

CELLULAR BASIS OF ANTI-HLA ANTIBODY REBOUND AND REJECTION IN KIDNEY TRANSPLANT PATIENTS DESENSITIZED WITH IMLIFIDASE

D. Kervella^{1,2}, C. Carrera^{1,2}, F. Casanova², N. Kamar³, M. Crespo⁴, F. Diekmann⁵, L. Couzi⁶, A. Torija², L. Donadeu², A. Martínez², E. Crespo², M. Meneghini^{1,2}, I.B. Torres^{1,2}, J.L. Caro⁷, E. Palou⁷, F. Moreso^{1,2}, O. Bestard^{1,2}

¹ H. U. Vall d'Hebron, Unitat de Trasplantament Renal, Vall d'Hebron Barcelona H. Campus, Barcelona

² Vall d'Hebron Institut de Reserca, Vall d'Hebron Barcelona H. Campus

³ Centre Hospitalier Universitaire de Toulouse, Toulouse, Francia

⁴ H. del Mar Barcelona, Unitat de Trasplantament Renal, Barcelona

⁵ H. Clínic Barcelona, Unitat de Trasplantament Renal, Barcelona

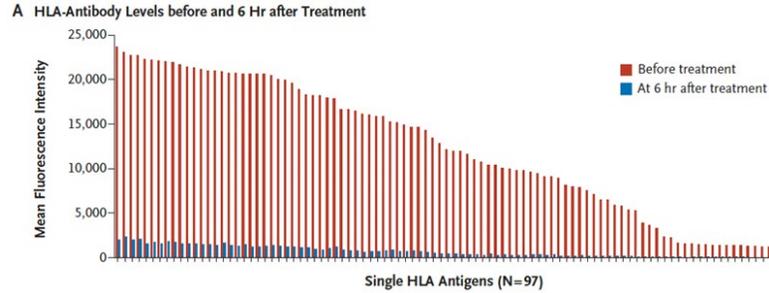
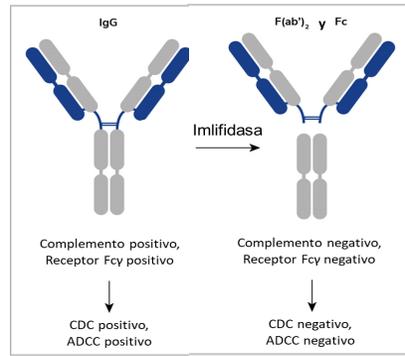
⁶ Centre Hospitalier Universitaire de Bordeaux, Bordeaux, Francia

⁷ H. Clínic Barcelona, Laboratorio de Inmunología, Barcelona



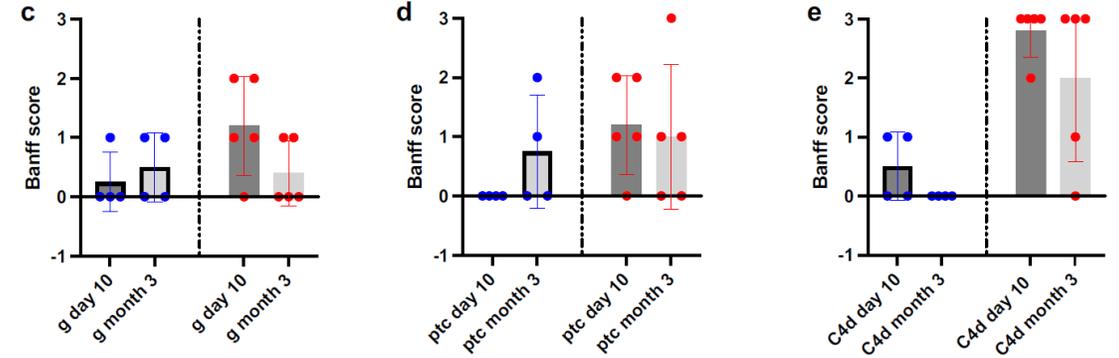
Imlifidase for transplantation of hypersensitized patients with positive cross-match

IgG Cleavage

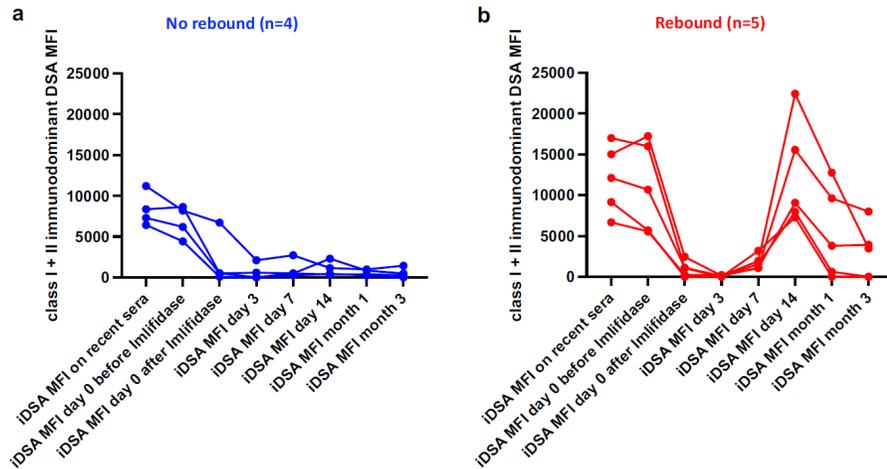


DSA rebound and ABMR

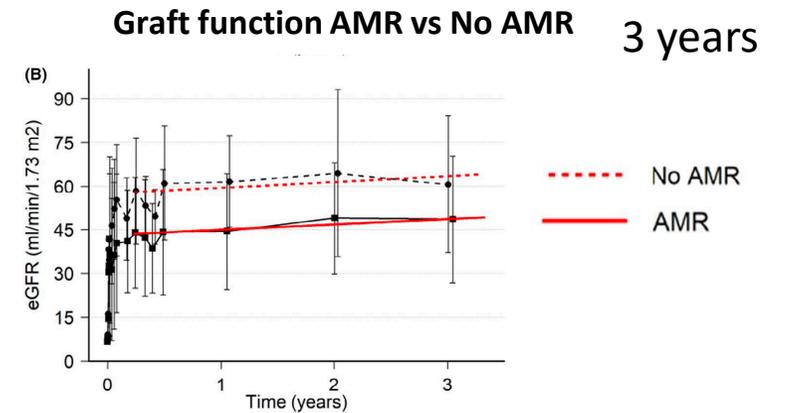
~40%



Post-transplantation DSA rebound



ABMR influences long-term outcomes



Jordan SC et al N Eng J Med 2017
 Kjellman C et al Am J Transplant 2021
 Kamar et al., KI reports 2024

OBJECTIVES

OBJECTIVE:

Decipher the biology of ABMR and anti-HLA antibody rebound after kidney transplantation with a positive cross-match and Imlifidase treatment to improve patient's (Antibodies) selection and post-transplantation care.

STUDY DESIGN AND METHODS

22 highly sensitized patients
KT with positive FCCM +/- CDC CM, and desensitization with
Imlifidase

5 Tx center
France (Toulouse, Bordeaux, n=6 patients)
Catalunya (HUVH, H Clinic, H del Mar, n=16 patients)

Protocol biopsy at day 10 and month 3 (France)
Protocol biopsy at month 6 (Catalunys)

Circulating Anti-HLA Ab (SAB)

1. Anti HLA Antibodies Pre-Tx (T0) and post-Tx



Serum



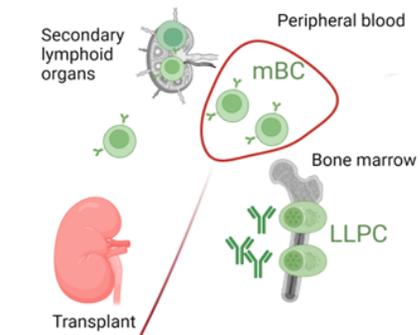
Luminex SAB assay



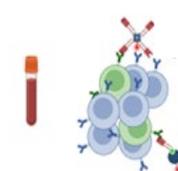
Output : number of positive HLA specificities, MFI

2. Cells (bulk phenotyping and HLA-sp mBC) Pre-Tx (T0)

Compartments of the humoral immune response



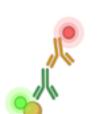
Bulk flow cytometry of PBMC



HLA-sp memory B cells



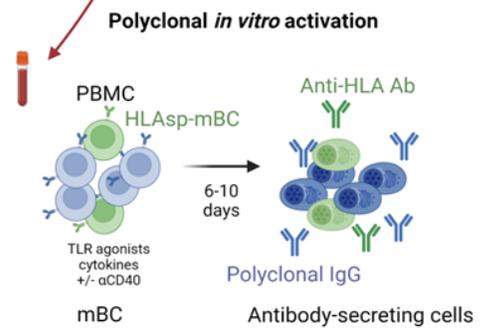
Culture supernatant



Luminex SAB assay



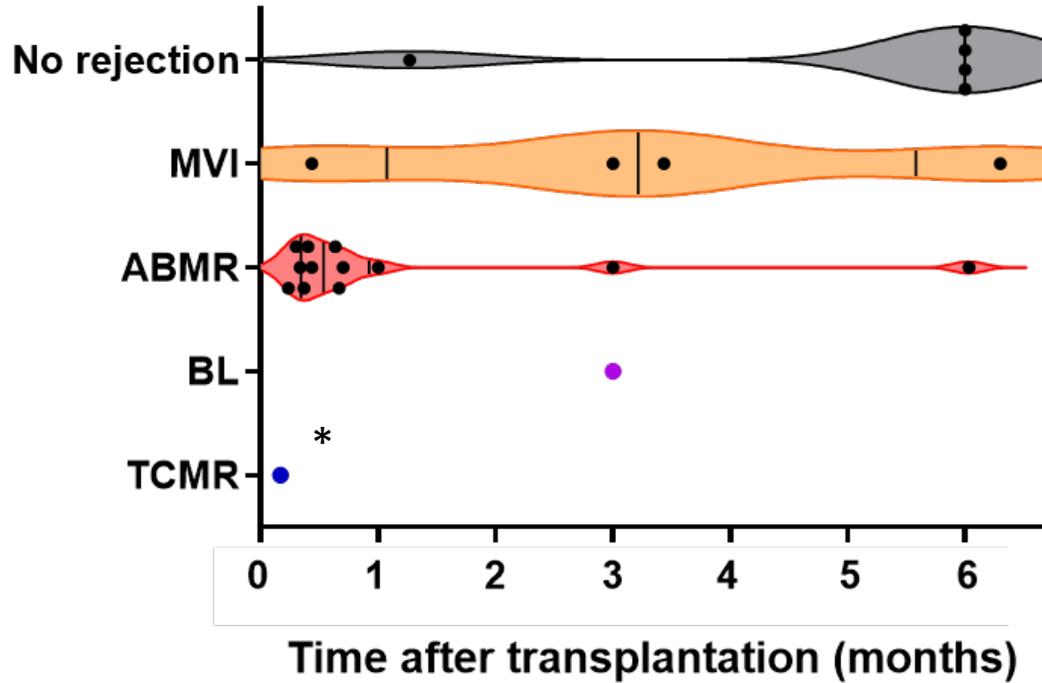
Output : number of positive HLA specificities, MFI



RESULTS: patients characteristics at transplantation

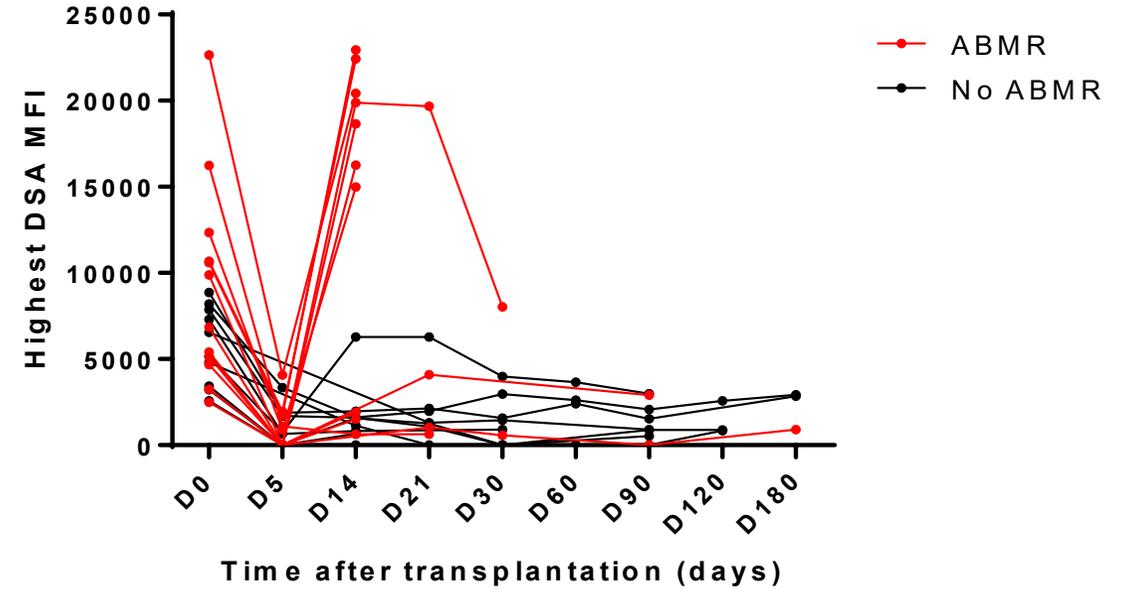
N=22 patients	
Male sex (n (%))	11 (50 %)
Age (median (IQR))	52.5 (45.3 – 58.0)
Time on dialysis (years, median (IQR))	8.0 (6.5 – 17.0)
Time on the waiting list (years, median (IQR))	7.0 (4.6 – 11.0)
Prior kidney transplants (n (%))	
0	2 (9 %)
1	11 (50 %)
>1	9 (41%)
Previous Kidney graft transplantectomy	14/33 (42 %)
Sensitization events (number of patients (%))	
Past pregnancy	7 (32 %)
Past blood transfusion	18 (82 %)
Pre-transplant sensitization	
cPRA (mean ± SD)	99.9 ± 0.3 %
Positive CDC CM (n (%))	1 (4.5 %)
Positive FC CM (n (%))	22 (100 %)
Number of DSA (MFI cut-off 500) (median (IQR))	4 (3 – 5)
Class I (n (%))	2 (2 – 3)
Class I (n (%))	2 (0.75 – 3)
Sum of DSA MFI	12 094 (7351 – 23 676)
Immunodominant DSA MFI (median (IQR))	6699 (4365 – 9120)
Class I (n (%))	11 (50 %)
Class II (n (%))	11 (50 %)

RESULTS: patients outcomes rejection 6 months



*acute TCMR at day 5 (before ATG initiation). During follow-up presented MVI

Immunodominant DSA MFI



Highest timepoint: Highest DSA MFI during first month

Day 5 n=2

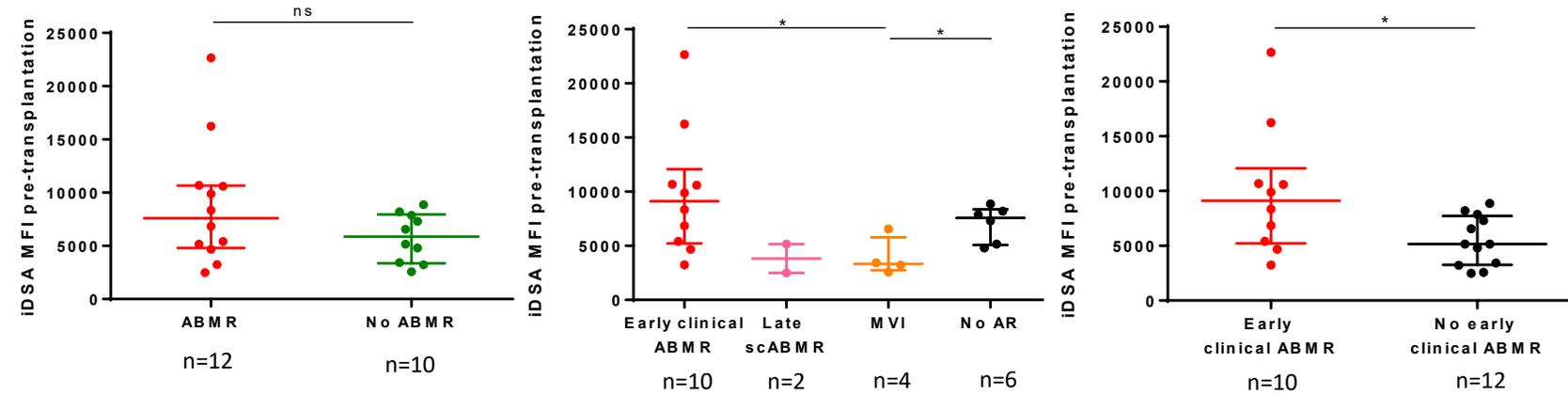
Day 10-14 n=14

Day 21 n=5

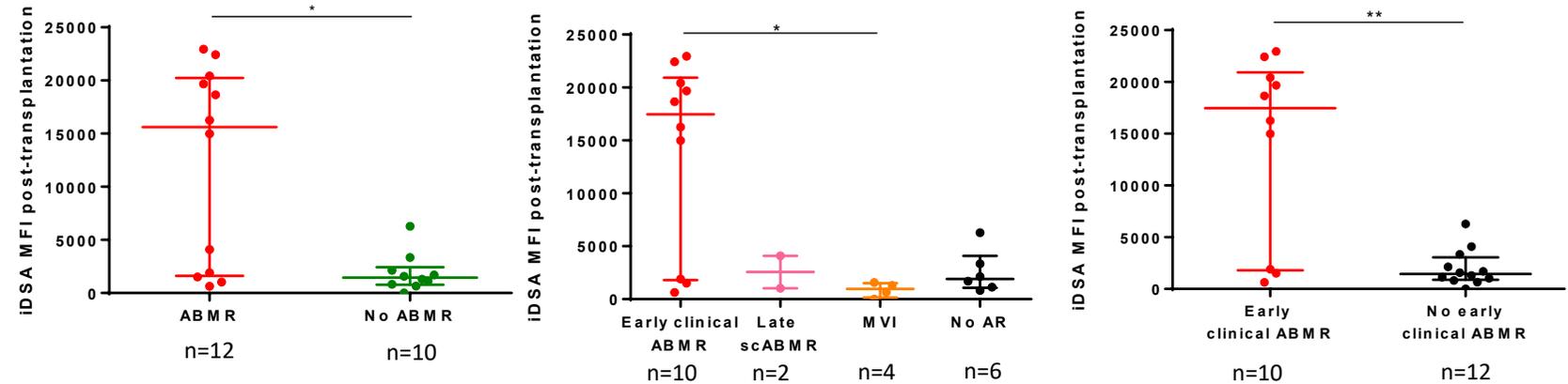
Occurrence of early (First month) clinical ABMR in 45 % of patients, associated with strong DSA rebound, and ABMR related phenotypes in other 27%.

RESULTS: DSA and Rejection

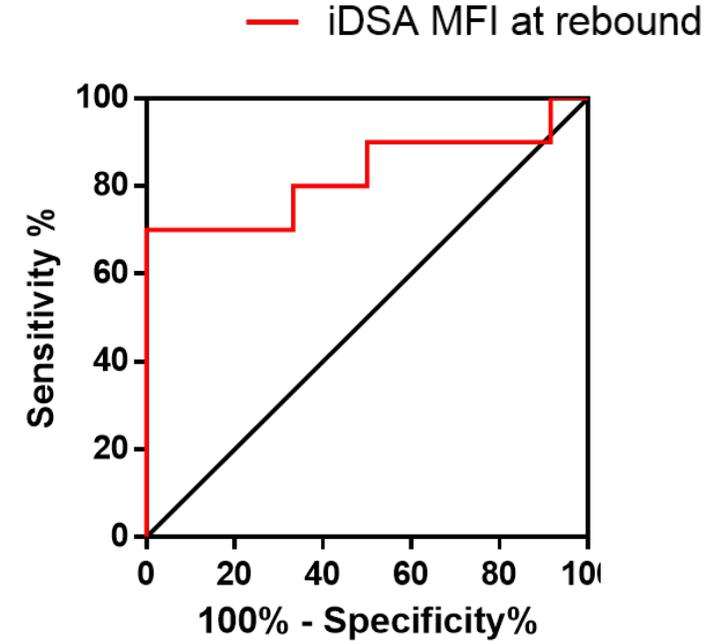
Prediction of ABMR with pre-Tx DSA



Prediction of ABMR with post-Tx DSA (highest timepoint during first month)



Early clinical ABMR



AUC 0.8250
 CI95% 0.6273 to 1.023
 P=0.01

Cut off MFI 5193 (rebound)

For early clinical ABMR vs all other diagnostics
 Se 70 % Sp 91.67 % PPV 87.5 % NPV 78.57 %

(Weak) significant association between iDSA MFI pre-transplantation and occurrence of early clinical ABMR
Significant association between iDSA MFI post-transplantation and occurrence of early clinical ABMR (rebound)

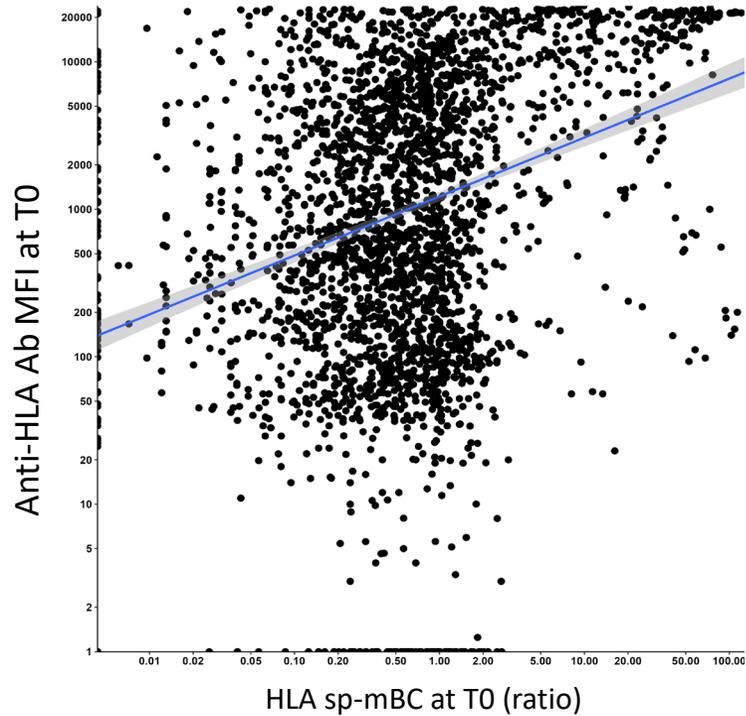
RESULTS: HLA-specific mBC associated with previous Tx

T0 anti-HLA Ab MFI (MM previous grafts)

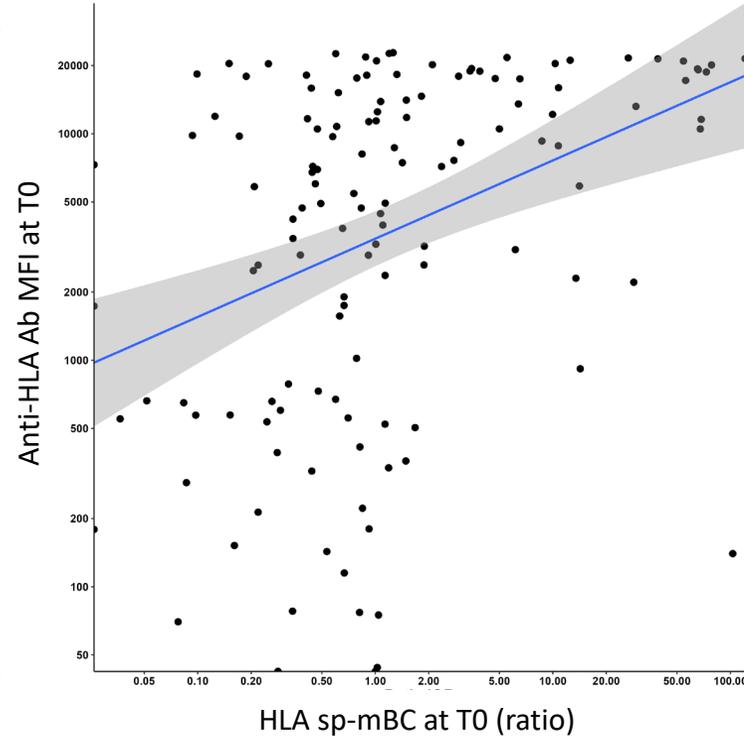
T0

All HLA specificities

Mismatch from previous grafts



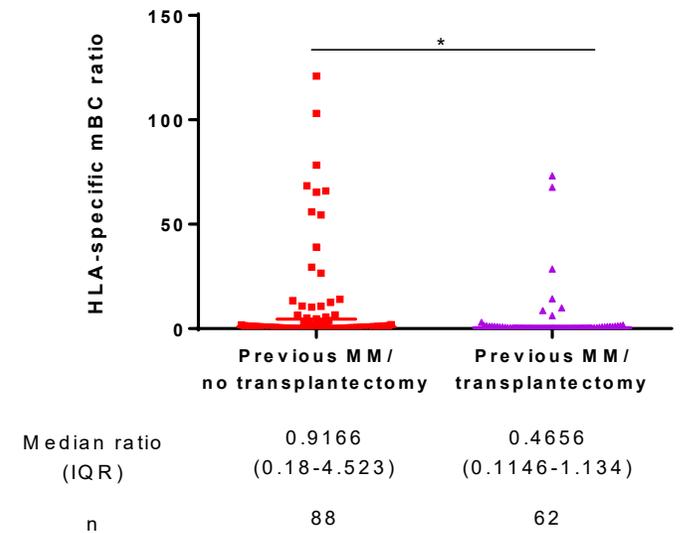
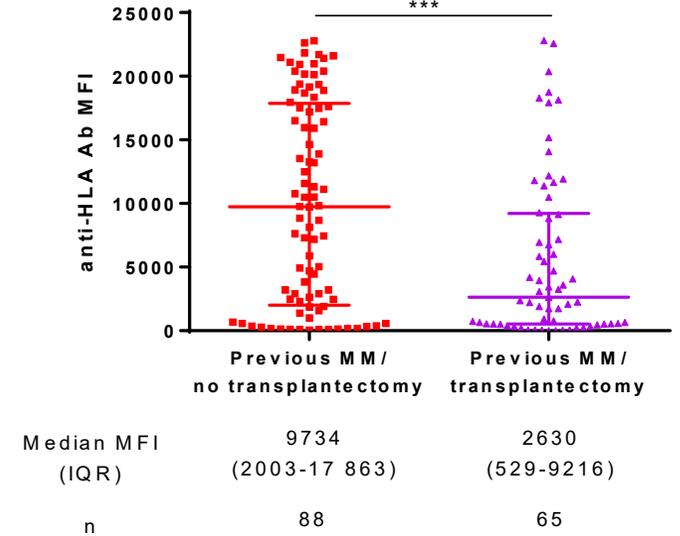
Correlation $r=0,29$ $p<0,0001$



$r=0,41$ $p<0,0001$

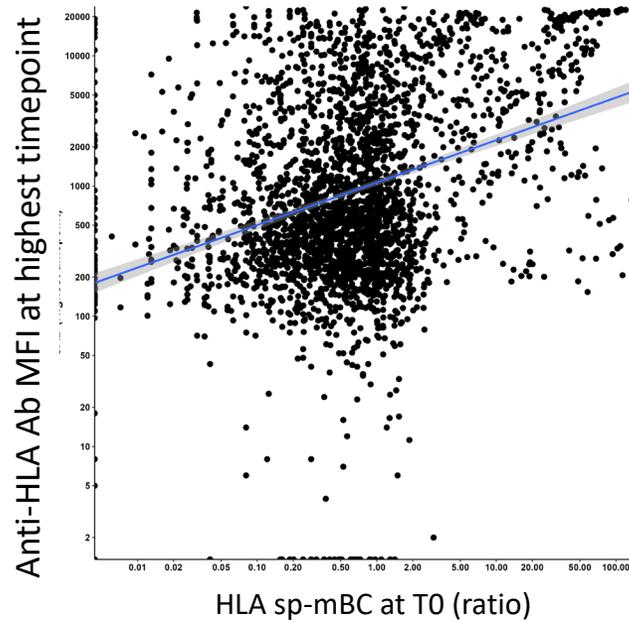
HLA specificities of previous grafts are associated with Higher anti-HLA Ab MFI and more frequent specific memory B cells pre-transplantation, particularly if the graft has not been retrieved.

T0 HLA-sp mBC (MM previous grafts)



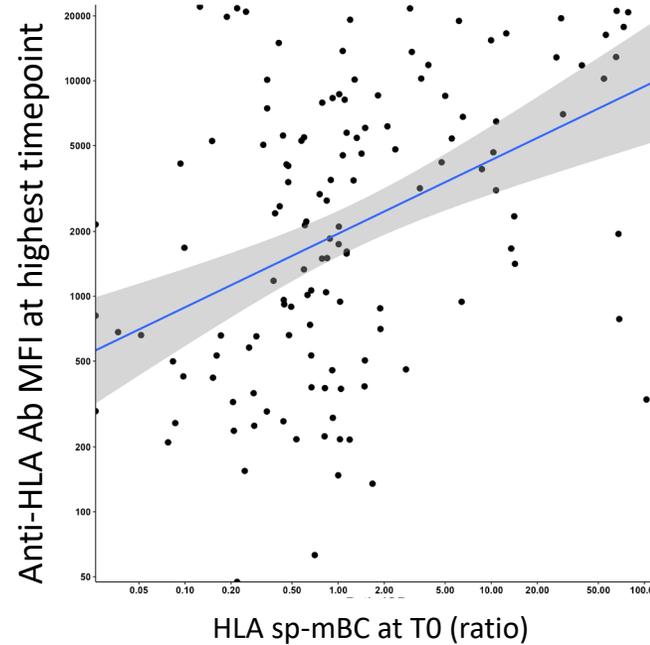
RESULTS: Preformed mBC and anti-HLA Ab rebound

All HLA specificities



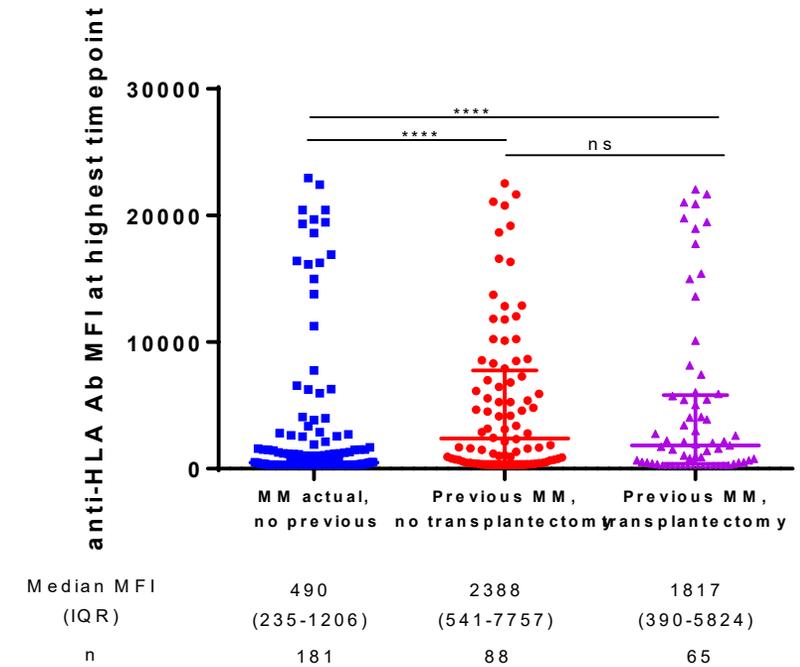
Correlation_r = 0.28 p value < 0.0001

Mismatch from previous grafts



r = 0,40 p value < 0.0001

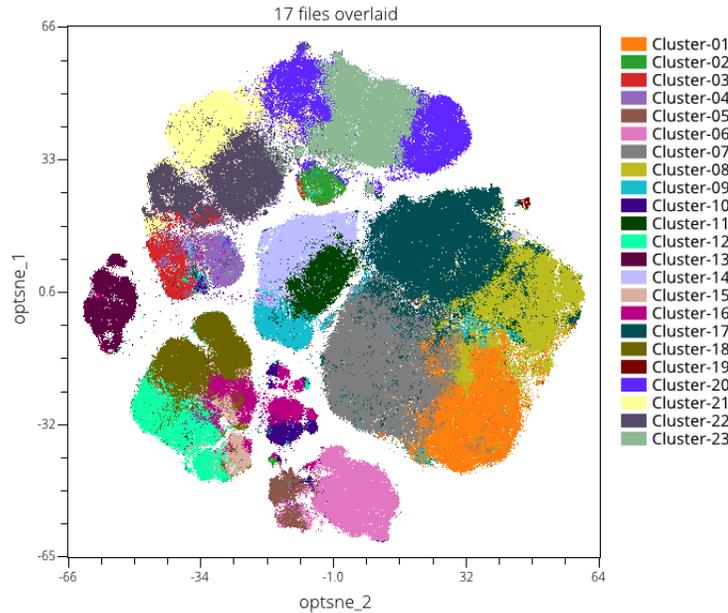
Anti-HLA Ab rebound (serum) and previous graft MM



Anti-HLA antibodies against previous grafts are associated with stronger rebound and associated with more frequent HLA-specific memory B cells.

RESULTS: Bulk cell phenotypes associated with outcomes

17 patients's PBMC samples at T0 (before IDES)
 Multiparameter spectral Flow cytometry
 Unsupervised analysis



B-cell phenotype

	% of file - Cluster-22	% of file - Cluster-12	% of file - Cluster-26	% of file - Cluster-21	% of file - Cluster-23	% of file - Cluster-17	% of file - Cluster-15	% of file - Cluster-08	% of file - Cluster-10	% of file - Cluster-11	% of file - Cluster-18	% of file - Cluster-13	% of file - Cluster-04	% of file - Cluster-29	% of file - Cluster-19	% of file - Cluster-09	% of file - Cluster-28	% of file - Cluster-14	% of file - Cluster-25	% of file - Cluster-07	% of file - Cluster-20	% of file - Cluster-05	% of file - Cluster-06	% of file - Cluster-27	% of file - Cluster-01	% of file - Cluster-24	% of file - Cluster-16	% of file - Cluster-02	% of file - Cluster-03
1	0.33	0	0.49	0	0	0.33	0.16	0.16	1.15	0	0.16	0	0	0.99	3.29	2.14	0.82	2.31	2.14	5.6	1.65	0	0	4.45	4.12	2.8	3.79	38.1	25
2	3.77	1.35	1.08	1.62	2.43	0.81	0.27	2.7	0.27	2.7	2.7	3.23	7.01	1.35	3.5	0.54	1.08	2.7	2.43	4.31	3.5	10.8	5.12	4.31	4.85	9.97	11.9	2.7	1.08
3	1.32	1.32	5.26	2.63	0	2.63	1.32	1.32	0	0	0	0	1.32	3.95	5.26	1.32	0	2.63	0	3.95	2.63	1.32	5.26	10.5	10.5	18.4	10.5	5.26	1.32
4	4.24	2.42	1.21	1.21	1.21	1.82	0	0	0	1.21	1.82	0.61	3.03	5.45	6.67	0.61	2.42	3.03	4.24	3.64	3.03	5.45	7.27	4.85	4.85	14.5	13.9	0	1.21
5	1.21	1.88	0.4	1.12	1.25	3	1.03	0.18	0.13	1.65	3.58	4.11	0.81	3.13	4.61	3.53	1.83	2.59	2.86	2.86	4.07	2.06	6.13	8.36	7.51	13.9	15.1	0.76	0.38
6	2.03	1.68	1.41	0.35	0.26	1.59	0.18	0.09	0.44	1.32	1.68	1.5	1.76	3.79	5.82	3.88	1.23	3.97	2.73	3.97	4.67	1.94	5.56	8.73	8.47	13.9	13	2.65	1.41
7	0.56	0.83	0.49	1.63	0.39	0.7	2.04	0.56	0.44	1.24	1.19	2.92	2.45	1.39	5.87	3.64	4.1	3.01	1.53	2.98	2.84	3.5	4.03	9.89	7.96	15.3	16.2	1.97	0.31
8	1.03	2.07	0.86	0.95	0.69	1.03	0.43	1.55	1.21	1.21	1.55	3.19	4.31	1.21	3.97	4.4	5.86	2.84	0.86	2.67	1.72	5.09	6.38	9.48	5.95	13	13.7	2.41	0.34
9	1.51	2.78	0.74	0.74	0.97	0.6	3.55	6.77	11.4	8.78	3.72	3.02	17	1.41	2.58	1.64	1.34	1.74	1.98	3.55	1.11	2.01	2.18	3.45	2.14	2.98	1.98	5.56	2.78
10	3.17	3.65	0.63	0.79	3.65	1.9	1.9	2.38	3.96	17	12.4	11.6	6.97	0.48	0.95	0.95	0.63	2.54	2.54	3.49	2.38	2.06	10.1	0.32	0.48	1.43	1.74	0	0
11	2.05	1.54	1.03	0.68	2.91	1.03	4.62	6.68	6.85	14.6	11.5	4.28	12.8	0.68	1.37	1.2	3.42	1.2	0.51	2.74	0.68	3.42	4.97	2.57	1.2	2.57	2.57	0.34	0
12	1.32	4.35	4.35	0.08	0.47	0.08	0.47	0.78	19.3	0.31	1.01	0.16	0.23	8	7.53	2.56	3.11	5.75	7.22	12.3	2.72	0	0.47	1.86	2.48	3.42	4.74	0.62	4.35
13	1.05	1.83	0.52	0.26	0.26	3.4	1.57	2.09	0	2.09	2.36	0.26	6.28	1.57	2.88	2.88	2.09	7.85	6.28	8.9	15.4	1.83	6.81	2.09	7.07	3.66	5.5	1.83	1.31
14	4.03	4.03	1.61	0	0	2.42	2.42	1.61	0.81	4.84	4.03	3.23	4.84	1.61	6.45	1.61	1.61	4.03	3.23	4.84	10.5	5.65	8.87	1.61	4.03	4.03	8.06	0	0
15	1.6	1.73	1.6	0.12	0.53	1.58	0.24	0.27	0.88	1.07	1.56	1.8	2.72	7.48	5.15	8.28	11.6	4.98	7.14	6.27	3.76	3.11	4.32	2.36	4.32	5.46	6.22	2.33	1.55
16	3.47	0.73	3.54	0	0.24	0.9	0.26	1.65	2.21	5.9	4.27	0.8	2.14	5.75	3.94	5.78	3.93	9.79	10.4	13.5	11.8	0.24	2.04	0.46	1.38	0.97	1.99	0.43	1.45
17	0.52	1.28	2.09	0	0.06	1.28	0	0.06	0.06	0.99	0.58	0.35	0.12	5.93	2.68	7.56	11.6	8.26	8.55	7.74	21.3	0.17	0.35	1.63	4.71	4.07	5.18	0.87	1.98

REBOUND
EARLY CLINICAL ABMR

No	No
No	No
No	Yes
No	No
Yes	Yes
No	No
No	No
No	No
No	Yes
No	Yes
No	No
Yes	Yes
Yes	Yes
Yes	Yes
Yes	No
No	No

DSA rebound



CD19+CD20+CD21+CXCR5+CD38+IgD+
 FCRL4+ ICOS+
 Germinal Centre Naïve activated B cells

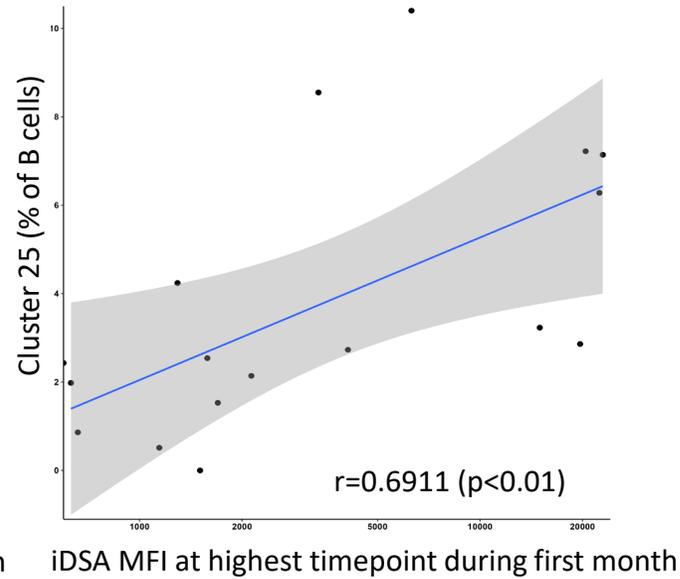
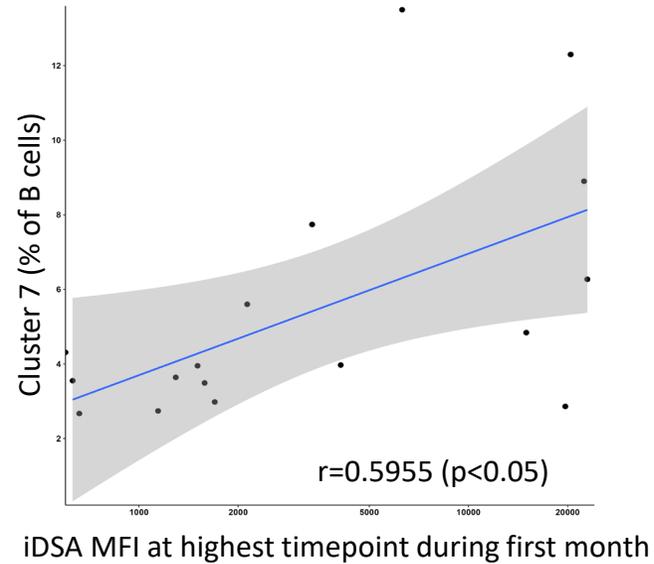
Association of DSA rebound with clusters of germinal center naïve activated B cells at T0

RESULTS: Bulk B cell phenotypes association with DSA rebound and mBC

T0 flow cytometry B cell clusters
Association with rebound of immunodominant DSA

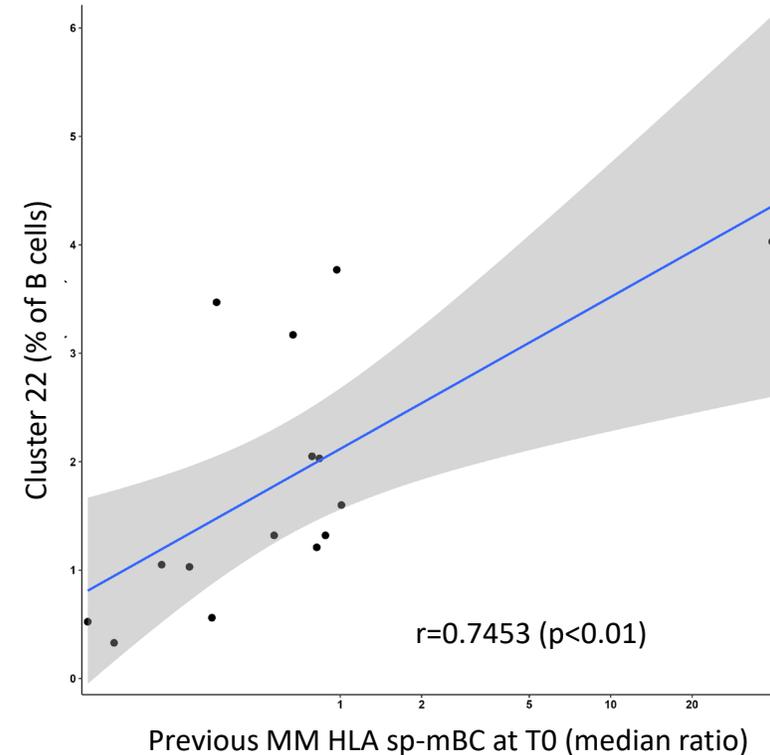
CD19+CD20+CD21+CXCR5+CD38+IgD+
FCRL4+ ICOS+
Germinal Centre Naïve activated B cells

CD19+CD20+CD10+CD21+CXCR5+CD38+IgM+IgD+
Germinal Centre Naïve activated B cells



T0 flow cytometry B cell clusters
Association with HLA-specific memory B cells against
previous graft MM

CD19+CD20-CD127+
Memory B cells transitioning to plasmablasts



Association of DSA MFI at rebound with clusters of germinal centre naïve activated B cells at T0.
Association of higher frequency of HLA-sp memory B cells against previous MM with memory B cell phenotypes.

SUMMARY

- Early clinical ABMR occurs in more than 40 % of hypersensitized patients transplanted with a positive cross-match, desensitized with Imlifidase.
- Early clinical ABMR is associated with strong rebound of DSA.
- HLA antigens from previous grafts (especially if the graft is still present) are associated with specific memory B cells that drives rapid antibody rebound.
- Bulk cell phenotyping before transplantation identifies cell populations associated with post-transplantation rebound (germinal center B cells).
- B cells driving DSA rebound (memory B cells, Germinal center B cells) may not be efficiently targeted by associated IS drugs (Rituximab).

Our study adds new insights in the understanding of mechanisms driving DSA rebound and ABMR after cross-match positive transplantation with Imlifidase, that could improve selection strategies of allowed DSA and pave the way to new associated therapeutic strategies.

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Nephrology Department (Hospital Clinic)

Fritz Dieckmann

Nephrology Department (CHU Toulouse)

Nassim Kamar

Nephrology department (Hospital del Mar)

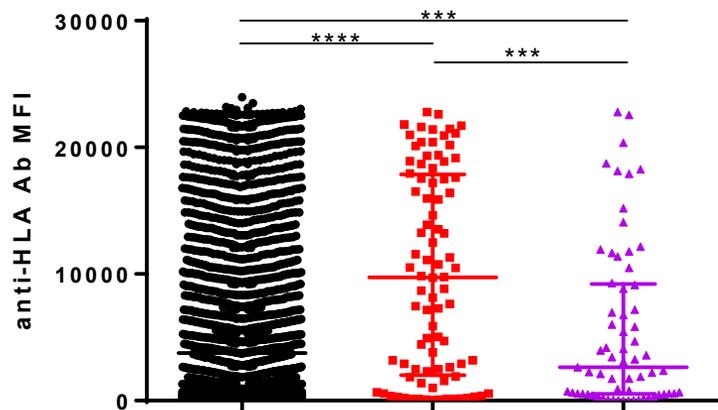
Marta Crespo

Nephrology department (CHU Bordeaux)

Lionel Couzi

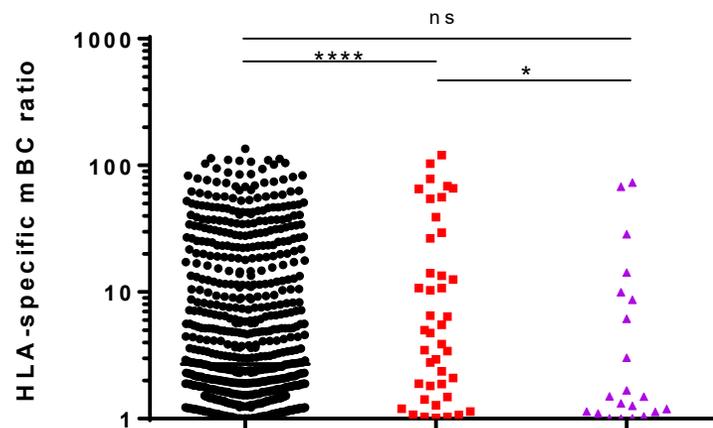
RESULTS: memory/role of transplantectomy?

Anti-HLA antibodies (SAB) at T0



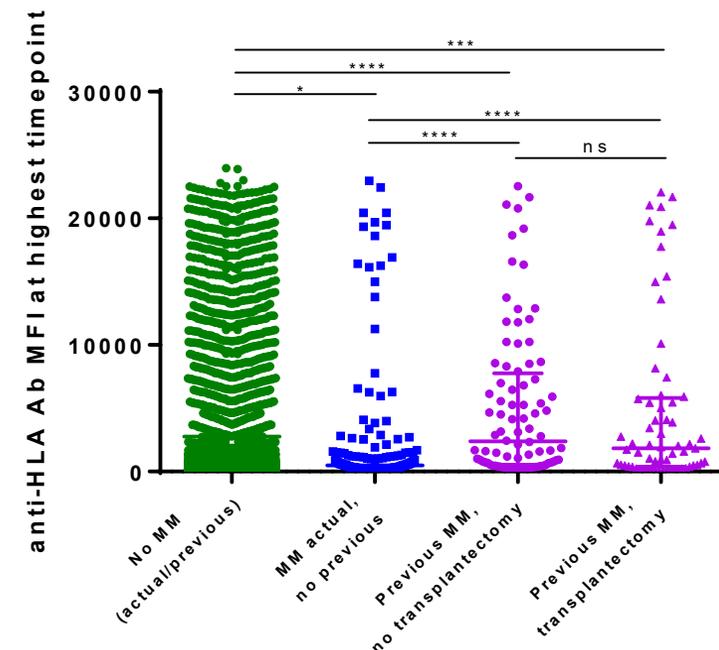
Median MFI (IQR)	674 (135-4817)	9734 (2003-17 863)	2630 (529-9216)
n	4072	88	65

HLA-specific mBC at T0



Median ratio (IQR)	0.3309 (0.0-0.9449)	0.9166 (0.18-4.523)	0.4656 (0.1146-1.134)
n	3660	88	62

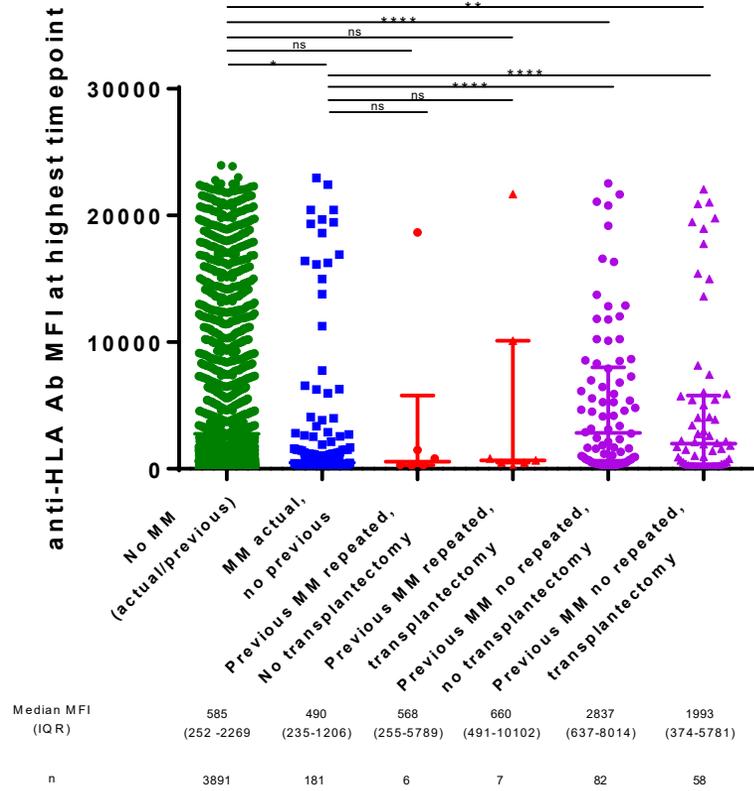
Anti-HLA antibodies (SAB) at rebound



Median MFI (IQR)	585 (252 -2269)	490 (235-1206)	2388 (541-7757)	1817 (390-5824)
n	3891	181	88	65

Anti-HLA Ab against previous MM show higher MFI at baseline, more frequent detection of specific mBC and higher rebound. Association between the presence of the previous graft and higher level of anti-HLA Ab and mBC versus transplantectomy

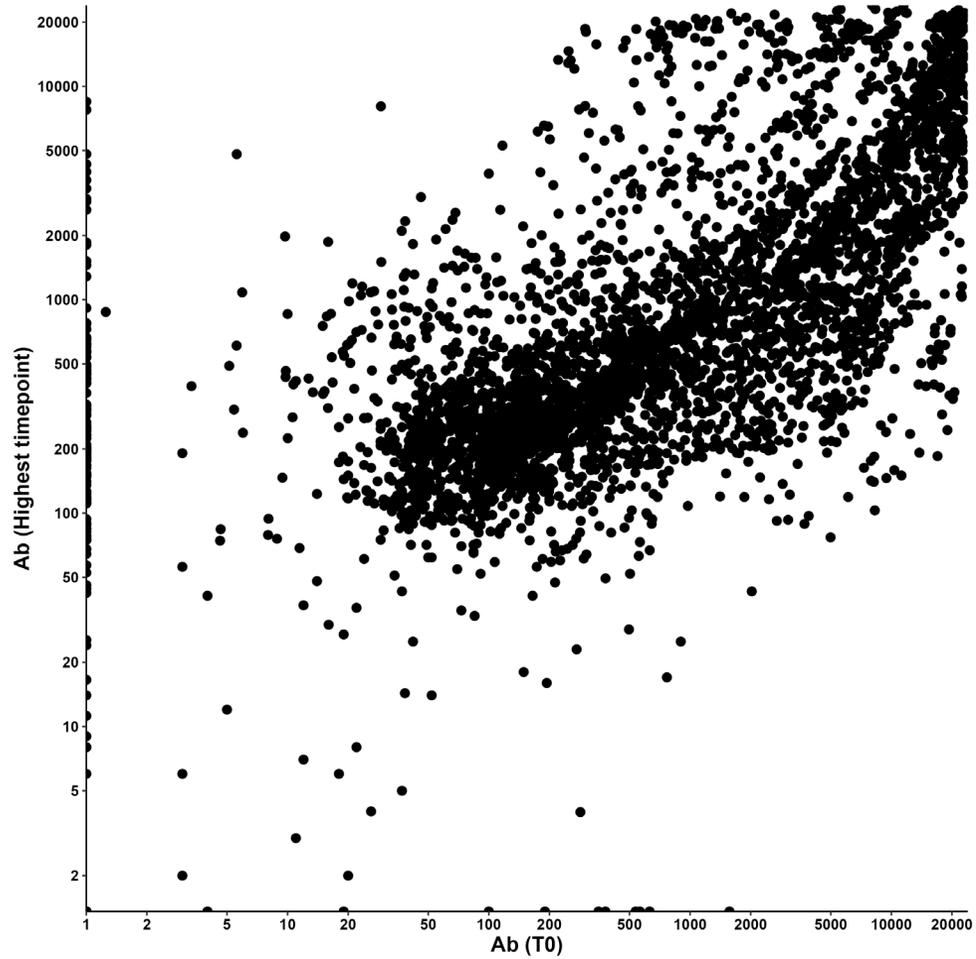
SAB highest timepoint previous MM/Txectomy



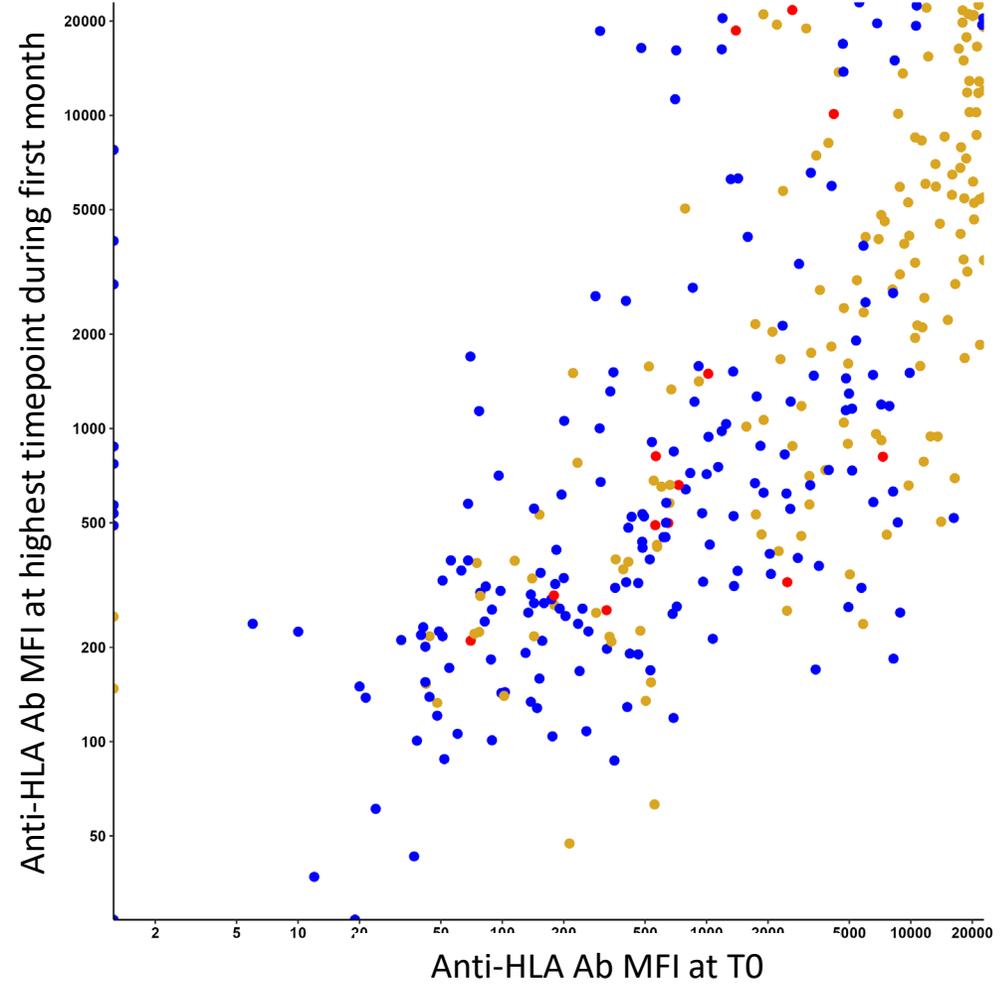
RESULTS: Anti-HLA antibodies evolution

MM groups

- Current MM (Non-repeated)
- Current MM (Repeated)
- Previous MM



Correlation $R = 0.72$ P value < 0.0001



Correlation for previous (incl. Repeated) MM $R = 0.75$ P value < 0.0001