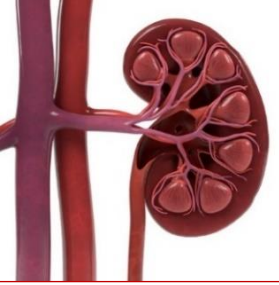
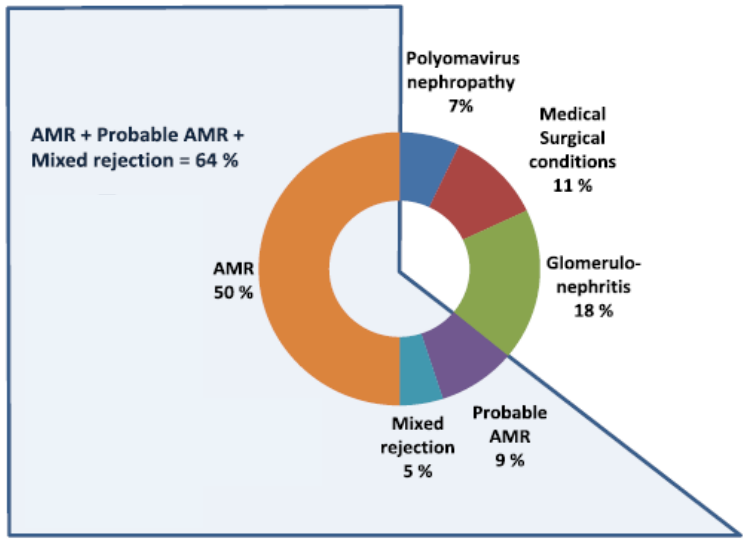


The impact of post-transplant non-HLA antibody burden on the occurrence of antibody-mediated rejection and graft loss in non-sensitized pediatric kidney recipients

Stella Muscianisi, M De Cicco, M Cioni, K Mebelli, B Ray, A Tagliamacco, J Hariharan, I Fontana, T De Feo, A Trivelli, A Magnasco, E Verrina, A Nocera, F Ginevri, P Comoli.

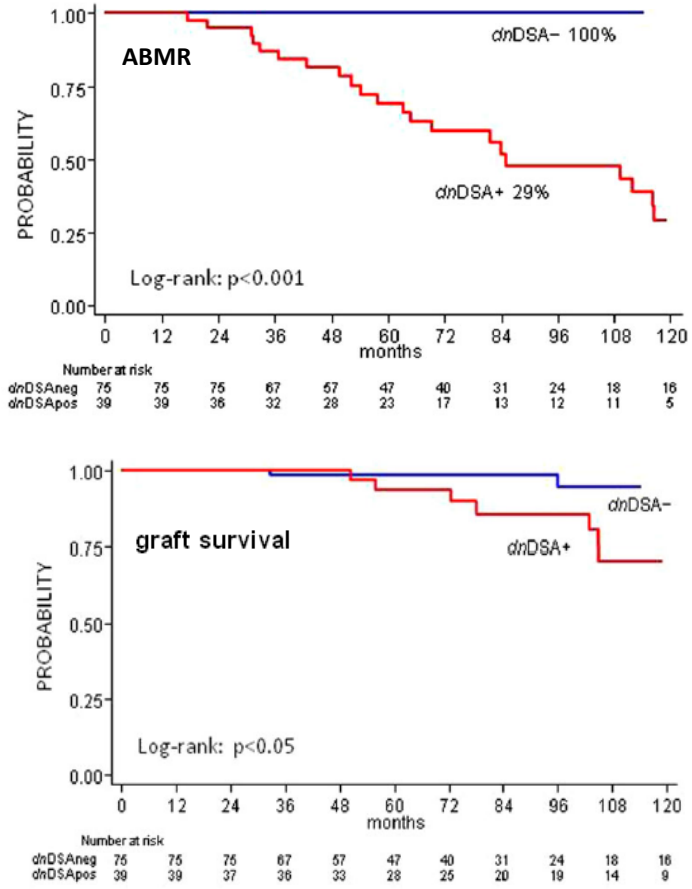
Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; Nephrology Unit, G. Gaslini Institute, Genova, Italy; Immucor Inc, Norcross, GA, USA; Transplantation Immunology, Fondazione Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italy; Kidney Transplant Surgery Unit, University of Genova, IRCCS San Martino University Hospital IST, Genova, Italy

Antibody-mediated rejection (ABMR) and kidney transplant loss



adapted from Sellares et al, *Am J Transplant* 2012

Antibody-mediated rejection (ABMR) is a major cause of renal allograft failure.



Ginevri et al, Am J Transplant 2012; Comoli et al, Am J Transplant 2016

Development of post-KTx **de novo HLA-DSAs** has been associated with:

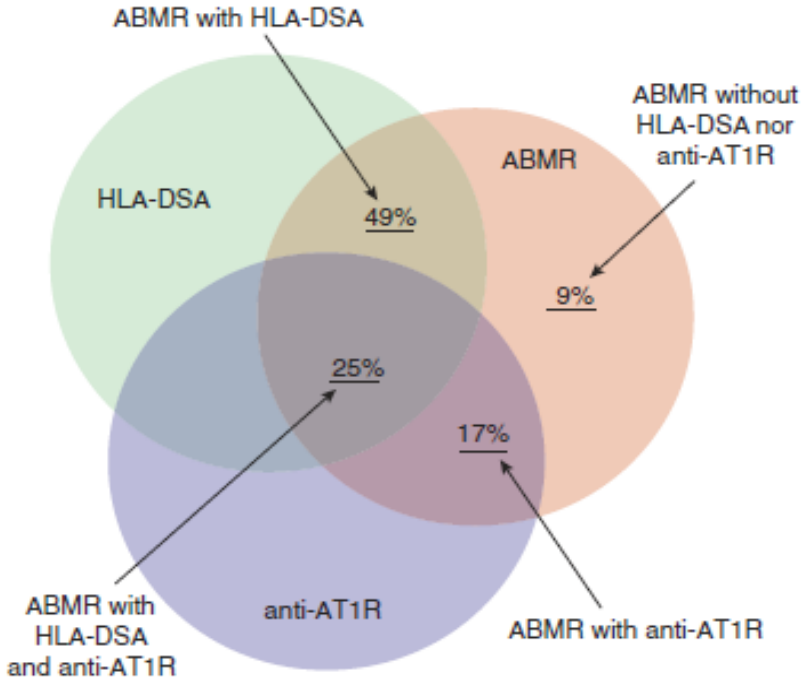
- highest risk of chronic ABMR[1,2,3] and allograft loss[2,3]
- subclinical ABMR at the time of first detection[6,7]

[1]Senev et al. *Am J Transplant* 2019;
[2]Aubert et al. *J Am Soc Nephrol* 2017;
[3]Haas et al. *Kidney Int* 2017;
[6]Wiebe et al. *Am J Transplant* 2017; [7]Bertrand et al. *Transplantation* 2020

ABMR: role of HLA and non-HLA Abs

Histologic ABMR in kidney allografts may exist in the absence of HLA-DSAs

- the association of non-HLA antibodies with ABMR lesions is increasingly reported.



Lefaucheur et al, *Kidney Int* 2021

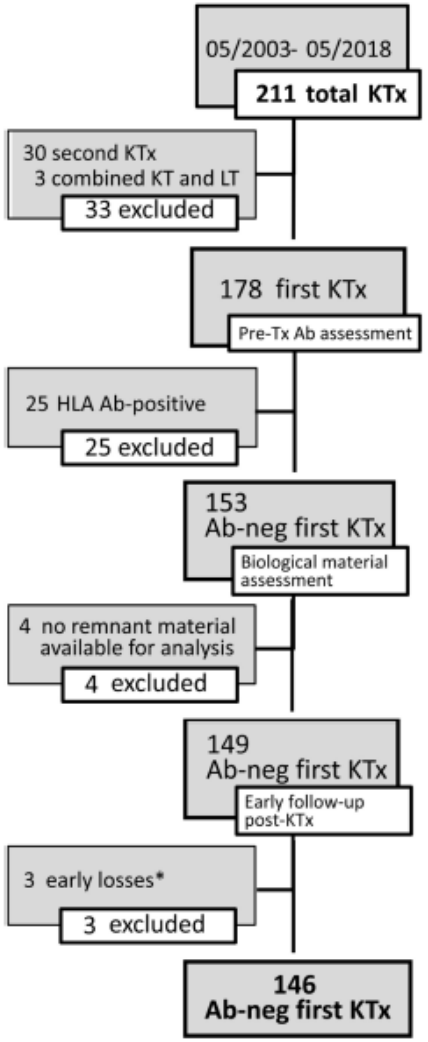
TABLE 3.

Non-HLA antigens associated with allo or autoantibodies in kidney transplant recipients or patients with kidney disease

Major histocompatibility class I-related chain A/B
Angiotensin II type 1 receptor
LIMS1
ARHGDIB (rho GDP-dissociation inhibitor 2)
Endothelin type A receptor
Peroxisomal trans-2-enol-CoA reductase
Vimentin
Agrin
Endorepelin (the C-terminal fragment of perlecan)
Lamin B1
Rho guanine nucleotide exchange factor 6
Glutathione-S-transferase T1
Keratin endoglin
FMS-like tyrosine kinase-3 ligand
EGF-like repeats and discoidin 1-like domain 3
Intercellular adhesion molecule 4
Protein kinase C zeta
Adipocyte plasma membrane-associated protein
LPLUNC1 (BPI fold-containing family B member 1)
Tubulin beta-4B
Phospholipase A2 receptor

Filippone & Farber, *Transplantation* 2021

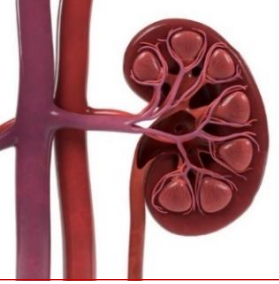
Impact of HLA and non-HLA Abs in low-risk pediatric KTx recipients



Single-center cohort of 146 Ab-negative pediatric recipients of first KTx

- sera for HLA Ab monitoring collected at Tx, 3-monthly for the first year, and annually thereafter
- follow-up: 28-220 months - median: 123 months
- a total of 1600 samples were analyzed for
  - **HLA-DSAs**: screen + SAB assays after EDTA treatment; C1qScreen™ (One Lambda) and Lifecodes C3d Detection kit (Immucor Inc) for identification of complement-binding Abs
  - **Non-HLA-Abs**: non-HLA multiplex bead panel (Immucor Inc).

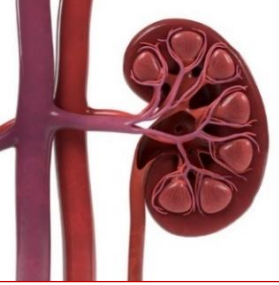
We evaluated the role of circulating DSAs and non-HLA Abs described as relevant for ABMR and graft loss: GSTT1, angiotensin II type 1 receptor (AT1R), Vinculin (VCL), Vimentin (VIM), Fibroblast Growth Factor-2 (FGF-2), Vascular Endothelial Growth Factor α (VEGFα), Epidermal growth factor-like 3 (EDIL-3).



Role of DSAs and non-HLA Abs in ABMR and graft loss

- 50 pts developed HLA-DSAs post-Tx, that persisted positive for the most part throughout the follow-up.
- 132 developed 1 or more non-HLA Abs:
  - 16 patients were found with GSTT1Abs at a MFI above the 75% quartile (Q4),
  - 51 with 1 or more of other non-HLA Abs (AT1R/VCL/VIM/FGF2/VEGFα/ EDIL3) at Q4, only 7 concomitantly positive for GSTT1.
- ABMR was diagnosed in 31 patients at a median follow-up of 5.0 years. Twenty-nine of the 31 patients had circulating dnDSAs, while two dnDSA-negative recipients were found positive for circulating anti-GSTT1 Abs but no other non-HLA Abs among those tested.
- Fifteen patients lost their graft at a median time of 6.7 years (range 2.3-14.4).

- Univariable analysis for ABMR and graft loss included:
  - clinical parameters: pts and donor age and sex, HLA mismatches, use of CNI, DGF, acute cellular rejection
  - immunological parameters: presence of dnDSAs and of Q4 MFI level GSTT1 Abs and Abs against AT1R, VCL, VIM, FGF2, VEGFα, and EDIL3 Abs, alone or in cluster.

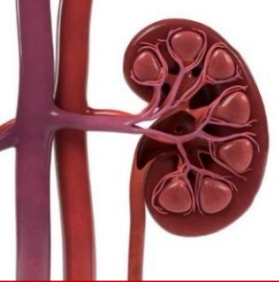


Role of DSAs and non-HLA Abs in ABMR: univariable analysis

31 pts developed ABMR:

- 2 pts were sHLA-DSA negative (both positive for GSTT1 Ab)

Variables	Patients (n)	HR	95% CI	P value
Immunologic factors				
dnDSA				
* no	2			
yes	29	31.95	7.62 – 133.98	**< 0.0001**
Anti-GSTT Ab (Quartile 4)				
* no	20			
yes	11	7.13	3.38 – 15.04	**< 0.0001**
Anti-AT1R Ab (Quartile 4)				
no	26			
yes	5	1.16	0.44 – 3.02	0.76
Anti-VCL Ab (Quartile 4)				
no	25			
yes	6	1.46	0.60 – 3.57	0.40
Anti-VIM Ab (Quartile 4)				
no	26			
yes	5	1.00	0.38 – 2.61	0.99
Anti-FGF2 Ab (Quartile 4)				
no	25			
yes	6	1.74	0.71 – 4.24	0.25
Anti-VEGFα Ab (Quartile 4)				
no	25			
yes	6	1.41	0.58 – 3.43	0.47
Anti-EDIL3 Ab (Quartile 4)				
no	25			
yes	6	1.52	0.62 – 3.70	0.36
Cluster AT1R/VCL/VIM/FGF2/VEGFα/ EDIL3 (Quartile 4)				
no	18			
yes	13	1.26	0.62 – 2.58	0.52



Role of DSAs and non-HLA Abs in graft loss: univariable analysis

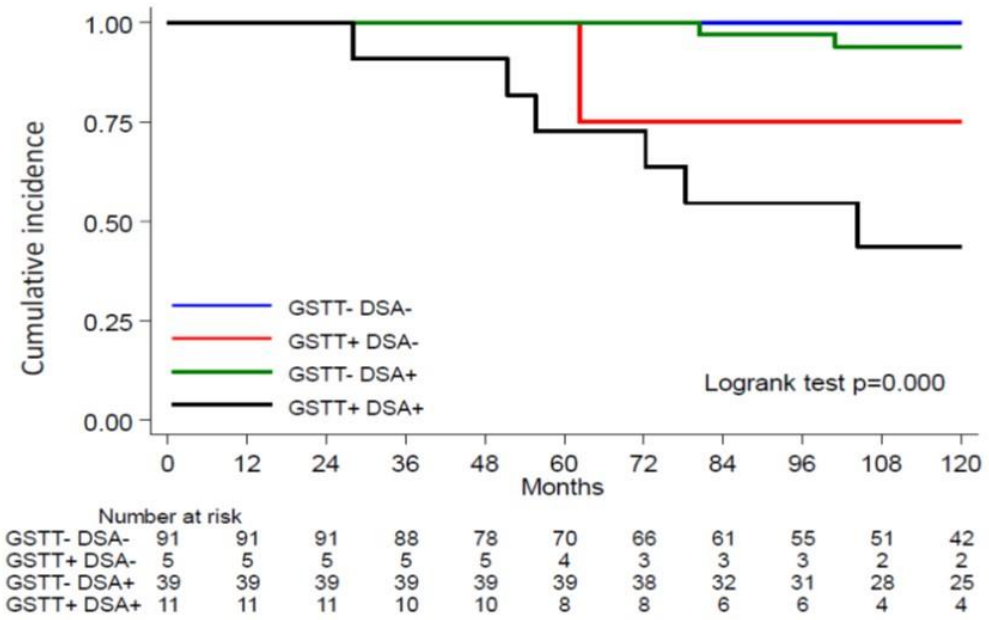
15 pts lost their graft due to ABMR:

- median 6.7 yrs

Variables	Patients (n)	Pts with GL (n)	HR	95% CI	P value
Immunologic factors					
* dnDSA					
no	96	1			
yes	50	14	20.10	2.64 – 153.20	< 0.0001
* Anti-GSTT Ab (Quartile 4)					
no	130	7			
yes	16	8	14.36	5.10 – 40.48	< 0.0001
Anti-AT1R Ab (Quartile 4)					
no	126	13			
yes	20	2	0.79	0.18 – 3.50	0.75
Anti-VCL Ab (Quartile 4)					
no	127	12			
yes	19	3	1.59	0.45 – 5.66	0.49
Anti-VIM Ab (Quartile 4)					
no	124	13			
yes	22	2	0.80	0.18 – 3.53	0.76
Anti-FGF2 Ab (Quartile 4)					
no	126	14			
yes	20	1	0.43	0.06 – 3.27	0.35
Anti-VEGFα Ab (Quartile 4)					
no	125	13			
yes	21	2	0.75	0.17 – 3.34	0.70
Anti-EDIL3 Ab (Quartile 4)					
no	127	12			
yes	19	3	1.34	0.38 – 4.78	0.66
Cluster AT1R/VCL/VIM/FGF2/VEGFα/ EDIL3 (Quartile 4)					
no	92	10			
yes	54	5	0.84	0.29 – 2.46	0.75

Graft loss: role of HLA and non-HLA Abs

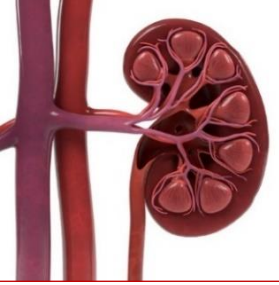
- The only 2 immunological variables independently associated with graft loss were the presence of DSAs and of anti-GSTT Ab.
- Graft survival was worse when both Q4 anti-GSTT and anti-HLA DSA antibodies were present, but not if anti-GSTT Abs were clustered with the other 6 Abs.



Comoli et al. *Front Med* 2022

Evaluated patients (n=146)	Patients (total n.)	Pts with graft loss	AIC*	P value*
Model with DSAs	50	14	111.17	<0.001
Model with Q4-GSTTAbs	16	8	108.06	<0.001
Model with DSA+Q4-GSTTAbs	11	7	99.54	<0.001

Evaluated patients (n=146)	Patients (total n.)	Pts with graft loss	AIC*	P value*
Model with DSAs	50	14	111.17	<0.001
Model with non-HLA Ab cluster	66	10	129.44	0.66
Model with DSA+non-HLA Ab cluster	23	7	113.01	<0.001



Role of DSAs and non-HLA Abs: conclusions

- In our pediatric cohort, HLA-DSAs and GSTT1 Abs are independent predictors of ABMR and graft loss
- According to our findings, the detection of antibodies against GSTT1 antigen, but not other non-HLA targets such as AT1R, VCL, VIM, FGF2, VEGFα, and EDIL3, increase immunologic risk and impact on graft survival
- These findings, that are apparently in contrast to other data present in the literature, may be related to
  - the peculiarities of the cohort: non-sensitized pediatric renal recipients
  - the methodology employed for Ab detection