antibodies are pos/neg; if pos, MFI evaluation
- It confirms the presence, even at low concentrations, of specific anti-HLA ab in the patient's serum
- It may give false positive and false negative results that may condition access to transplantation if virtual crossmatch is performed.

Luminex : what is MFI?
How do we interpret MFI?

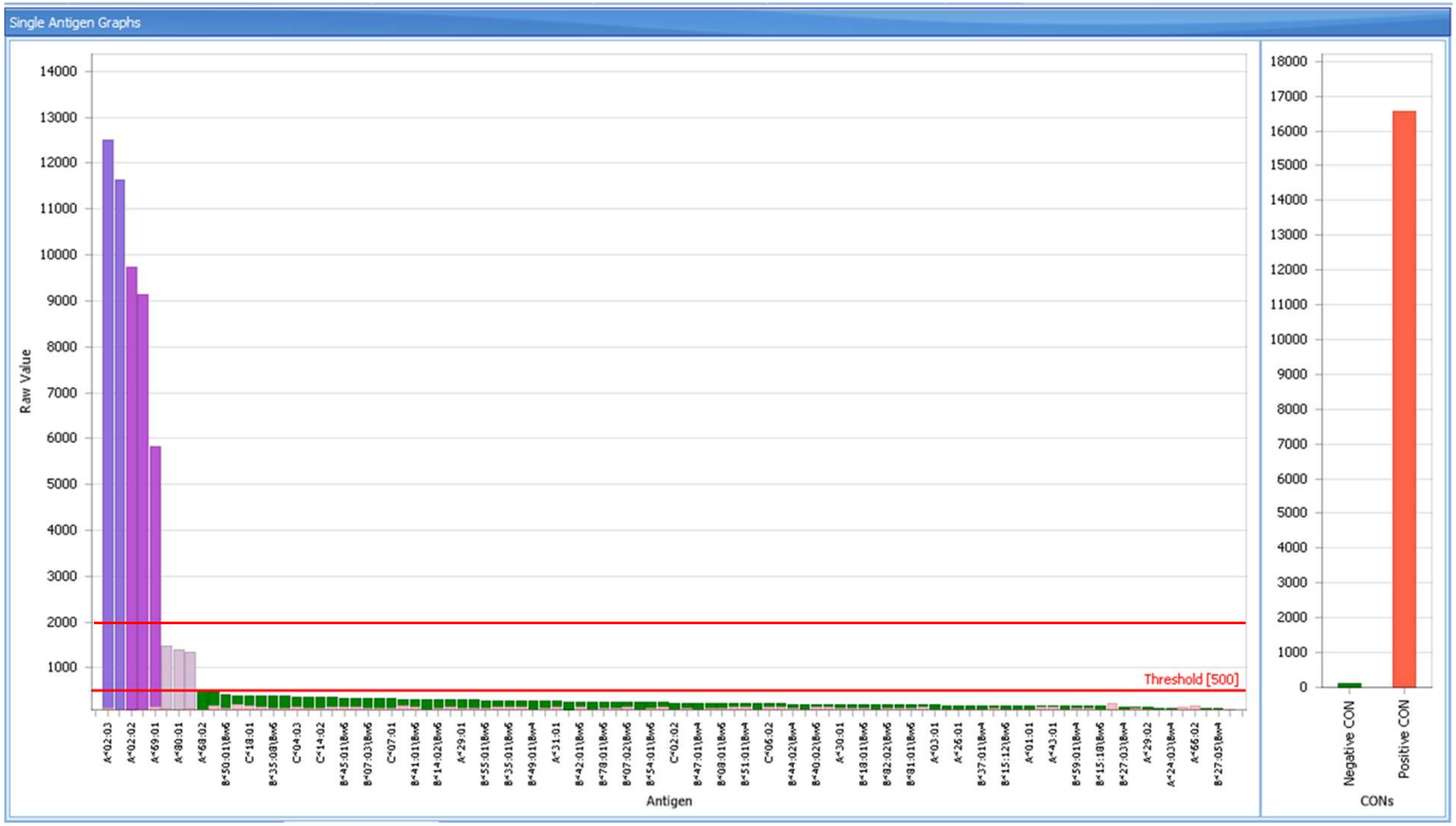
- Mean Fluorescence Intensity is the result of many factors:



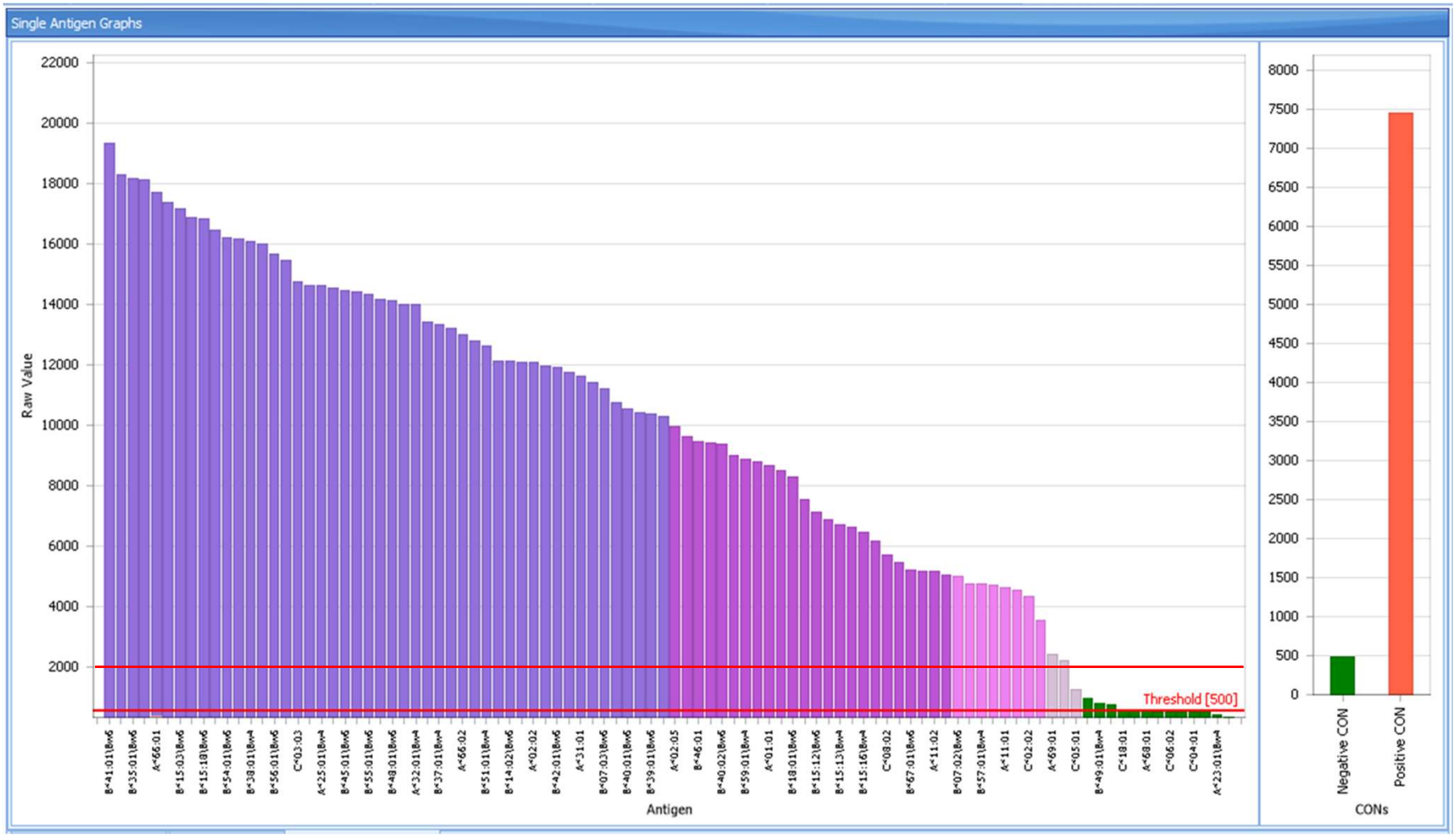
- Ab concentration in the serum
- Ab affinity

MFI does not mean Antibody strength!!!

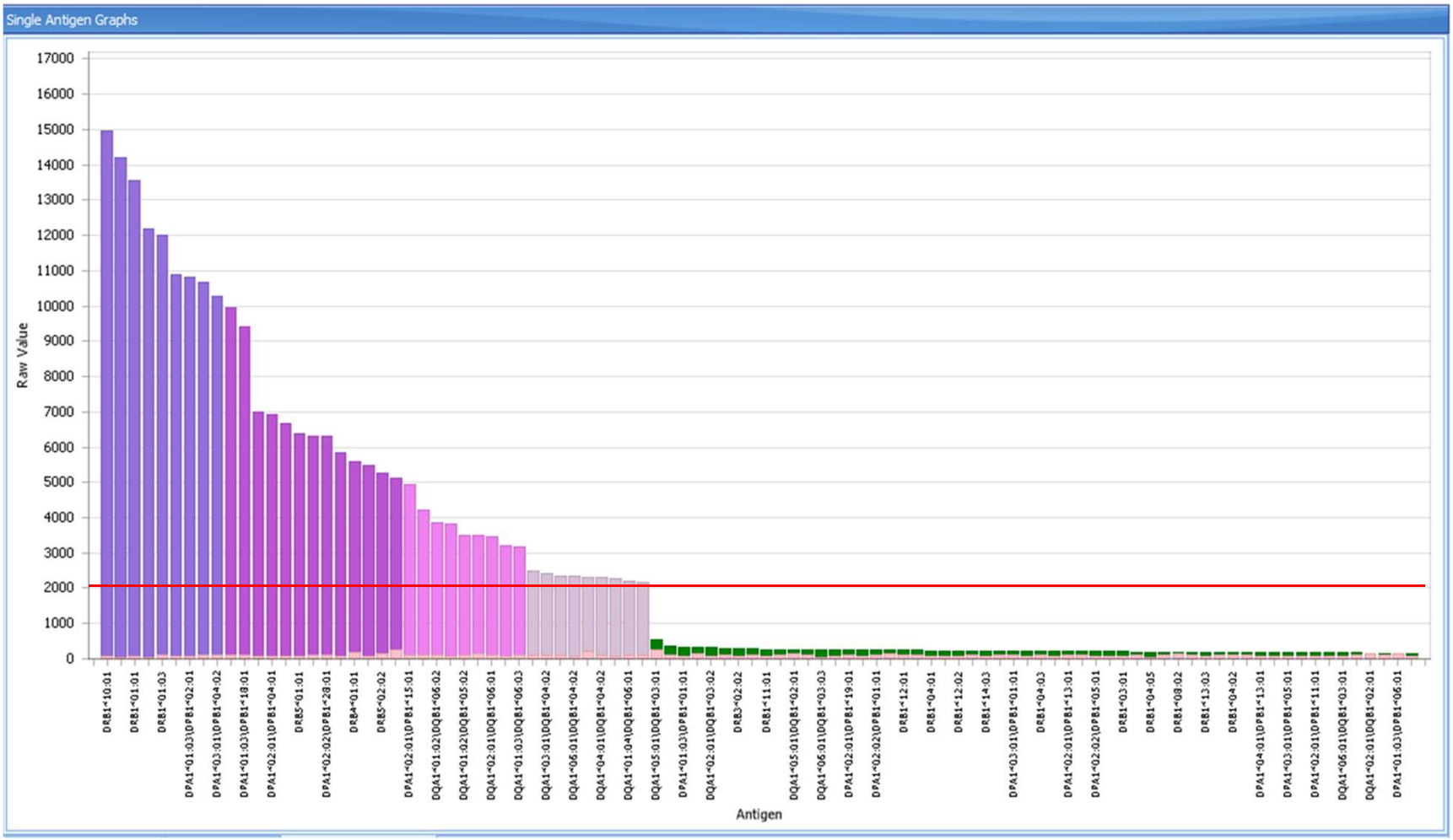
Sensitization against class I : few specificities



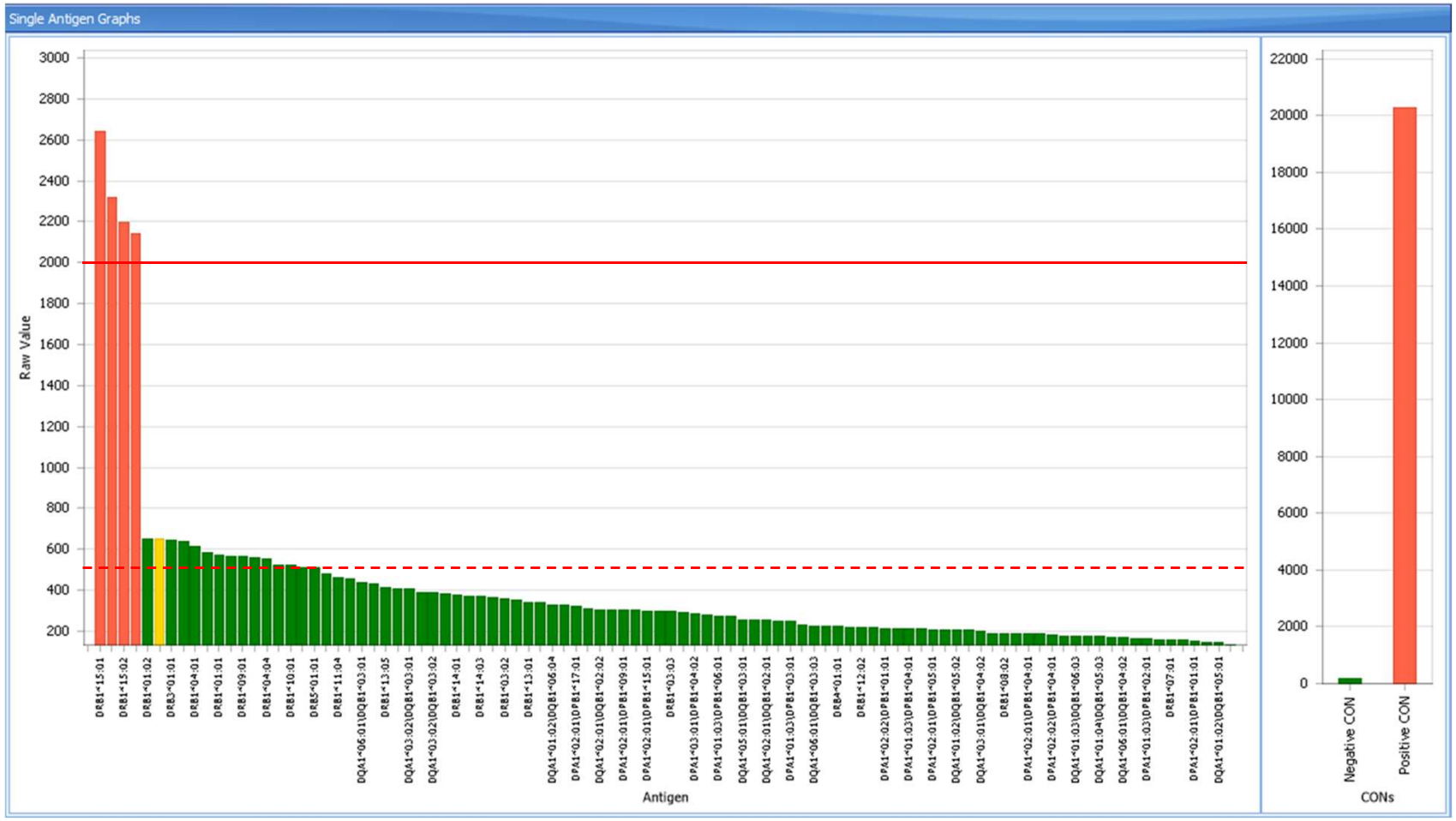
Sensitization against class I : many specificities



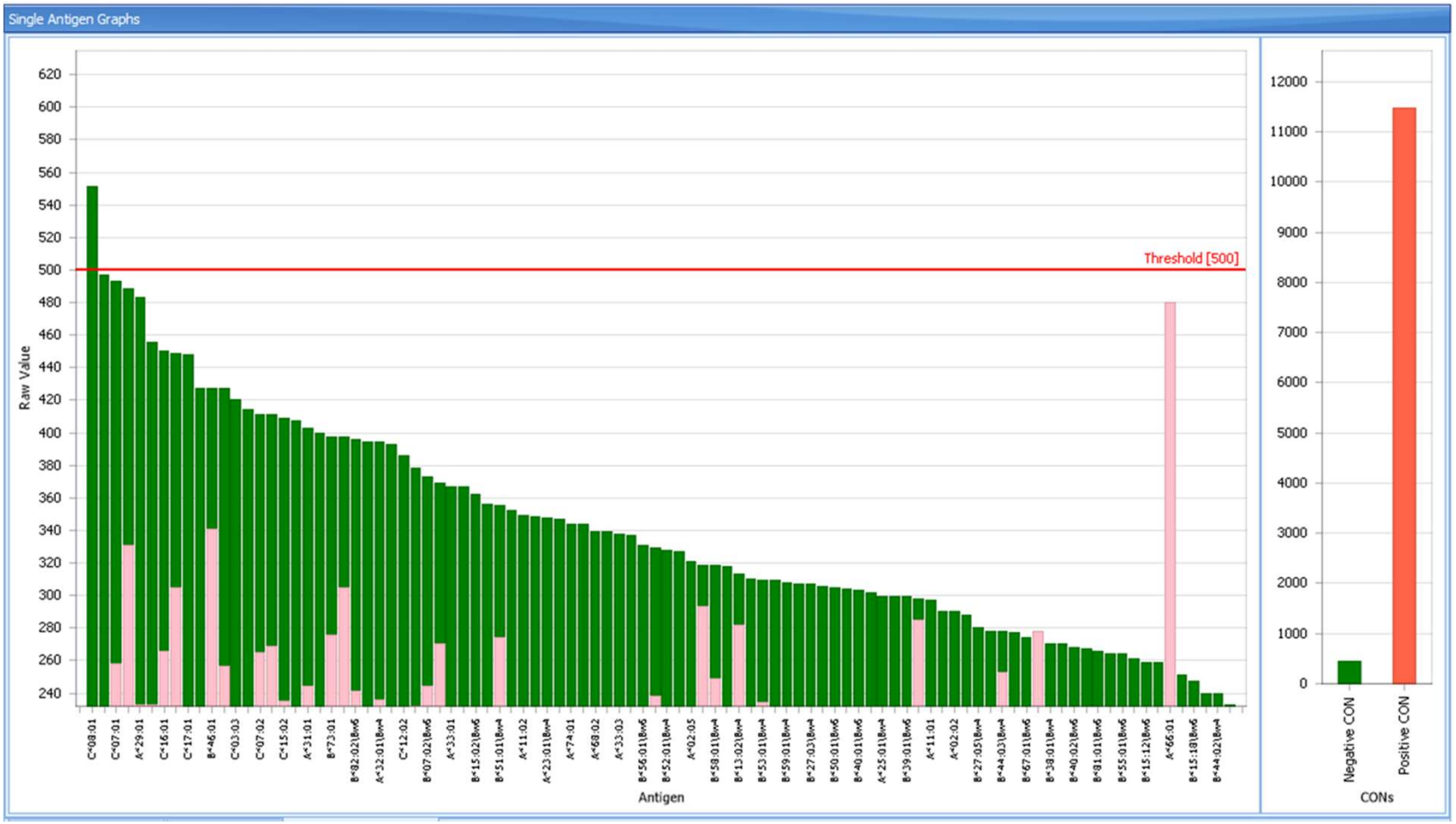
Sensitization against class II :many specificities



Sensitization against class II : few specificities



No sensitization against class I antigens

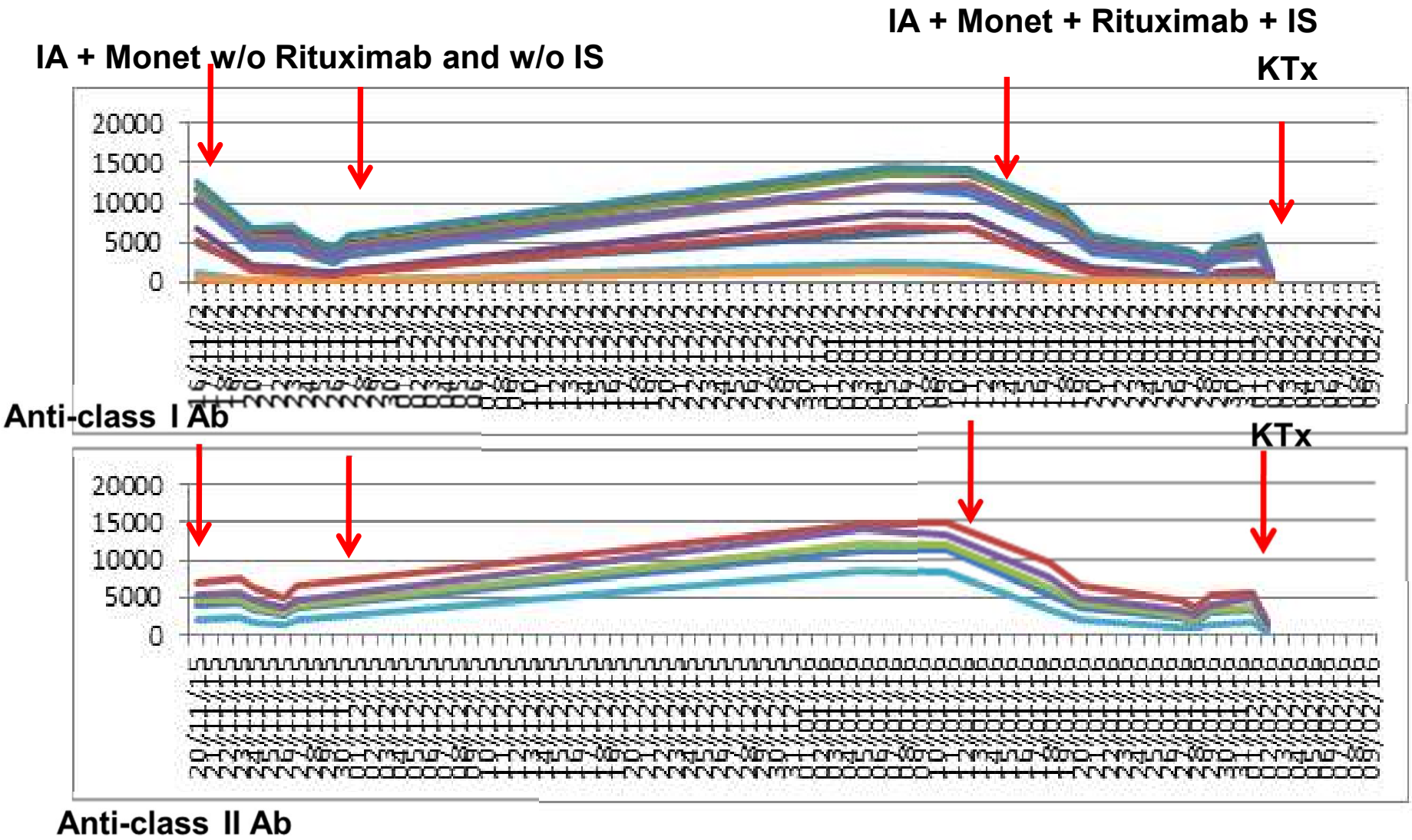


MFI follow-up

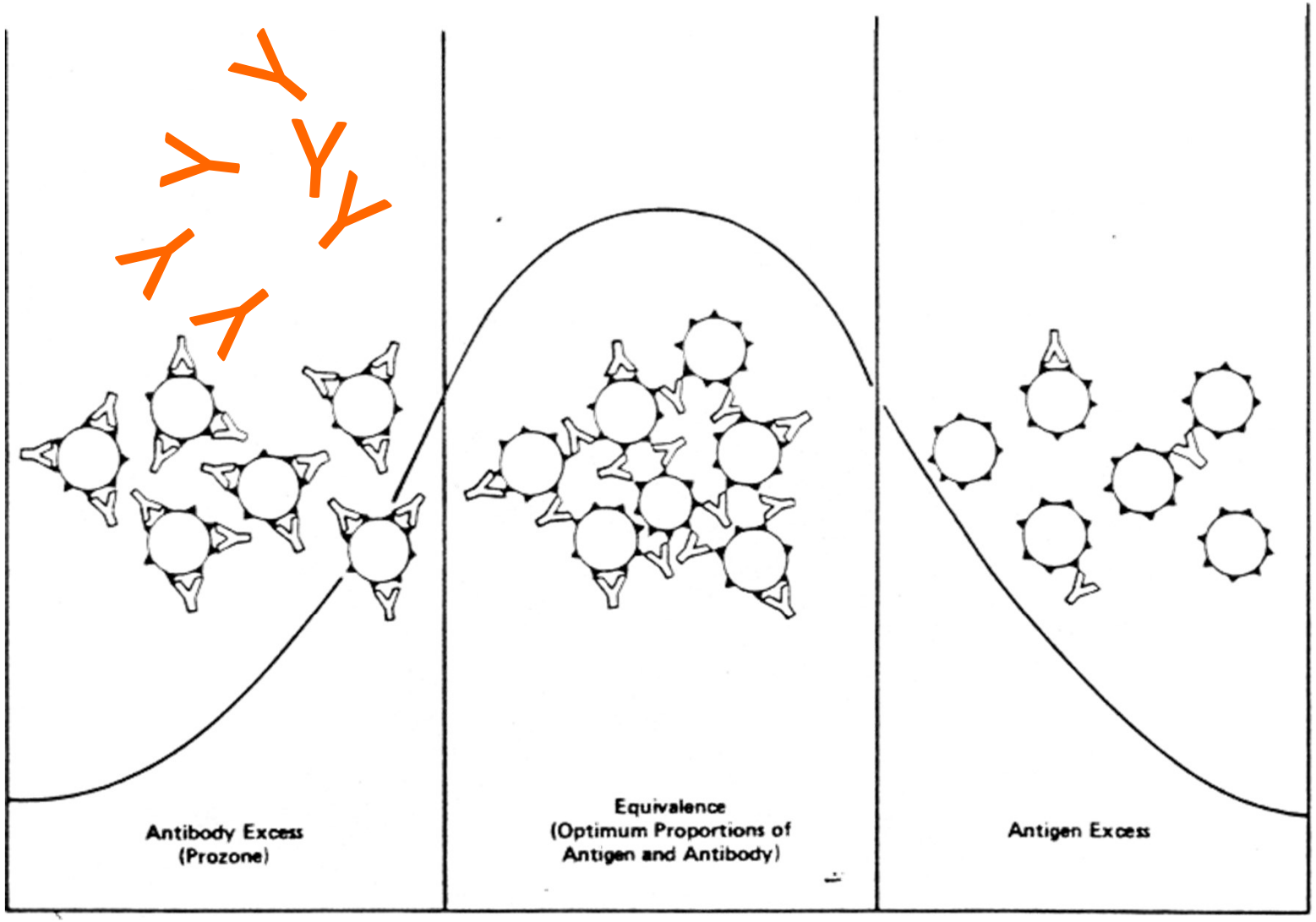
fait le 15/02/19 cb				greffe foie rein 15/09/20	Masque	244246 du 28/09/2020				243938 du 21/09/2020				243857 du 16/09/2020 SD2 après GREFFE FOIE				243793 du 16/09/2020 SD1 après IA, avant GREFFE FOIE DILUE 1/10				243793 du 16/09/2020 SD1 après IA, avant GREFFE FOIE A REFAIRE DILUE 1/10				243792 du 15/09/2020 avant séances d'IA DILUE 1/10				243792 du 15/09/2020 avant séances d'IA PROZONE? A REFAIRE DILUE 1/10			
A/B/Cw	Serology		soi	allo	CRISTAL 14/09/20	RawValue	MFI/LRA	Eplets Identifiés	Ass	RawValue	MFI/LRA	Eplets Identifiés	Ass	RawValue	MFI/LRA	Eplets Identifiés	Ass	RawValue	MFI/LRA	MFI/LRA	Eplets Identifiés	Ass	RawValue	MFI/LRA	Eplets Identifiés	Ass	RawValue	MFI/LRA	Eplets Identifiés	Ass	RawValue	MFI/LR	Eplets Identifiés
A*01:01	A1				i	2051	25,6	144KR	Pos	2583	30,0	144KR	Pos	993	24,8	163RG	Pos	6784	173,95	116D, 66NM,	Positive	Pos	16947	232,1	193PI, 138MI	Pos	14865	309,7	193PI, 138MI	Pos	14327	52,0	193PI, 138MI
A*02:01	A2				i	5444	68,1	62GK	Pos	5731	66,6	62GK	Pos	2352	58,8	62GK	Pos	871	22,33		Positive	Pos	5156	70,6	62GE	Pos	3940	82,1	62GE	Pos	9740	35,4	116Y, 62GE
A*02:02	A2				i	7354	91,9	62GK	Pos	7467	86,8	62GK	Pos	3202	80,1	62GK	Pos	1199	30,74	43R	Positive	Pos	6245	85,5	62GE	Pos	4919	102,5	62GE	Pos	11407	41,4	116Y, 62GE
A*02:03	A2				i	3455	43,2	62GK	Pos	3946	45,9	62GK	Pos	1012	25,3	62GK	Pos	444	11,38		Negative	Pos	2840	38,9	62GE	Pos	2020	42,1	62GE	Pos	8703	31,6	116Y, 62GE
A*02:05	A2				i	4107	51,3	62GK	Pos	4110	47,8	62GK	Pos	1273	31,8	62GK	Pos	564	14,46	43R	Positive	Pos	3340	45,8	62GE	Pos	2350	49,0	62GE	Pos	8815	32,0	116Y, 62GE
A*03:01	A3			foierein C182026	i	1083	13,5	144KR	Pos	1960	22,8	144KR	Pos	684	17,1	161D	Pos	8057	206,59	116D, 144KR	Positive	Pos	18421	252,3	193PI, 138MI	Pos	17040	355,0	193PI, 138MI	Pos	10085	36,6	193PI, 138MI
A*11:01	A11			foierein C182026	i	1104	13,8	144KR	Pos	2161	25,1	144KR	Pos	667	16,7	151AHA	Pos	8292	212,62	116D, 144KR	Positive	Pos	19710	270,0	193PI, 138MI	Pos	18817	392,0	193PI, 138MI	Pos	7732	28,1	193PI, 138MI
A*11:02	A11			foierein C182026	i	1066	13,3	144KR	Pos	1990	23,1	144KR	Pos	655	16,4	151AHA	Pos	8284	212,4	116D, 144KR	Positive	Pos	18799	257,5	193PI, 138MI	Pos	17289	360,2	193PI, 138MI	Pos	9695	35,2	193PI, 138MI
A*23:01	A23	Bw4	soi		p	80	1,0		Neg	86	1,0		Neg	40	1,0		Neg	56	1,44		Negative	Neg	86	1,2	193PI, 138MI	Neg	48	1,0	193PI, 138MI	Neg	281	1,0	193PI, 138MI, 116Y
A*24:02	A24	Bw4			i	560	7,0	144KR	Pos	1013	11,8	144KR	Pos	312	7,8		Neg	5352	137,23	144KR	Positive	Pos	15768	216,0	193PI, 138MI	Pos	14554	303,2	193PI, 138MI	Pos	15306	55,6	193PI, 138MI, 116Y
A*24:03	A24	Bw4			i	570	7,1	144KR	Pos	1135	13,2	144KR	Pos	370	9,3		Neg	6067	155,56	144KR	Positive	Pos	17779	243,6	193PI, 138MI	Pos	16695	347,8	193PI, 138MI	Pos	12753	46,3	193PI, 138MI, 116Y
A*25:01	A25	Bw4			i	365	4,6		Neg	415	4,8		Neg	235	5,9		Neg	1779	45,6	76ES, 116D	Positive	Pos	9391	128,6	138MI	Pos	7744	161,3	138MI	Pos	16563	60,1	138MI
A*26:01	A26				i	497	6,2		Neg	785	9,1		Neg	340	8,5		Neg	966	24,77	116D	Positive	Pos	6063	83,1	138MI	Pos	4384	91,3	138MI	Pos	13117	47,6	138MI
A*29:01	A*29				i	273	3,4		Neg	253	2,9		Neg	118	3,0		Neg	539	13,82	116D	Positive	Pos	4108	56,3	138MI	Pos	2960	61,7	138MI	Pos	10945	39,7	138MI
A*29:02	A29				i	347	4,3		Neg	363	4,2		Neg	191	4,8		Neg	883	22,63	116D	Positive	Pos	6609	90,5	138MI	Pos	4765	99,3	138MI	Pos	14161	51,4	138MI
A*30:01	A30				i	251	3,1		Neg	270	3,1		Neg	183	4,6		Neg	757	19,4	152W	Positive	Pos	5826	79,8	193PI, 138MI	Pos	3801	79,2	193PI, 138MI	Pos	12811	46,5	193PI, 138MI
A*31:01	A31				i	216	2,7		Neg	223	2,6		Neg	177	4,4		Neg	390	10	116D	Negative	Pos	3416	46,8	138MI	Pos	2072	43,2	138MI	Pos	9511	34,5	138MI
A*32:01	A32	Bw4			i	331	4,1		Neg	344	4,0		Neg	179	4,5		Neg	1623	41,62	76ES, 116D	Positive	Pos	9124	125,0	138MI	Pos	6842	142,5	138MI	Pos	14560	52,9	138MI
A*33:01	A33				i	649	8,1	186R	Pos	2083	24,2	186R	Pos	528	13,2	186R	Pos	2189	56,13	116D	Positive	Pos	10250	140,4	138MI	Pos	8215	171,2	138MI	Pos	14239	51,7	138MI
A*33:03	A33				i	255	3,2		Neg	255	3,0		Neg	148	3,7		Neg	657	16,83	116D	Positive	Pos	4994	68,4	138MI	Pos	3384	70,5	138MI	Pos	11858	43,0	138MI
A*34:02	A34				i	352	4,4		Neg	504	5,9		Neg	291	7,3		Neg	1001	25,67	116D	Positive	Pos	6972	95,5	138MI	Pos	5009	104,4	138MI	Pos	13697	49,7	138MI
A*36:01	A36				i	728	9,1	144KR	Pos	959	11,2	144KR	Pos	392	9,8		Neg	7262	186,21	116D, 66NM,	Positive	Pos	18056	247,3	193PI, 138MI	Pos	16475	343,2	193PI, 138MI	Pos	11921	43,3	193PI, 138MI
A*43:01	A43				i	455	5,7		Neg	502	5,8		Neg	278	6,9		Neg	817	20,95	116D	Positive	Pos	5960	81,6	138MI	Pos	4377	91,2	138MI	Pos	13208	47,9	138MI
A*66:01	A66				i	350	4,4		Neg	415	4,8		Neg	258	6,5		Neg	981	25,14	116D	Positive	Pos	6879	94,2	138MI	Pos	4914	102,4	138MI	Pos	15108	54,8	138MI
A*66:02	A66				i	336	4,2		Neg	412	4,8		Neg	250	6,3		Neg	1368	35,08	116D	Positive	Pos	8302	113,7	138MI	Pos	6280	130,8	138MI	Pos	15393	55,9	138MI
A*68:01	A68		x		p	87	1,1		Neg	87	1,0		Neg	57	1,4		Neg	51	1,29	116D	Negative	Neg	73	1,0		Neg	70	1,5		Neg	335	1,2	
A*68:02	A68		soi		p	85	1,1		Neg	107	1,2		Neg	71	1,8		Neg	39	1		Negative	Neg	78	1,1		Neg	59	1,2		Neg	276	1,0	116Y
A*69:01	A69				i	181	2,3		Neg	185	2,2		Neg	98	2,5		Neg	92	2,36		Negative	Neg	303	4,2		Neg	213	4,4		Neg	1873	6,8	116Y
A*74:01	A74				i	298	3,7		Neg	324	3,8		Neg	164	4,1		Neg	1132	29,03	116D	Positive	Pos	7639	104,6	138MI	Pos	5457	113,7	138MI	Pos	14682	53,3	138MI
A*80:01	A80				i	1135	14,2	144KR	Pos	2221	25,8	144KR	Pos	715	17,9	152RR	Pos	7513	192,63	116D, 144KR	Positive	Pos	18176	249,0	193PI, 138MI	Pos	16320	340,0	193PI, 138MI	Pos	12883	46,8	193PI, 138MI
B*07:02	B7	Bw6		foierein C182026	i	961	14,7		Pos	1911	23,0		Pos	893	20,8		Pos	11948	204,23	76ES	Positive	Pos	19439	262,7	193PI	Pos	19982	314,7	193PI	Pos	5612	20,5	193PI, 116Y
B*07:03	B7	Bw6		foierein C182026	i	682	10,4		Pos	1238	14,9		Pos	651	15,1		Pos	8989	153,65	76ES	Positive	Pos	18482	249,8	193PI, 71TTS	Pos	17165	270,3	193PI, 71TTS	Pos	16482	60,2	193PI, 116Y, 71TTS
B*08:01	B8	Bw6			i	2178	33,3		Pos	4126	49,7	177DT	Pos	1256	29,2		Pos	7202	123,11	76ES	Positive	Pos	19016	257,0	193PI, 113HN, 71	Pos	17018	268,0	193PI, 113HN, 71	Pos	19900	72,6	193PI, 116Y, 71TTS
B*13:02	B13	Bw4			i	112	1,7		Neg	121	1,5		Neg	82	1,9		Neg	1515	25,9	76ET	Positive	Pos	7203	97,3	193PI, 113HN	Pos	5399	85,0	193PI, 113HN	Pos	11357	41,5	193PI
B*14:01	B64	Bw6			i	1003	15,3		Pos	3240	39,0		Pos	1084	25,2		Pos	7202	123,11	76ES	Positive	Pos	17647	238,5	193PI, 71TTS	Pos	16975	267,3	193PI, 71TTS	Pos	16655	60,8	193PI, 71TTS
B*14:02	B65	Bw6			i	1000	15,3		Pos	3037	36,6		Pos	937	21,8		Pos	5873	100,38	76ES	Positive	Pos	15720	212,4	193PI, 71TTS	Pos	14003	220,5	193PI, 71TTS	Pos	17643	64,4	193PI, 71TTS

21/09/2023

MFI follow-up (2) after desensitization



Prozone effect



Prozone effect: how to get rid of it

- Too much antibody is present in the serum and interferes with the detection system.
- Too much antibody is present and complement and/or IgM present in the serum interferes with the detection system.

To prevent this phenomenon a few methods are useful:

- Heat inactivation (56° for 30’)
- Dilution of patient serum
- Use of EDTA or DTT in order to destroy complement bonds

Anti-HLA ab detection: considerations

- Excellent instruments to detect anti-HLA ab
  - Lymphocytotoxicity-complement dependent test (LCT): assays to determine *in vivo* cytotoxicity of the antibodies
    - *Low sensitivity, but inexpensive and will allow to avoid hyperacute rejection*
  - Luminex Single Antigen assay: to detect and identify all circulating HLA antibodies
    - *Extremely sensitive, expensive,* ***needs a good lab with experience***
    - *Will allow to determine whether patient is at risk for antibody-mediated rejection*

cPRA vs. DSA

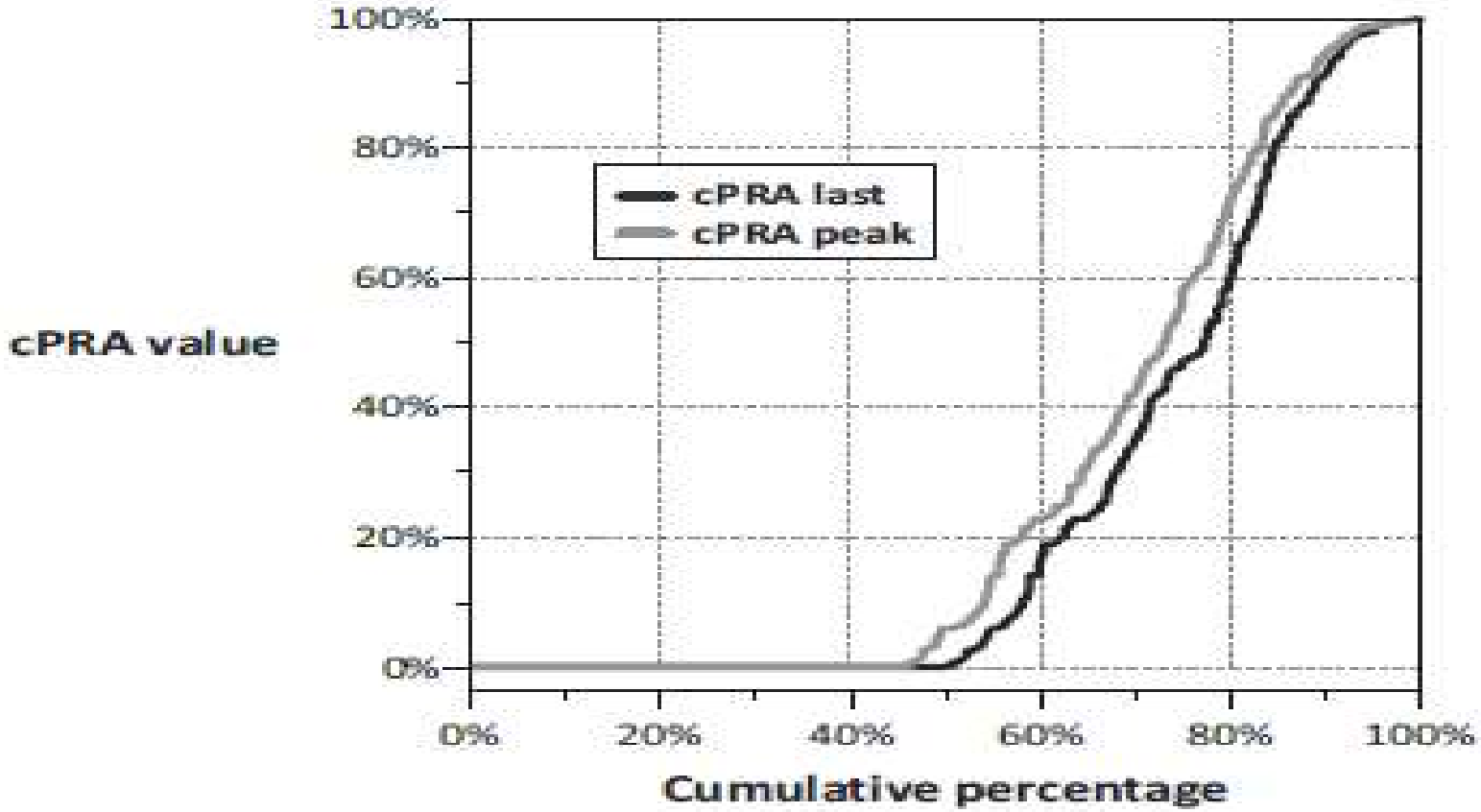
Donor Specificity but Not Broadness of Sensitization Is Associated With Antibody-Mediated Rejection and Graft Loss in Renal Allograft Recipients (1)

- Single center study (Basel, Switzerland); 527 KTx patients
- calculated PRA (c PRA) at pretransplant:
  - cPRA 0% (n=250) ; cPRA 1-50% (n=124) ; 51-100% (n=43) and DSA (n=105)
- In the absence of DSA = standard risk → IS = basiliximab, Tac, MPA and steroids (for 3 months posttransplant)
- In the presence of DSA: ATG induction + IVIg (2 g/kg) + Tac +MPA + steroids
- Surveillance kidney biopsies = M3 and M6
- Median follow-up = 5.7 years

Wehmeier C. et al. AJT 2017; 17: 2092–2102

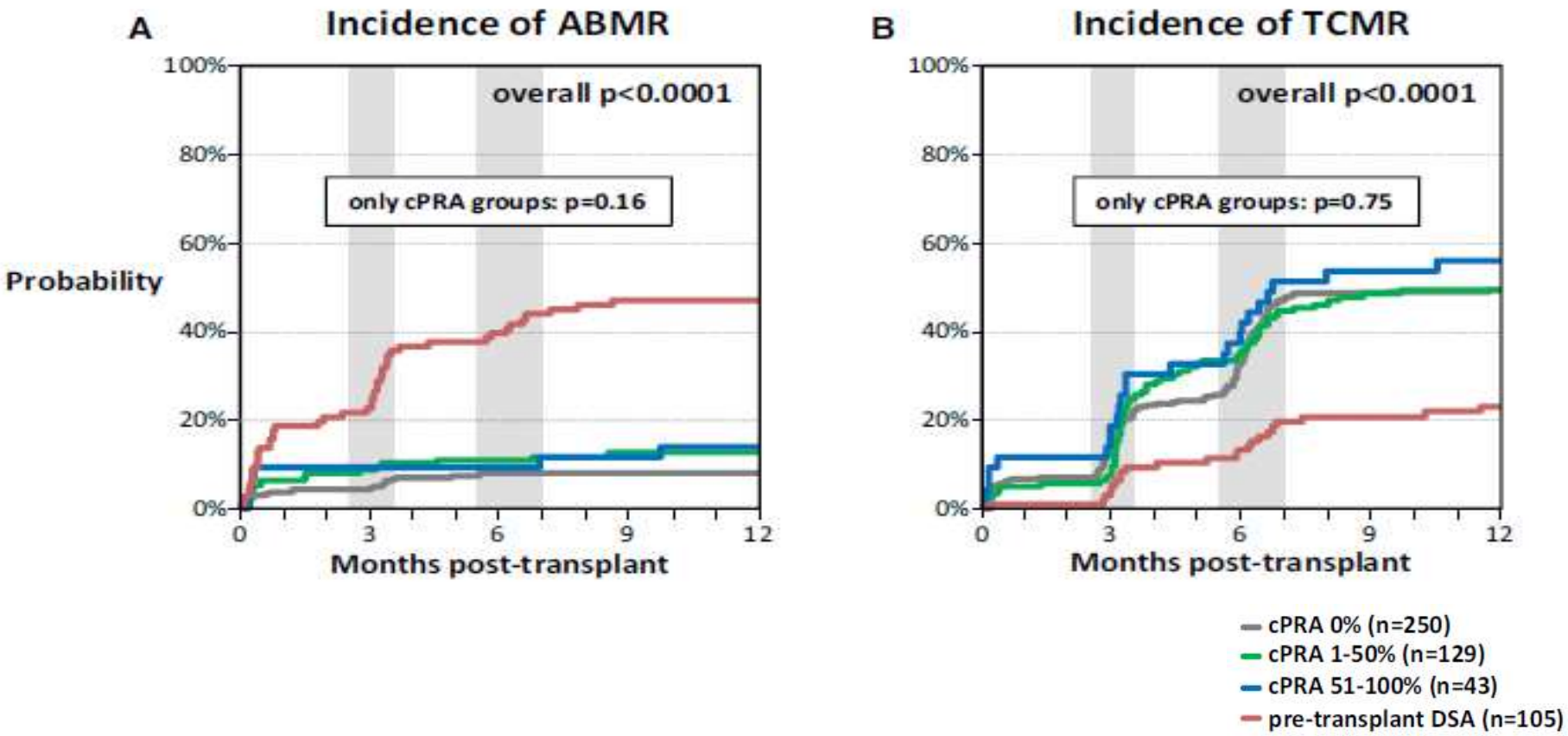
Donor Specificity but Not Broadness of Sensitization Is Associated With Antibody-Mediated Rejection and Graft Loss in Renal Allograft Recipients (2)

Distribution of calculated population-reactive antibody values among the 527 patients.



Wehmeier C. et al. AJT 2017; 17: 2092–2102

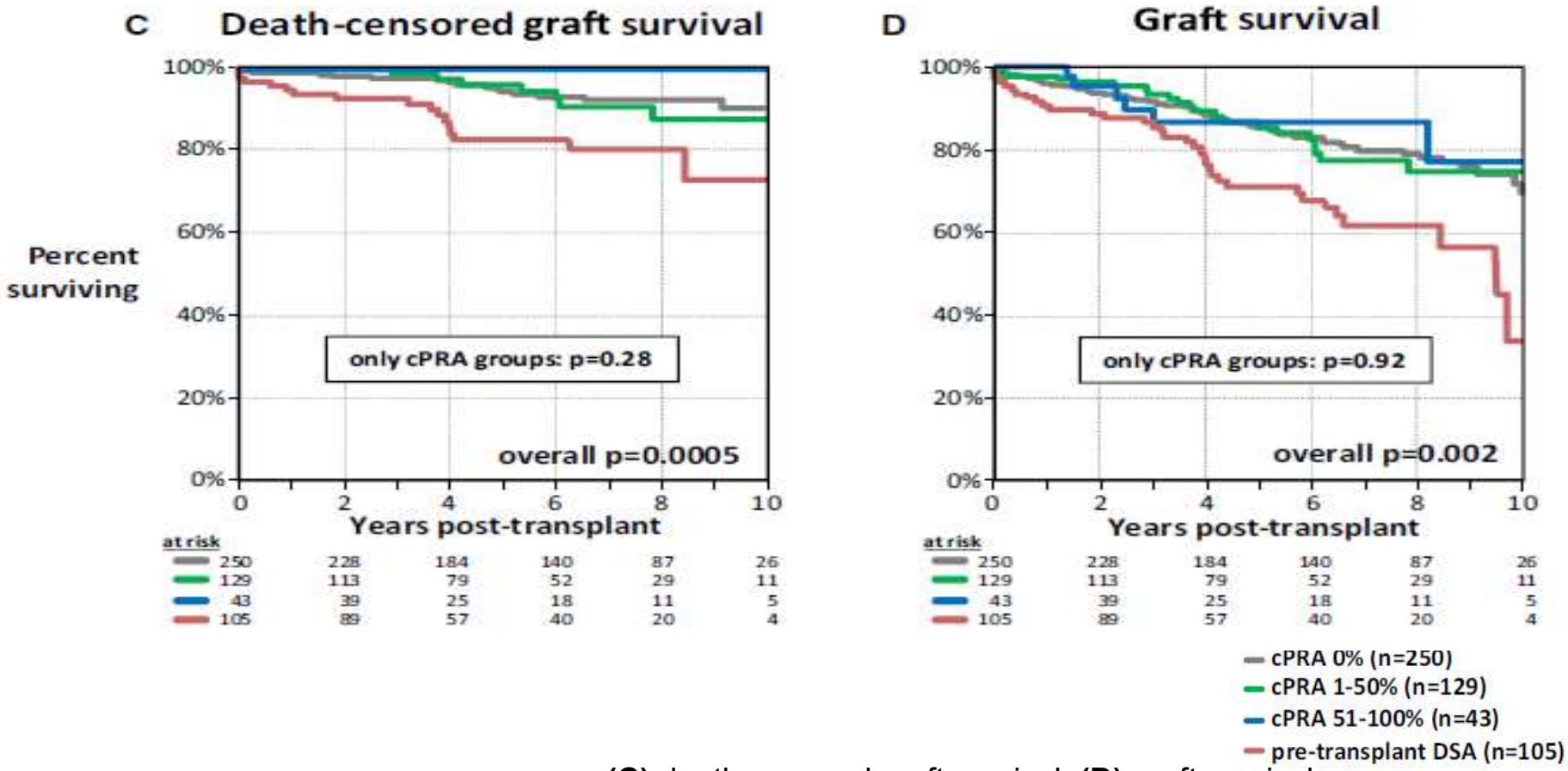
Donor Specificity but Not Broadness of Sensitization Is Associated With Antibody-Mediated Rejection and Graft Loss in Renal Allograft Recipients (3).



Major outcomes among the cPRA and the DSA groups. **(A)** Incidence of ABMR; **(B)** incidence of TCMR; antibody; DSA, donor-specific antibody; ABMR, antibody-mediated rejection; TCMR, T cell–mediated rejection.

Wehmeier C. et al., AJT 2017;17:2092-2102.

Donor Specificity but Not Broadness of Sensitization Is Associated With Antibody-Mediated Rejection and Graft Loss in Renal Allograft Recipients (4).



Major outcomes among the cPRA and the DSA groups. **(C)** deathcensored graft survival; **(D)** graft survival. The gray shades in (A) and (B) represent the time frames, in which surveillance biopsies were performed. Borderline changes were included in the calculation of the incidence of TCMR. cPRA, calculated population-reactive antibody; DSA, donor-specific antibody; ABMR, antibody-mediated rejection; TCMR, T cell–mediated rejection

Wehmeier C. et al., AJT 2017;17:2092-2102.

Immunological work-up pretransplant

- Male patient, never transfused, 1st transplant candidate:
  - ZERO immunological risk; NO work-up
- Transfused patient; women patient

  INTERMEDIATE immunological risk
  - Assess HLA sensitization with Luminex; Flow cytometry cross-match
- Patient with preformed DSA : HIGH immunological risk
  - Low level DSA (=low MFI < 3000) kidney transplant is possible provided pretransplant rituximab infusion
  - DSA > 3000 : pretransplant desensitization is needed

Patients at high immunological risk (DSA=0)

- Female patients with pregnancies and PRA >0
  - when the potential donor is the husband or one of the offsprings or a deceased donor
- Retransplant patients and PRA > 0
- Polytransfused patients with WBC-containing red packed cells and PRA >0
- **In these settings** the risk of ACR and / or AAMR

  is high : **this implies pretransplant Luminex test and PRA**

PRA < 50%: ATG low dose + Rituximab + Tacrolimus + MPA + Steroids.

PRA > 50%: ATG high dose + Rituximab + Tacrolimus + MPA + Steroids.

Patients at intermediate immunological risk (DSA=0)

- Female patients with previous pregnancies with PRA = 0
  - when the potential donor is the husband or one of the offsprings or a deceased donor
- Retransplant patients with PRA = 0
- Polytransfused patients with WBC-containing red packed cells with PRA = 0
- **In these settings** the risk of ACR and / or AAMR

  is moderate : **this implies pretransplant Luminex test and PRA**

IS: low ATG + Tacrolimus + MPA + Steroids.

Patients at low immunological risk (DSA=0; PRA=0)

- First kidney transplant
- No history of blood transfusions
- Female patients without pregnancy
- Living (un)related or deceased donor
  - In these settings the risk of ACR is low and the risk of AAMR is almost nil
  - Induction therapy with Basiliximab OR **no induction**
  - Maintenance therapy ; CNI + MPA + Steroids **OR**

low CNIs (CsA or Tac)+ low Everolimus + Steroids

HLA fully matched patients (DSA=0; PRA=0 or > 0)

- Start immunosuppression 8-10 days pretransplant
  - Tacrolimus (trough levels : 6-8 ng/mL) plus MMF (500 mg bid) plus prednison (0.5 mg/kg/day)
  - NO induction therapy
  - Post-transplant :
    - ✓ Tacrolimus trough levels :6-8 ng/mL
    - ✓ MMF : 1 g bid 2 weeks than 500 mg big
    - ✓ Prednison
- Perform kidney allograft biopsy at Month 3 post-transplant : if normal consider weaning-off tacrolimus over a period of 3 months

Post-transplant anti-HLA monitoring

- It is advised to monitor anti-HLA alloantibodies at 3 and 12 months post-transplant and then every other year
  - Should a *de novo* DSA appear this might lead to perform a kidney transplant biopsy and to reinforce immunosuppression
  - Rituximab injection could be done (a single injection of 1 gr)
- Beyond the first year post-transplantation anti-HLA alloantibodies should be monitored yearly

In case of suspected ACUTE antibody-mediated rejection

- It means EARLY onset at post-transplantation (days or weeks)
  - Drop in urine output
  - Suddeen rise in serum creatinine
- After checking for patency of vessels and ureter
  - Make a kidney biopsy
  - Perform CDC cross-match (or better flow cross match) in order to detect DSA)
  - If available perform Luminex test (*de novo* DSA or rebound DSA)
- Treatment relies on plasmapheresis and Rituximab +/- IVIG

Thank you for your attention