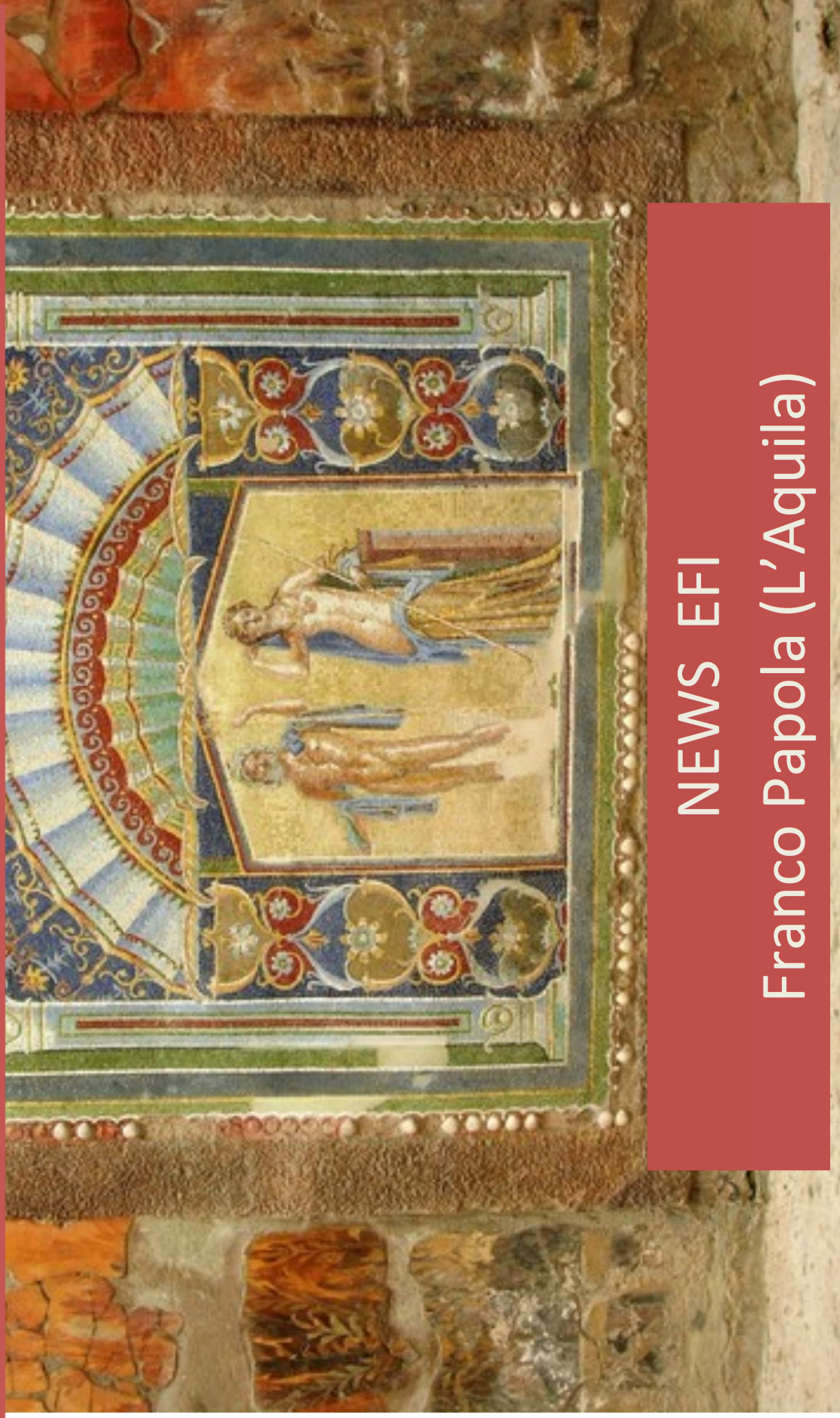


SUMMER SCHOOL AIBT 2019

ERCOLANO (NA) 13-15/06/2019



NEWS EFI

Franco Papola (L'Aquila)

The Human Microbiome

10^{14} microbes /
person

3% human body
mass

1:1 ratio bacterial to
human cells

10X microbial :
human genes

99% of microbes
reside in the gut

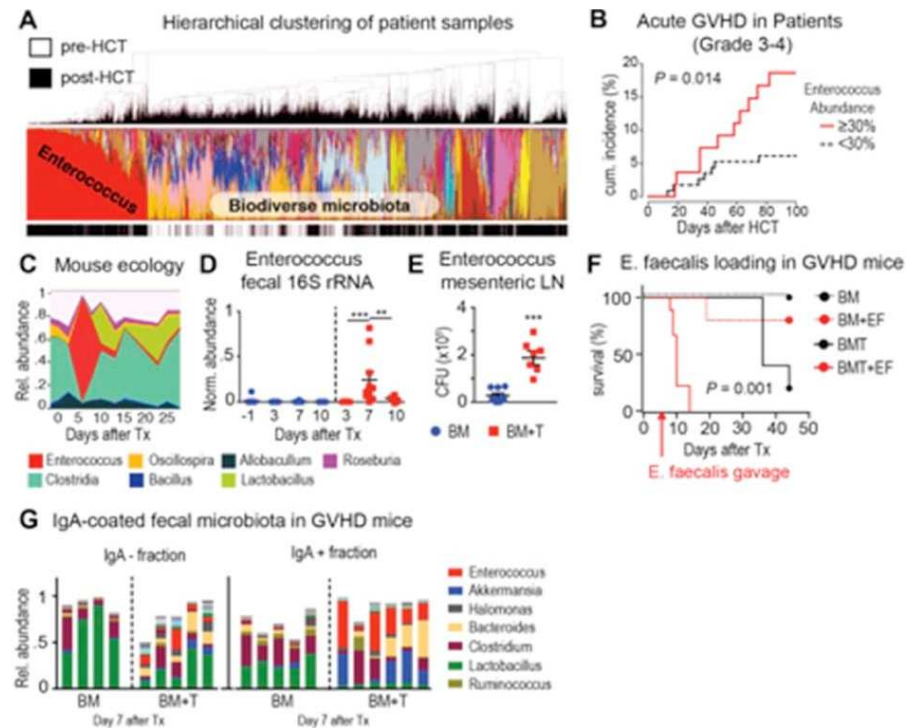
10^4 species across
human hosts

10^3 species
/person
(Firmicutes, Bacteroidetes and
Actinobacteria)

Associations With

- Obesity
- Atherosclerosis
- Diabetes
- Malnutrition
- Carcinogenesis
- Arthritis
- Eczema
- Allergies
- Asthma
- Autism

Domination of the Gut Microbiota with *Enterococcus* Species Early after Allogeneic Bone Marrow Transplantation is an Important Contributor to the Development of Acute Graft-Versus-Host Disease (GVHD) in Mouse and Man



(A) Hierarchical clustering of stool samples from 637 allo-HCT patients demonstrates posttransplant *Enterococcus* domination (predom. *E. faecium*). (B) Patients with monodomination by *Enterococcus* (defined as $\geq 30\%$ abundance, $n = 55$) have a significantly higher cumulative incidence of grade 3-4 acute GVHD than those below this threshold ($n = 115$). (C) In mice 16S rRNA sequencing from a MHC-matched (C57BL/6>129S1) transplant (Tx) demonstrates an *Enterococcus* bloom at day 5-10 (predom. *E. faecalis*). (D) The *Enterococcus* bloom is recapitulated in an MHC-disparate (C57BL/6>BALB/c) Tx, and (E) is accompanied by significant translocation of *Enterococcus* into mesenteric lymph nodes (LN) of GVHD mice. (F) Spiking of the host gut flora with *E. faecalis* OG1RF (EF) after transplant aggravates acute GVHD in the MHC-disparate (C57BL/6>BALB/c) model. (G) Using IgAbug 16S sequencing to analyze IgA-coated (IgA+) vs. non-coated (IgA-) bacteria in the intestinal microbial community at day 7 in an MHC-disparate (C57BL/6>BALB/c) Tx model. ** $P < .01$, *** $P < .001$. BM, no T cells transplanted; BMT, T cells transplanted.

Microbiota Profiling Methods

Weekly (or daily) stool samples
4° ASAP, frozen aliquots without additives at -80° within 24 hrs
Inpatient & outpatient (outpatients given 4° chill packs)
Timepoint-driven & event-driven

Extract bacterial DNA
(Bead beating phenol/chloroform)

Amplify 16S rRNA gene PCR
(V4-V5 hypervariable regions)

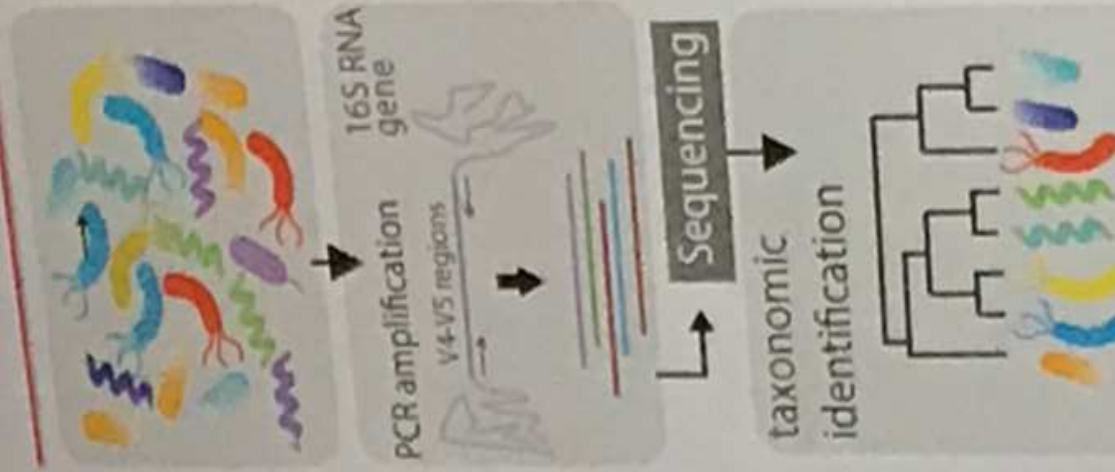
Sequence (MiSeq paired end 250bp)

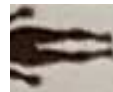
qiime and vparse
Taxonomic classification by
- Greengenes
- NCBI 16S database

Abundance

OTU

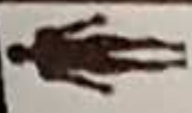
(Operational Taxonomic Unit)





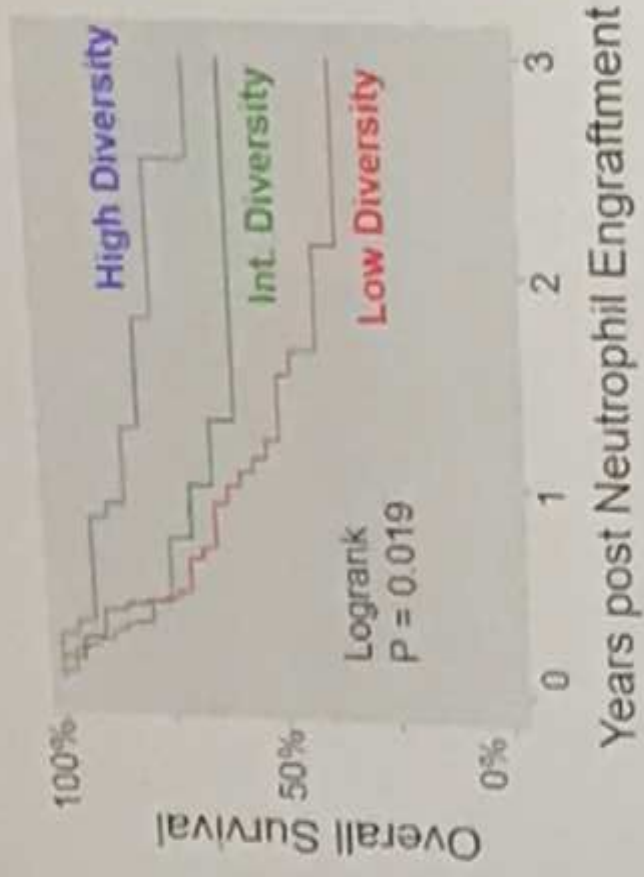
Hierarchical clustering of allo-HCT patient samples shows loss of diversity and increased bacterial domination after transplantation





High microbiota diversity predicts overall survival and protection from lethal acute GVHD

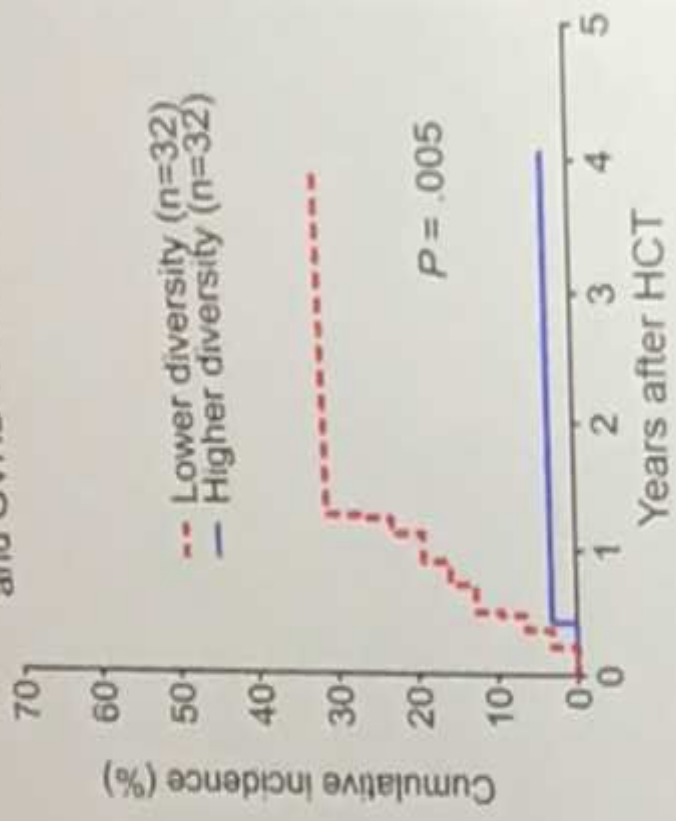
Overall Survival
sampled within 7 days post engraftment



Taur Blood 2014, n = 80

Lethal GVHD

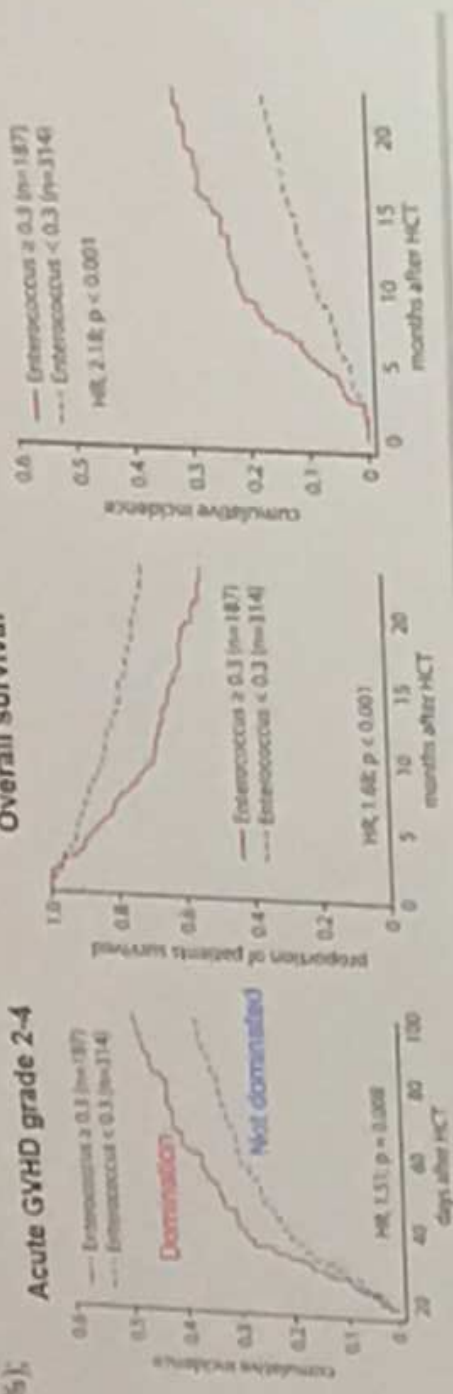
Inverse Simpson diversity index
and GVHD-related mortality



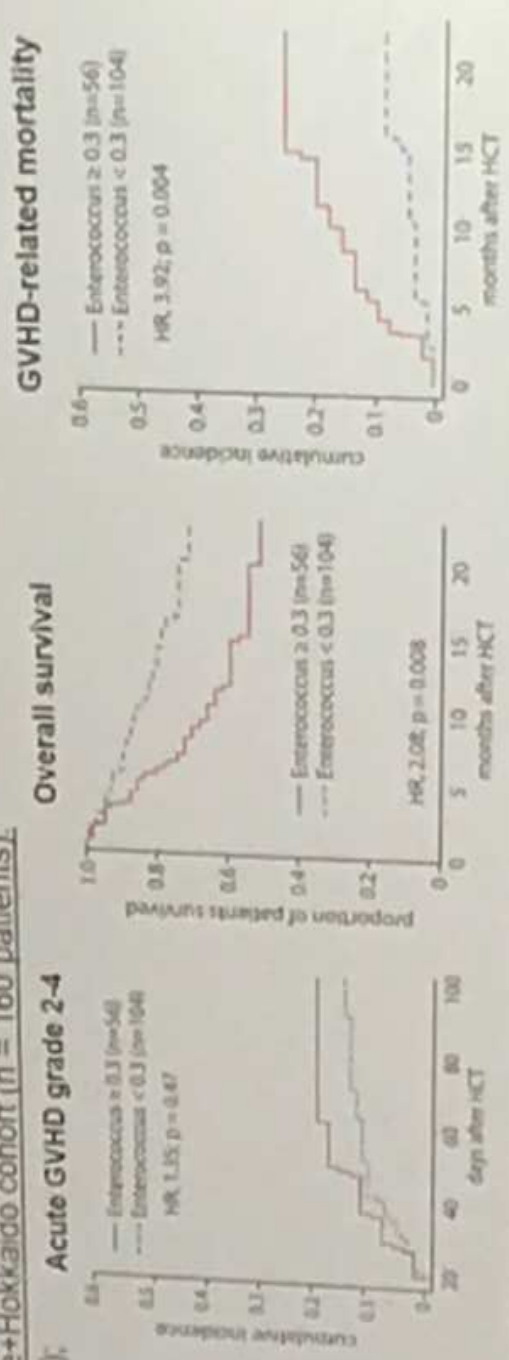
Jenq BBMT 2015, n = 64

Enterococcus domination of gut flora increases risk for acute GVHD and reduces survival

MSKCC cohort (n = 520 patients): Enterococcus mono-domination: rel. abundance > 0.3, days 0 - 21 after HCT
 cord grafts (27.8%);
 unmodified BM/PBSCs (72.2%)



Regensburg+Duke+Hokkaido cohort (n = 160 patients):
 cord grafts (11.8%);
 unmodified BM/PBSCs (88.2%)



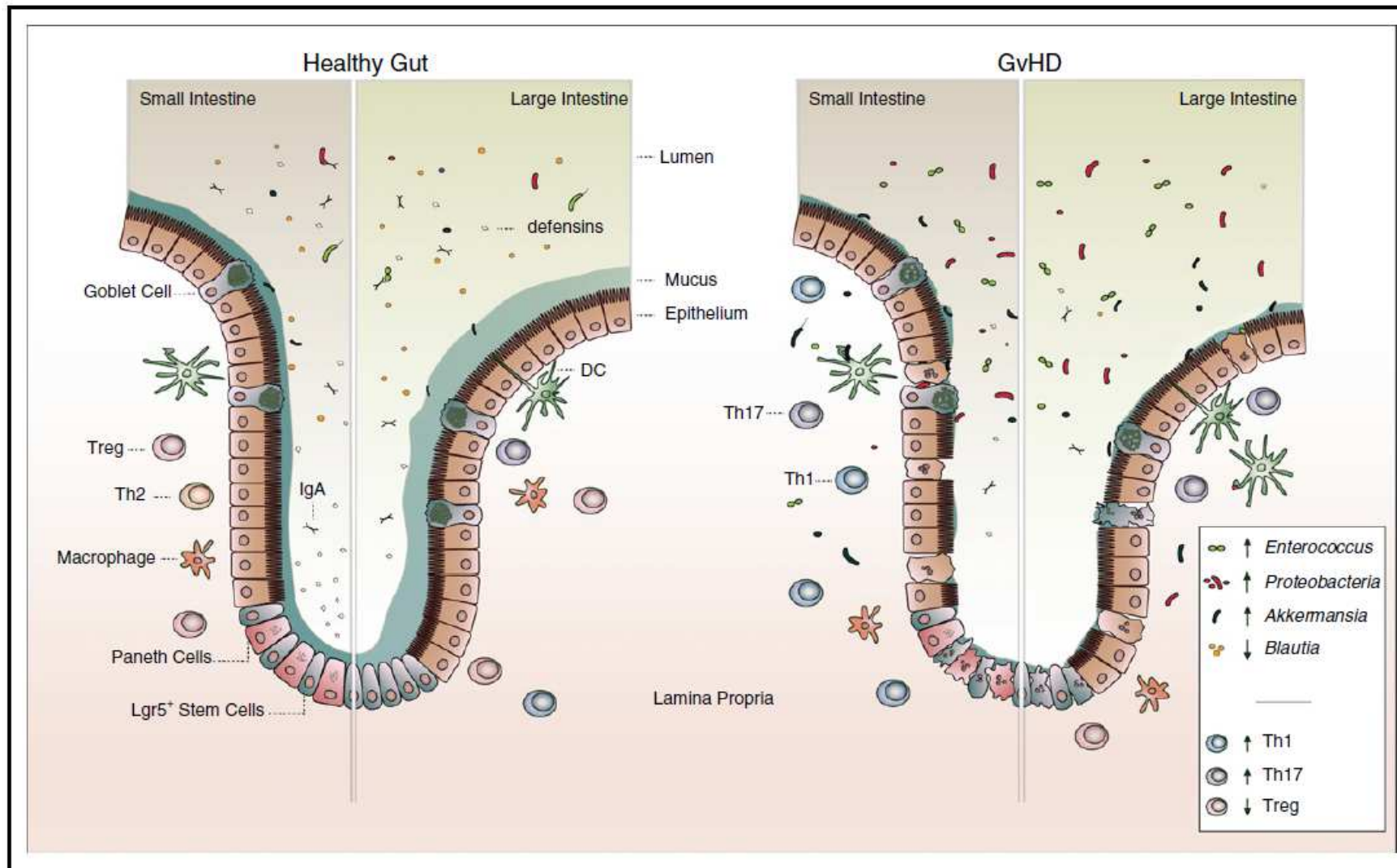
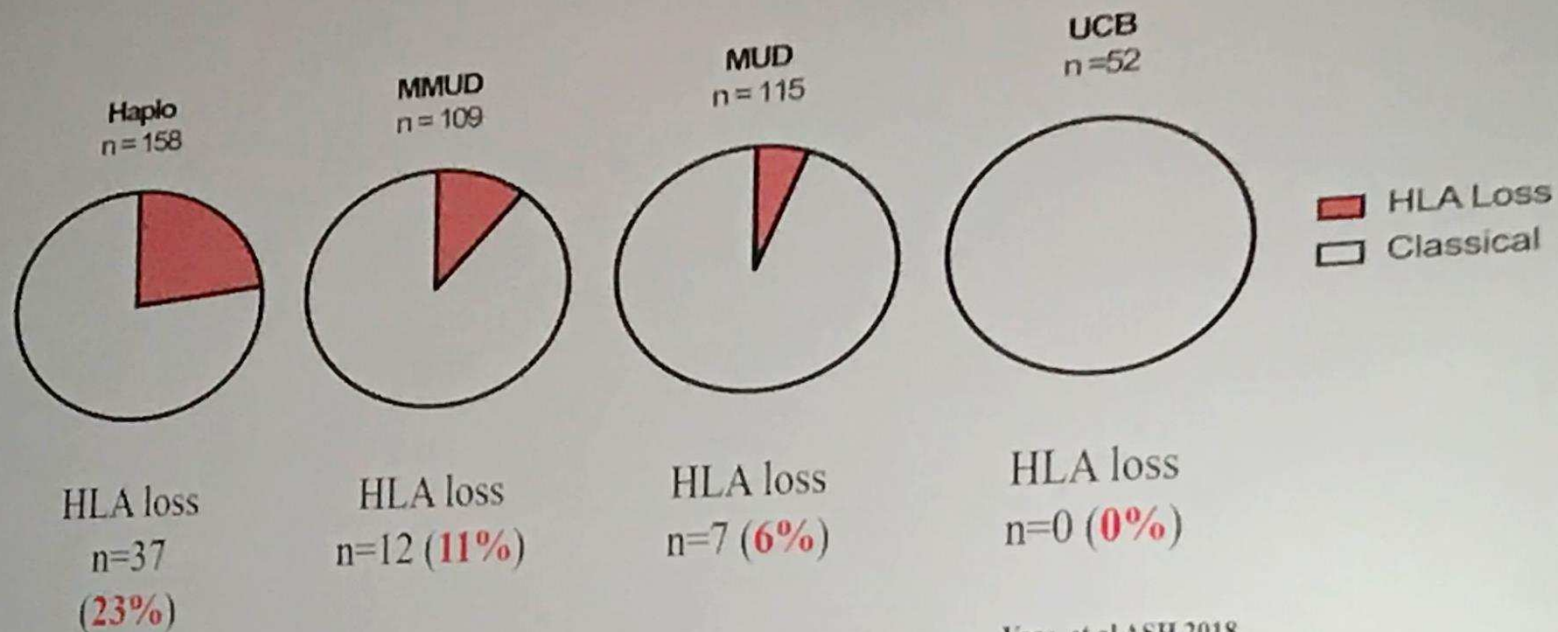


Figure 1. Intestinal damage and dysbiosis linked to GVHD. Upon HCT, multiple factors such as conditioning toxicity, antibiotic treatment, and immune activation mediate GVHD. GVHD progression associates with injury to stem cell compartments along with Paneth cells (small intestine) and goblet cells. This leads to increased intestinal permeability, inflammation, and reduction of the mucus layer and antimicrobial products (eg, defensins). Antibiotic treatment and a limited amount of nutrients in the gut also promote gut dysbiosis, furthering gastrointestinal damage and disease. DC, dendritic cell; IgA, immunoglobulin A.

Results: Incidence of HLA Loss (Vago, Fleischauer)



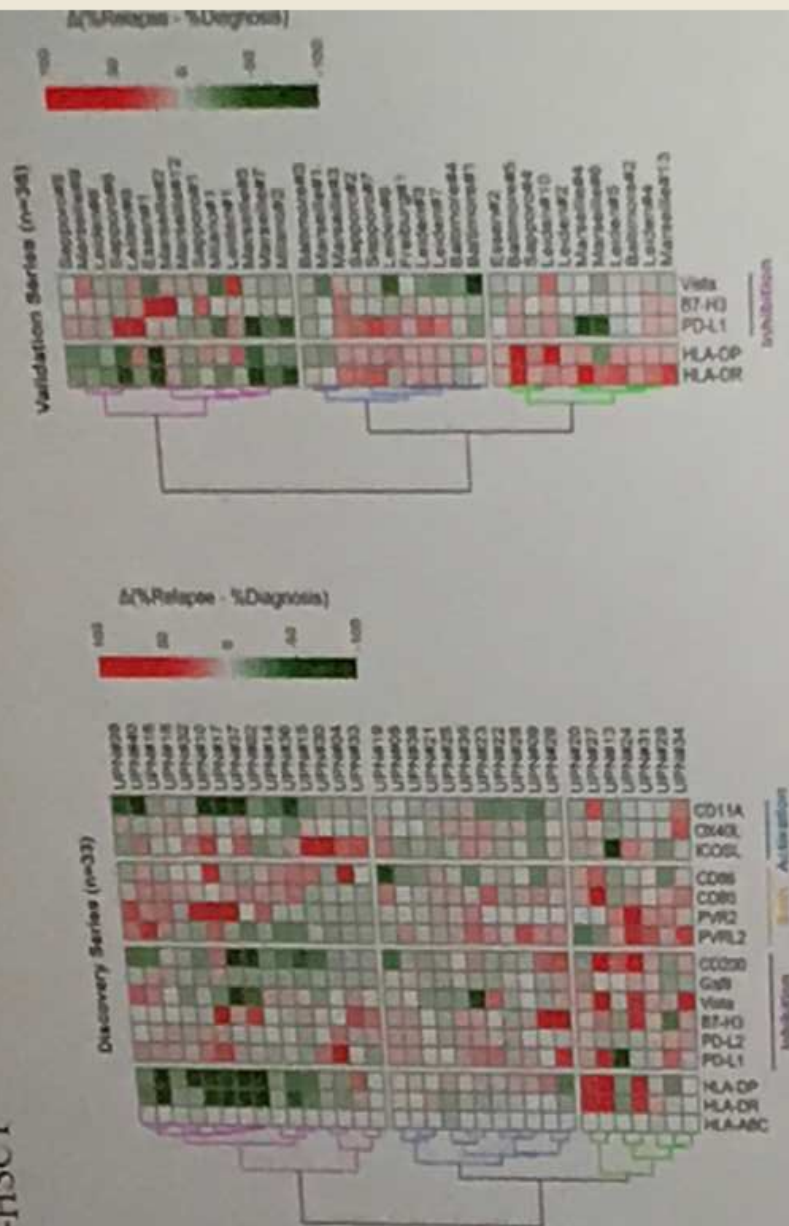
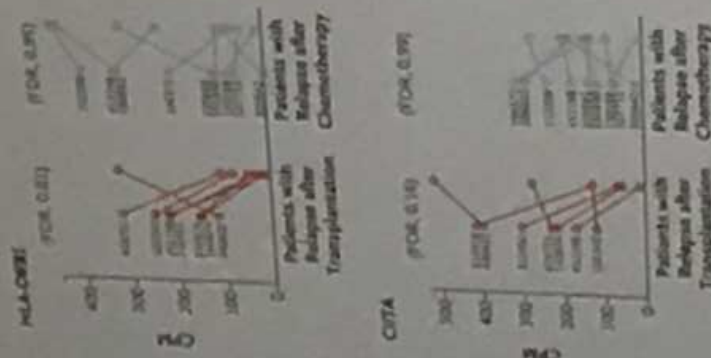
Vago, et al ASH 2018

Economic Loss of HLA Class II

Non-Genomic Loss of HLA Class II molecules

Loss of surface expression of HLA Class II molecules is observed in a considerable proportion of AM patients relapsing after matched allo-HSCT

Group	Loss of surface expression	Proportion
Validation Series (n=38)	Loss of surface expression	~0.45
Validation Series (n=38)	Supportive Care	~0.25



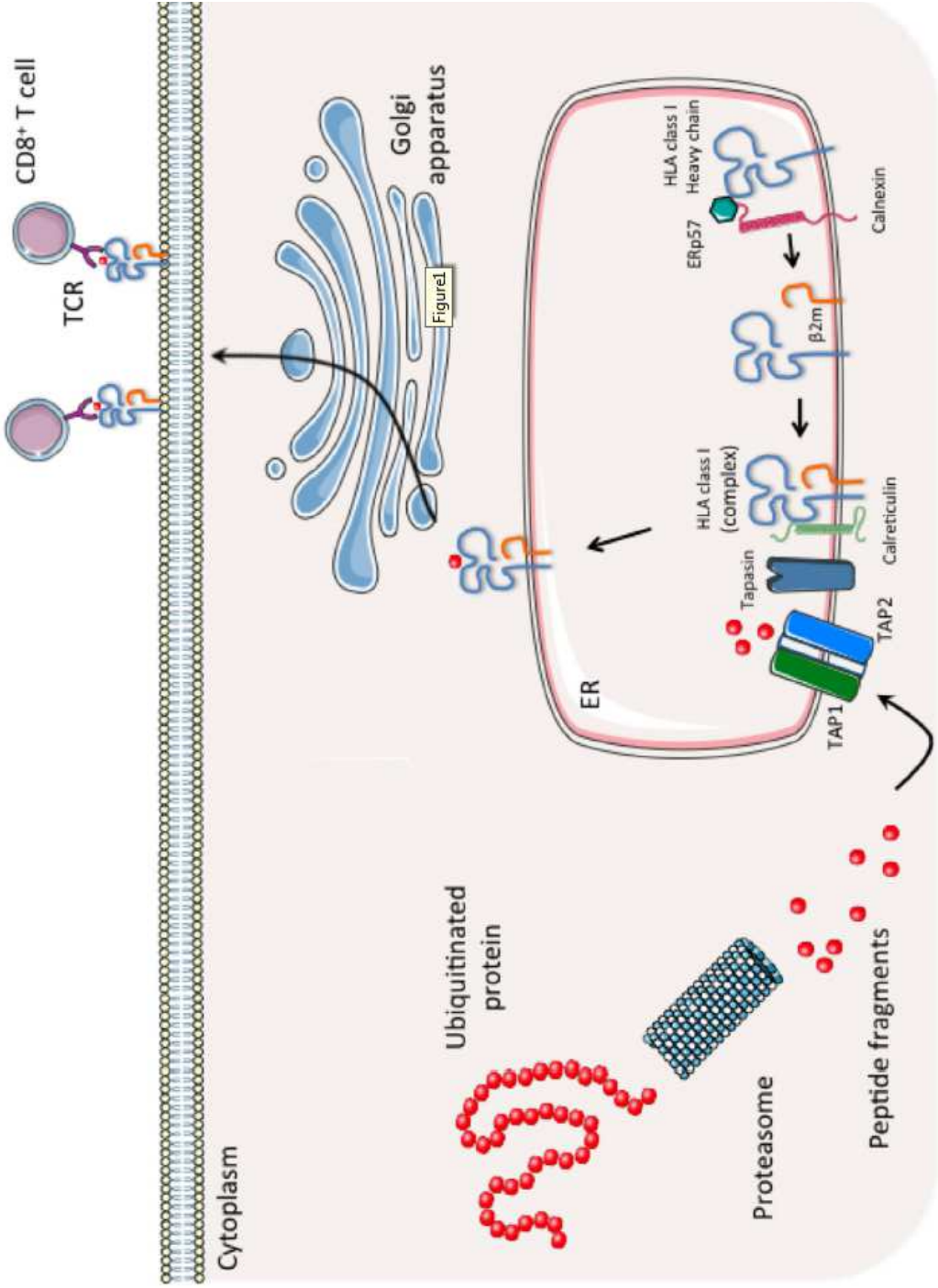


Fig. 1.

Antigen processing machinery (APM) components. Normal cells process intracellular ubiquitinated proteins tagged for degradation via the proteasome generating peptide fragments that are loaded onto nascent HLA class I molecules inside the endoplasmic reticulum (ER). Antigen presentation on the cell surface requires intact APM machinery in

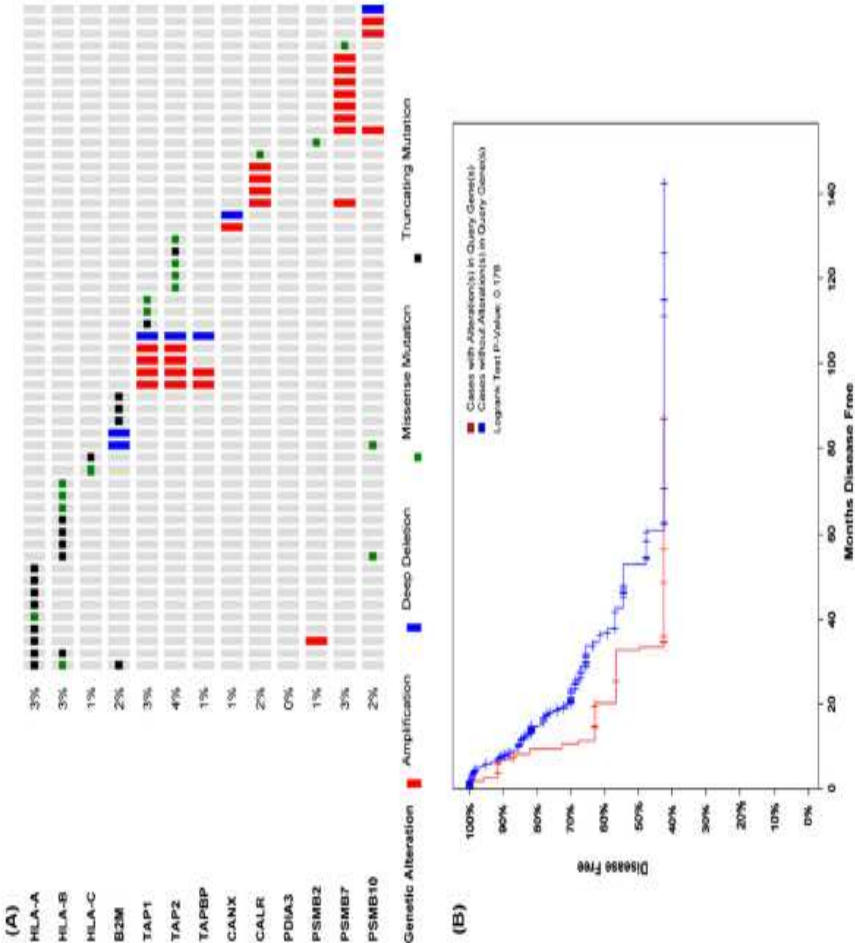
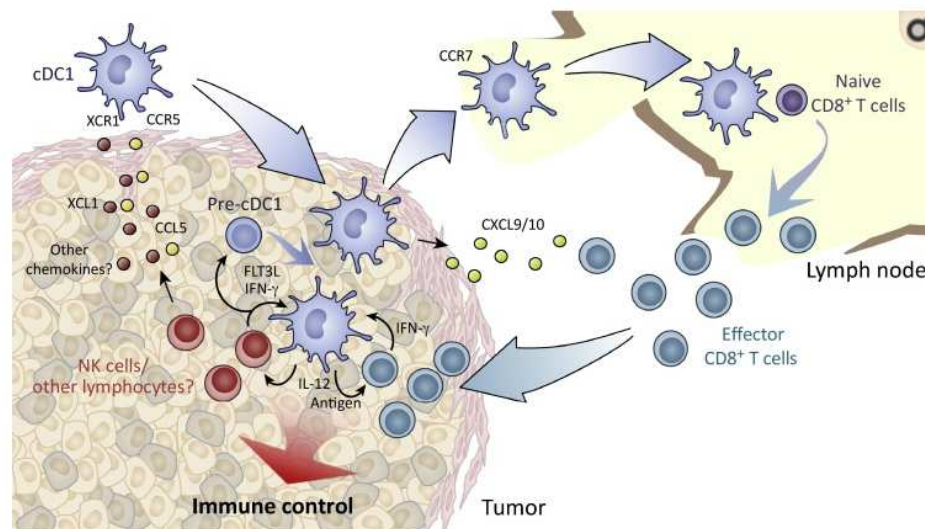


Fig. 2. The cancer genome atlas (TCGA) mutational landscape of APM components in head and neck cancer. (A) TCGA data from 279 HNC specimens with whole sequencing and copy number alterations (CNA) was accessed via the cBio portal (www.cbioportal.org). 20% of specimens (55/279) harbored genetic alterations in the APM gene sequences queried. An OncoPrint view was generated for each gene queried, the most altered APM genes were TAP2 (4%), TAP1 (3%), HLA-A and HLA-B (3% respectively) and PSMB7 (LMP7, 3%). Interestingly the mutations found in the HLA-A and HLA-B loci were almost exclusively

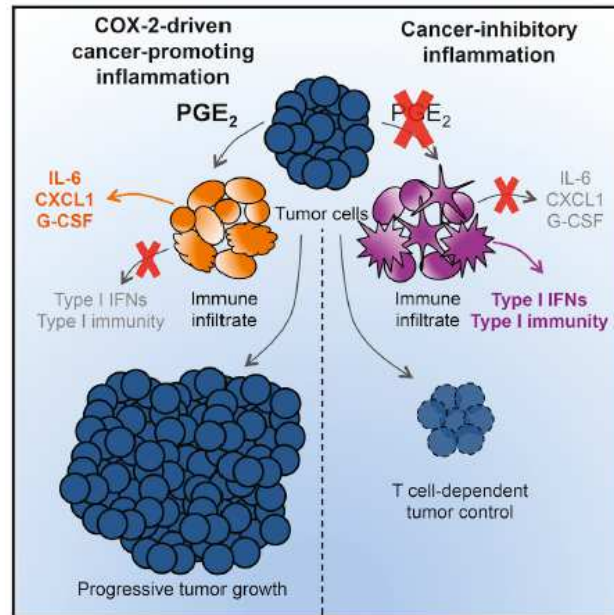


Trends in Cancer

Orchestration of Cancer Immune Control by cDC1. Conventional type 1 dendritic cells (cDC1s) are recruited into the tumor microenvironment by chemokines such as XCL1 and CCL5, produced by intratumoral natural killer (NK) cells (and potentially other lymphocytes). NK cells further secrete the growth factor FLT3L, which supports the survival of cDC1s and might enhance local cDC1 differentiation from DC precursors. Within the tumor, cDC1s take up material from (dead?) tumor cells and are uniquely able to transport tumor antigens to tumor-draining lymph nodes for presentation to naive CD8⁺ T cells, priming cytotoxic effector CD8⁺ T cells. In addition, cDC1s within the tumor microenvironment produce the chemokines CXCL9/10 that can recruit CD8⁺ effector T cells into tumor tissue and can locally present tumor antigens to restimulate recruited T cells. Finally, the anti-tumor activity of T cells and NK cells within the tumor might further be boosted by cytokines made by cDC1, for example, interleukin-12 (IL-12), that, in turn, is amplified by T- and NK cell-derived cytokines such as interferon-γ (IFN-γ).

Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity

Graphical Abstract



Authors

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In Brief

Cyclooxygenase-driven prostaglandin E₂, produced by a variety of tumors, drives malignant growth through successful evasion of type I interferon and/or T-cell-dependent tumor elimination. A remarkable synergy between cyclooxygenase inhibitors and checkpoint blockade immunotherapy results in tumor eradication.

Highlights

- Cyclooxygenase in tumors induces PGE₂ that subverts myeloid cell function
- COX ablation in tumors enables immune control
- COX inhibition synergizes with checkpoint blockade therapy
- A COX inflammatory signature is conserved across mouse and human cancer biopsies

COX-deficient tumor

COX-sufficient tumor

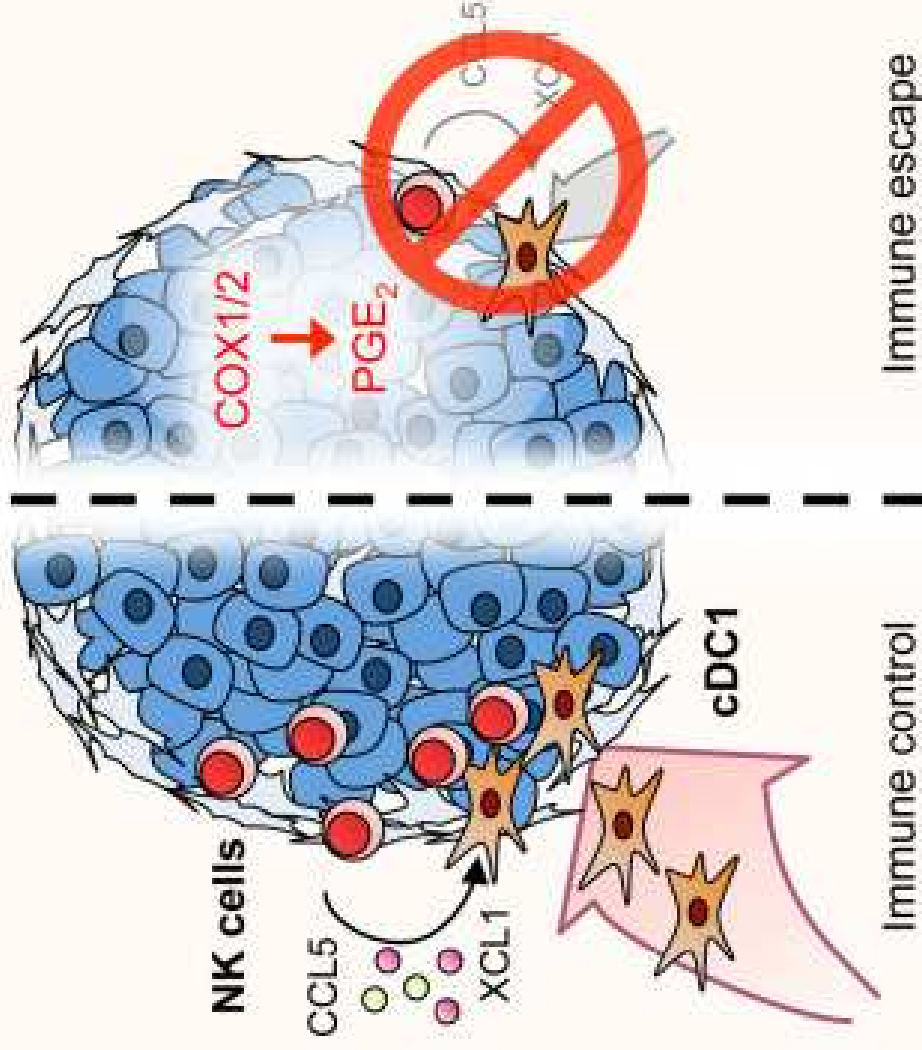
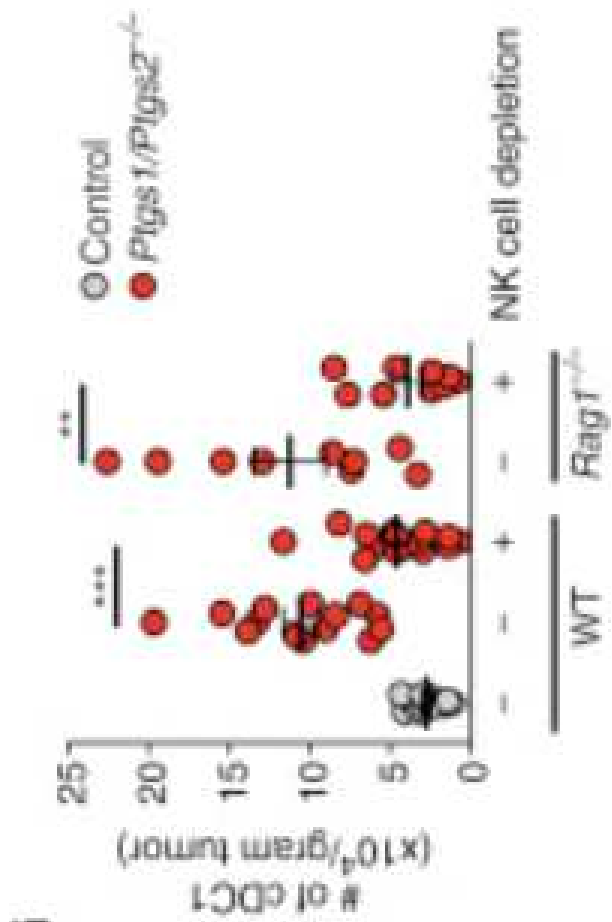


Figure 2. Intratumoral cDC1 Accumulation Depends on NK Cells



Plasmacytoid DC producers of IFN-I

Table 1. The phenotype and functions of mouse and human DC subsets.

	Mouse DC Subsets		Human DC Subsets	
	Phenotype	Functions	Phenotype	Functions
pDC	CD45R, CD45RA, CD317	Anti-viral immunity Tolerance induction	CD123, CD303, CD304, CD45RA	Anti-viral immunity Tolerance induction
cDC1	CD8 α or CD103, DEC205, Clec9A, XCR1	MHC I cross-presentation	CD141, DEC205, Clec9A, XCR1	MHC I cross-presentation MHC II presentation
cDC2	CD11b, Sirp α	MHC II presentation	CD1c, CD1a (skin), CD103 (mucosa)	MHC I cross-presentation MHC II presentation
Inf-DC	F4/80, Ly6C, CD64, Fc ϵ R1	MHC I cross-presentation MHC II presentation	CD1c, CD1a, Fc ϵ R1, CD14, CD206	MHC I cross-presentation Th17 induction

While human cDC1 are superior at cross-presentation of cell-associated antigens, both cDC1 and cDC2 have been shown to cross-present soluble antigens at comparable levels. Human cDC1 and cDC2 are equally competent at MHC II presentation and CD4+ T cell priming

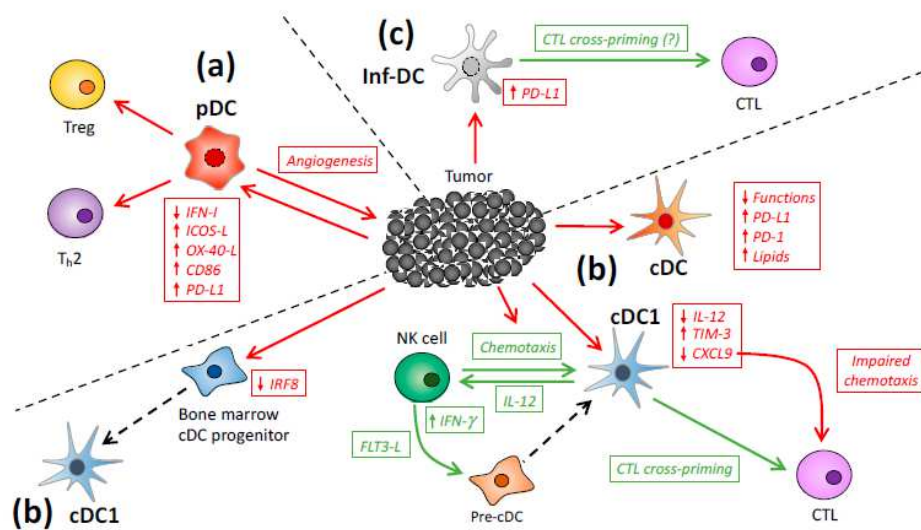


Figure 1. The biology of DC in the tumor microenvironment: (a) pDC; (b) cDC; and (c) inf-DC subsets infiltrate the tumor microenvironment and either support the anti-tumor immune response or promote tumorigenesis. Tumors frequently develop strategies to alter DC development, tumor infiltration and function. The mechanisms that promote anti-tumor immunity are shown in green, while those that act to promote tumorigenesis are displayed in red.

←

→

↺

↻

http://www.transplanttoolbox.org/


TransplantToolbox

ALLAN

VICTOR

CPRA Calculator

Gragert Lab



TransplantToolbox

Informatics Tools for HLA Data in Solid Organ Transplantation

ALLAN

Map Molecular HLA Typing Data to UNOS Serologic Equivalents

CLICK HERE !

VICTOR

Compute Virtual Crossmatch for Molecular HLA Typing Data

CLICK HERE !

CPRA Calculator

Compute CPRA with NMDP Allele Frequencies Data and Allele Specific Unacceptables

CLICK HERE !

ALLAN: IPD-IMGT/HLA ALLELE to UNOS ANTIGEN conversion tool

Automatically convert molecular HLA typing data into UNOS antigen equivalents for entry into UNet

IPD-IMGT/HLA release 3.35.0

x

HLA Typing Data Input Formats (choose one):

Unambiguous HLA Typing

Single Allele

Alleles List

Ambiguous HLA Typing

Genotype List String

NMDP Multiple Allele Codes

Reverse Mapping

Convert a UNOS Antigen to IPD-IMGT/HLA Alleles List

VICTOR: Virtual CrossmaTch for mOleculaR typing data

This prototype tool computes virtual crossmatch using the candidate's unacceptable antigens including high resolution alleles and molecular HLA typing of the donor.

IPD-IMGT/HLA release 3.35.0

X

Virtual Crossmatch Algorithm and Donor HLA Typing Input Format (choose one:)

Current UNOS Match Run Logic

UNOS Antigen Equivalents High Resolution Alleles

Proposed Algorithm for Interpreting Ambiguous HLA Typing

UNOS Antigen Equivalents Genotype List Strings NMDP Multiple Allele Codes

Donor HLA Typing as Unambiguous High Resolution HLA Alleles

Enter Donor's HLA typing data as a list of HLA alleles. Alleles entered should be resolved to specific HLA protein field (2nd field) or fully resolved (full IMGT/HLA allele name). Protein expression specifying characters ("L", "N", "S", "Q") can be used. Characters that specify antigen recognition domain equivalents ("G", "P", "g") can also be used. HLA loci supported are A, B, C, DRB1, DQA1, DQB1, DRB3/4/5. Enter Candidate's Unacceptable Antigens in the second text area and click on "SUBMIT" to run Virtual Cross Match.

Donor's HLA Typing as Alleles

A*03:01 A*24:02 B*08:01 B*41:01 C*07:02 C*17:01 DRB1*13:01 DRB1*15:01 DRB3*01:01 DRB5*01:01 DQB1*06:02 DQB1*06:03

Candidate's Unacceptable Antigens

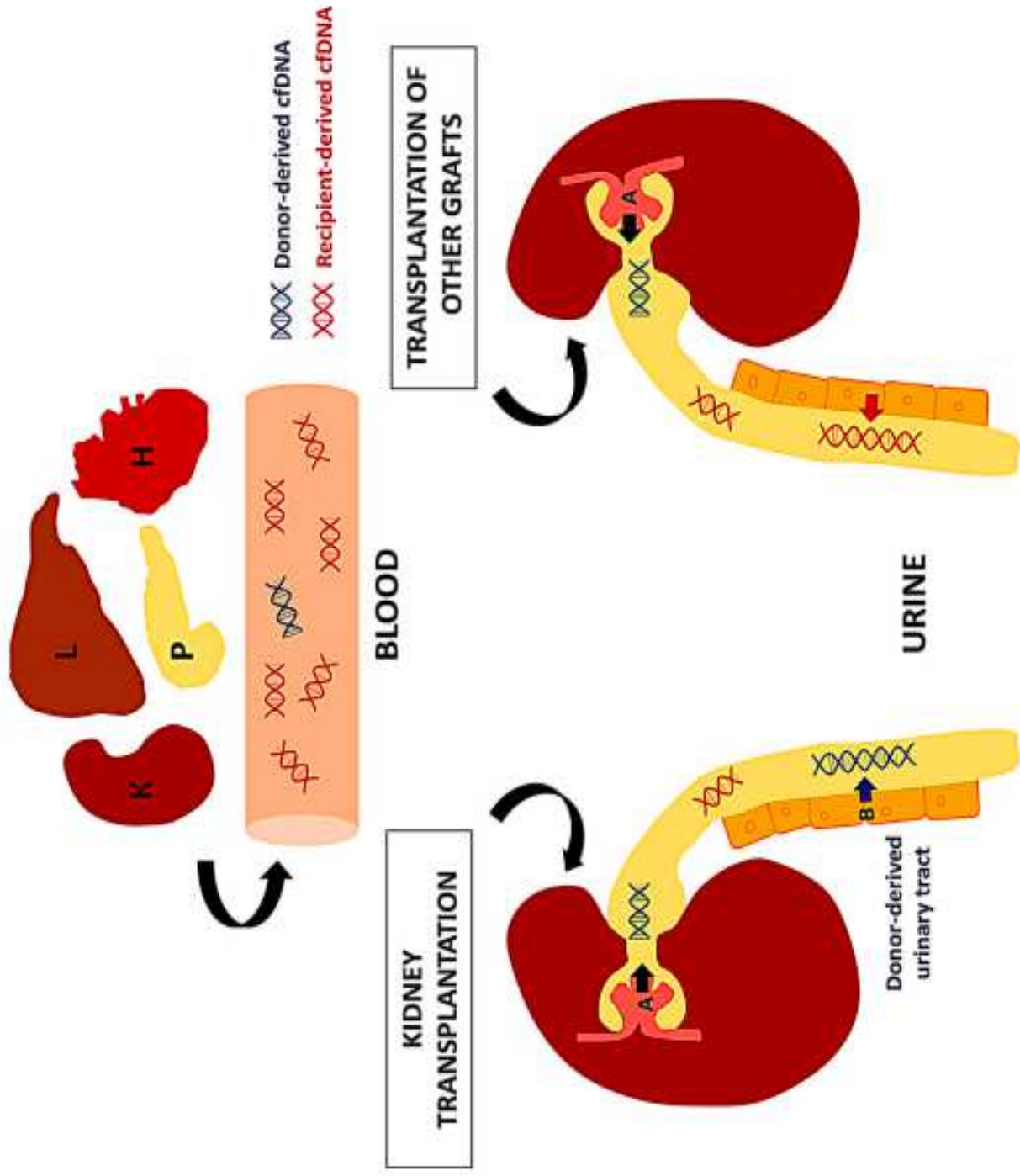
B18 B35 B39 B51 B53 B57 B58 B71 B75 B76 B77 B78 DR7 DR12 A*02:03 B*44:03 DRB1*09:01 DRB1*14:01 DRB3*01:01 DRB3*03:01 DQB1*05:01

SUBMIT

Virtual Crossmatch results using VICTOR algorithm

Donor Typing as Unambiguous HLA Alleles

Locus	Donor's HLA Alleles	Donor's UNOS Equivalents	Donor Specific Antibodies	OPTN Equivalents of Unacceptable Antigens	Candidate's Unacceptable Antigens
A	A*03:01, A*24:02	A24, A3		A*02:03	A*02:03
B	B*08:01, B*41:01	B41, B8	B8	B*3905, B*44:03, B18, B39, B3901, B3902, B51, B53, B57, B58, B71, B75, B76, B77, B78, B8	B*44:03, B18, B39, B51, B53, B57, B58, B71, B75, B76, B77, B78, B8
Bw		Bw6			
C	C*07:02, C*17:01	C07, C07			
DR	DRB1*13:01, DRB1*15:01	DR13, DR15		DR12, DR7, DRB1*09:01, DRB1*14:01	DR12, DR7, DRB1*09:01, DRB1*14:01
DRB3/4/5	DRB3*01:01, DRB5*01:01	DR51, DR52	DRB3*01:01	DRB3*01:01, DRB3*03:01	DRB3*01:01, DRB3*03:01
DQ	DQB1*06:02, DQB1*06:03	DQ6, DQ6		DQB1*05:01	DQB1*05:01



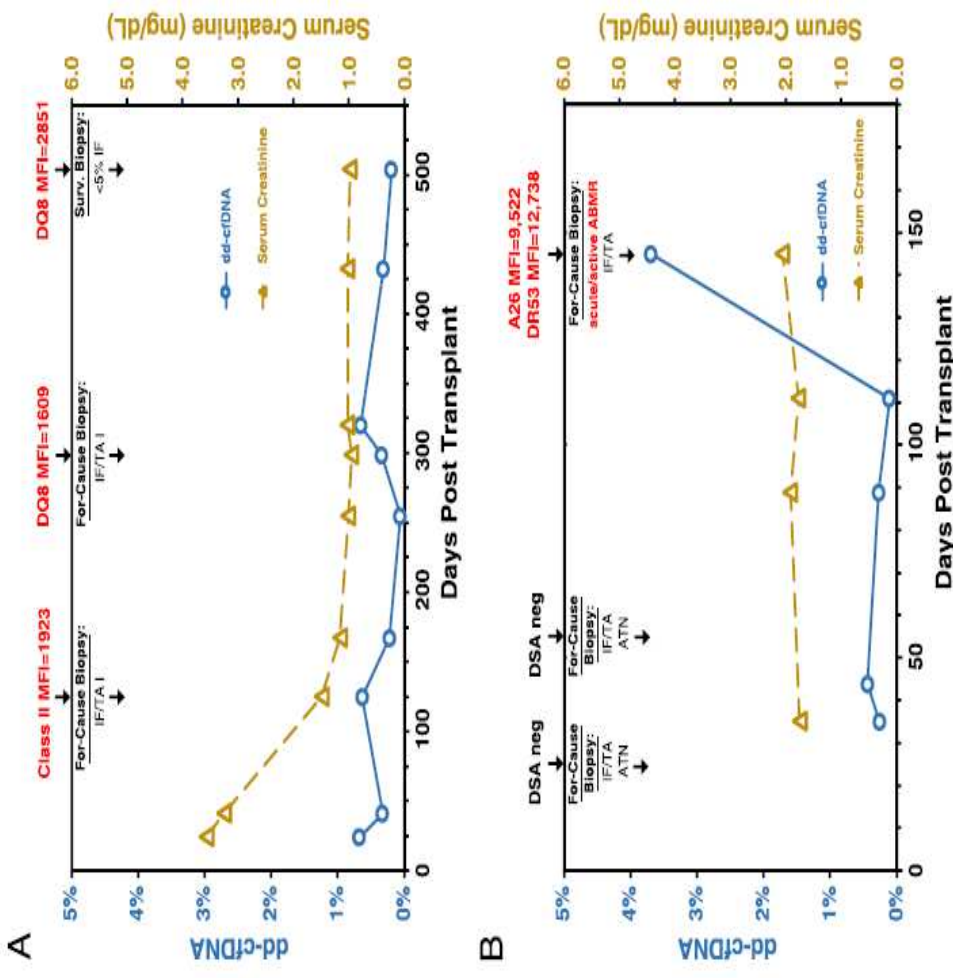


FIGURE 2. Case studies. A, This patient had 3 consecutive DSA+ results and no ABMR found in associated biopsies. Serial dd-cfDNA were all negative (below 1%). B, A significant rise in dd-cfDNA was associated with acute/active ABMR in a renal allograft recipient. dd-cfDNA level (3.7%) on day 145 posttransplantation significantly increased compared to 0.2% on day 111. Serum creatinine rose from 1.77 to 2.06. Allograft biopsies on posttransplant days 30 and 60 (see arrows) revealed mild IF/TA and focal areas of ATN. Biopsy on day 145 revealed ABMR. DSAs were neg at days 30 and 60 and pos on day 145. IF/TA, interstitial fibrosis/tubular atrophy; ATN, acute tubular necrosis; negative, neg; positive, pos.

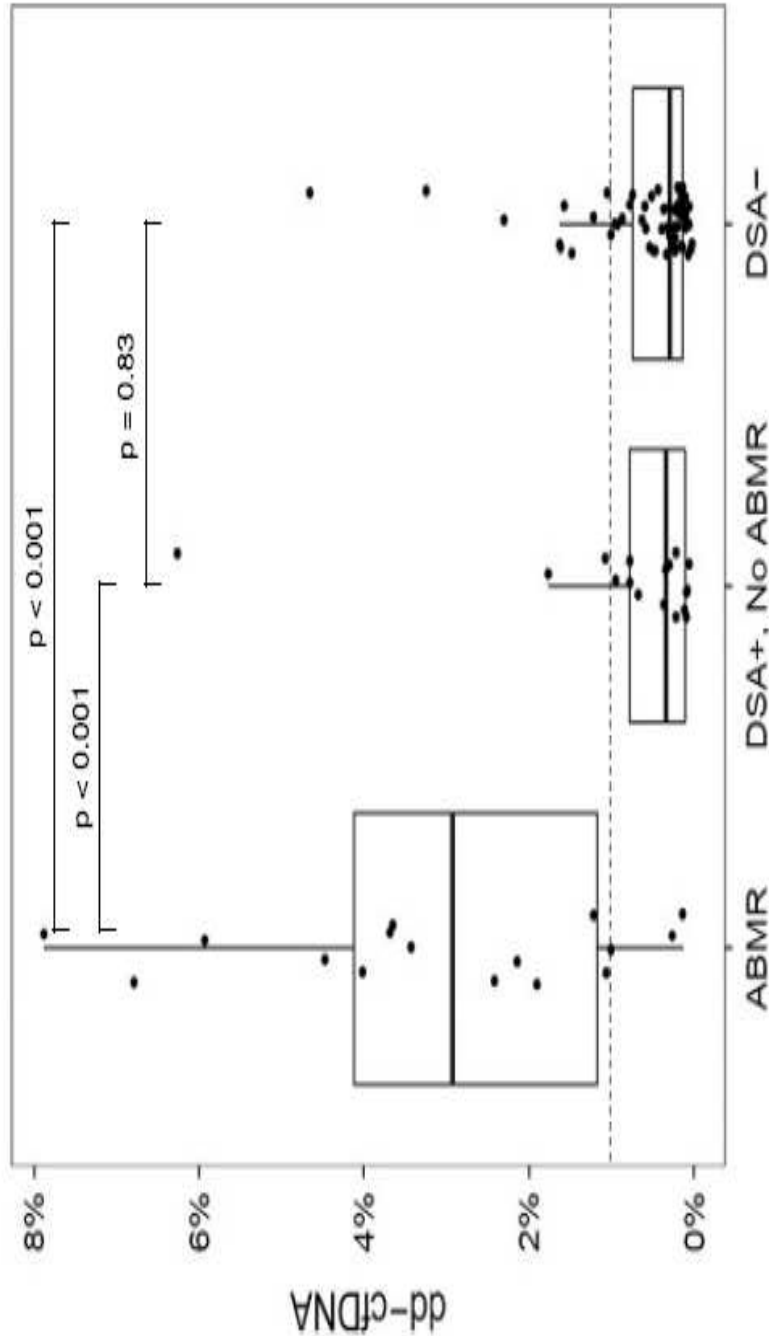
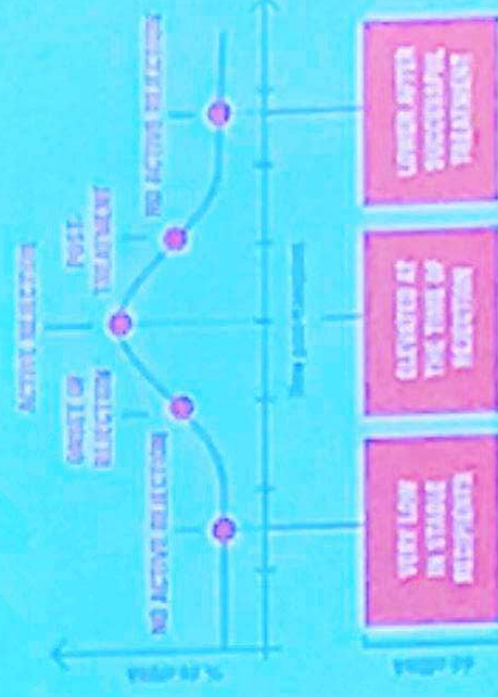


FIGURE 1. dd-cfDNA level is significantly higher in patients with active ABMR. Patients with positive DSA and a biopsy diagnosis of active ABMR had significantly elevated dd-cfDNA when compared to DSA-positive patients who are biopsy negative for ABMR (DSA+, No ABMR). dd-cfDNA for DSA- samples are not significantly different from DSA+, No ABMR. TCMR are included in the No ABMR and DSA- data. Medians are shown by the horizontal bar, 25th and 75th percentiles (interquartile range) are shown as the top and bottom of the boxes and are 2.9 [1.18-4.13] for active ABMR; 0.34 [0.11-0.78] for DSA+, No ABMR; and 0.29 [0.14-0.74] for the DSA-.

Summary

- dd-cfDNA is a non-invasive marker of allograft injury in solid organ transplant recipients
- Stable kidney transplant recipients have low levels of dd-cfDNA
- High levels of dd-cfDNA are associated with rejection
- The combined use of dd-cfDNA and DSA may improve the non-invasive diagnosis of active ABMR



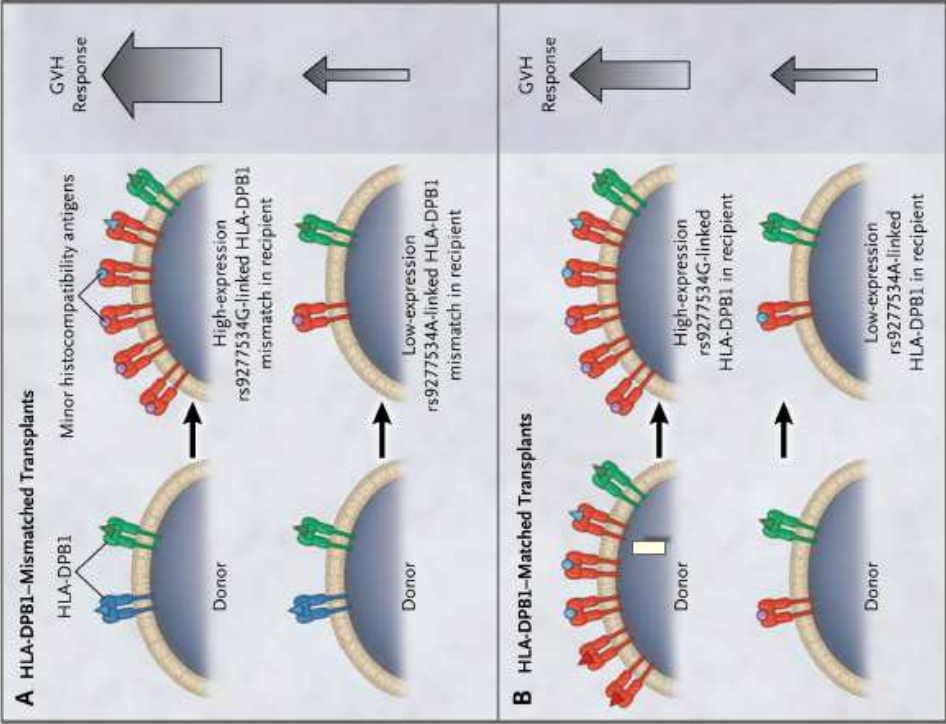


Figure 1. Proposed Schema for the Role of HLA-DPB1 Expression in Graft-versus-Host Recognition

In the context of an HLA-DPB1 mismatch between a recipient and a donor (Panel A), an rs9277534G-linked (high-expression) mismatch is hypothesized to be a viable target for donor-mediated graft-versus-host (GVH) recognition. Red HLA molecules represent the mismatched HLA-DPB1 in the recipient, blue molecules represent the donor's mismatched HLA-DPB1, and green molecules are shared (matched) between the recipient and the donor. Black arrows pointing from the donor toward the recipient represent the GVH vector of allorecognition. The size of the upward-pointing gray arrows indicates the magnitude of the

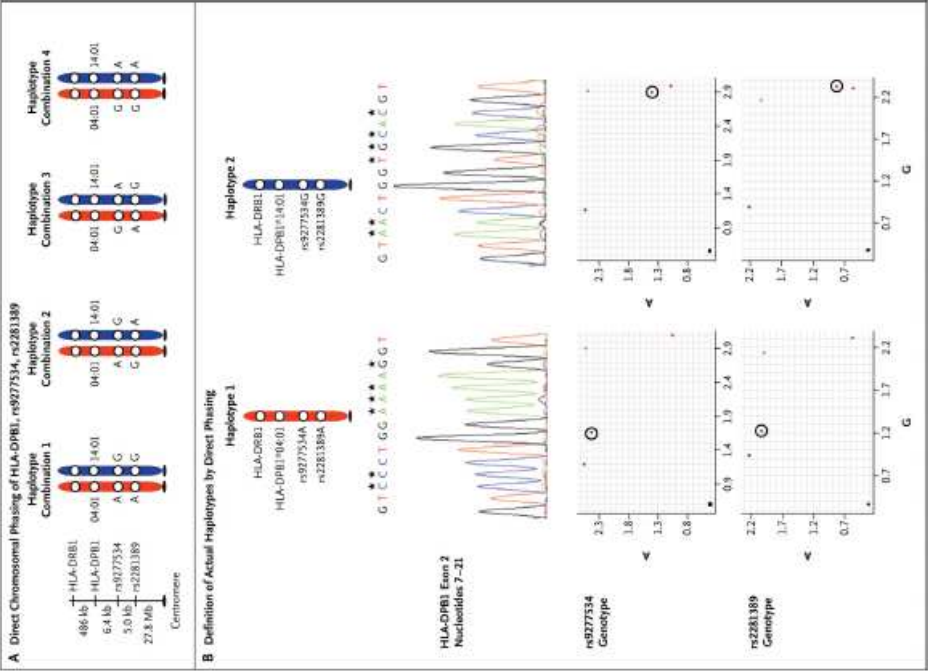


Figure 2. Direct Chromosomal Phasing of HLA-DPB1, rs9277534, rs2281389

A person with the genotype DPB1*04:01.1:4:01; rs9277534G; rs2281389A is heterozygous at all three genetic markers. The diploid genotype (both chromosomes together) can be produced by four theoretical haplotypes (Panel A). The physical linkage of HLA-DPB1, rs9277534, and rs2281389 alleles on each haplotype in a diploid sample requires separation of the chromosomes (a process known as phasing) (Panel B). The DNA was phased with the rs9277534A probe (red chromosome) and the rs2281389A probe (blue chromosome). The haploid DNA (one chromosome by itself) captured by the rs9277534A

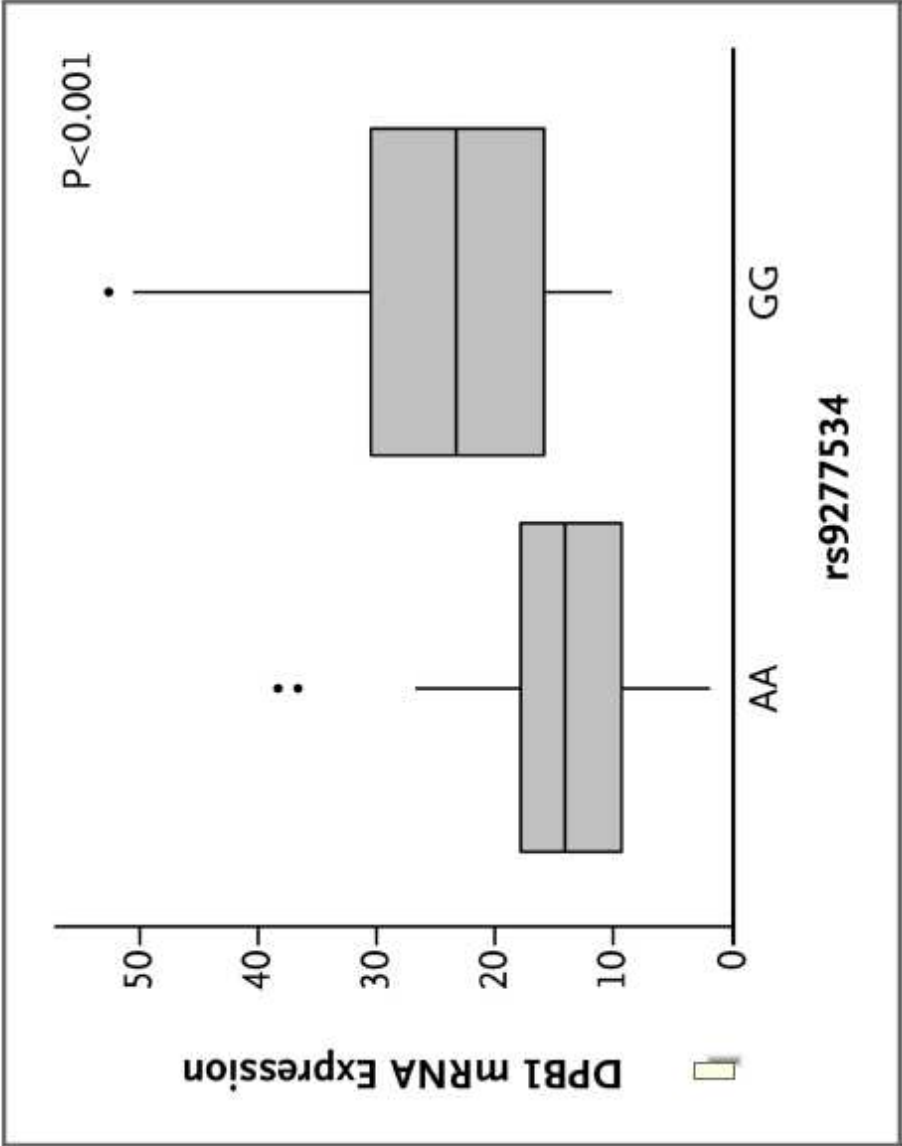
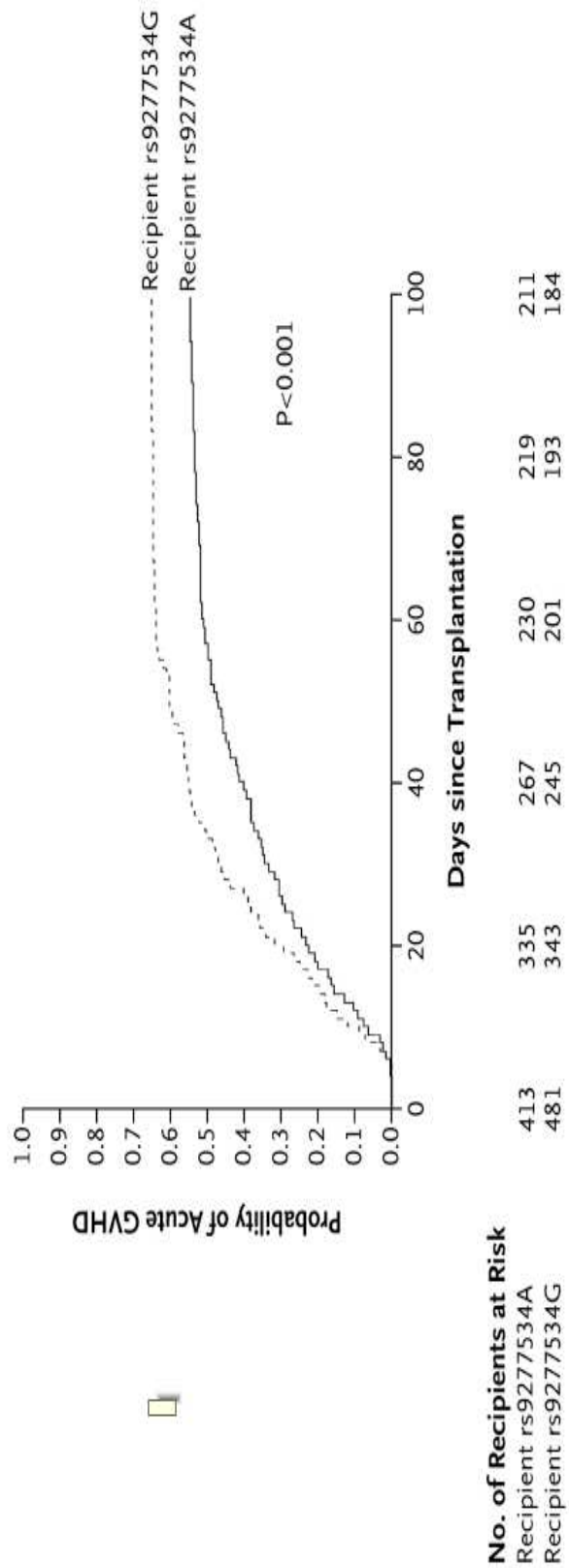
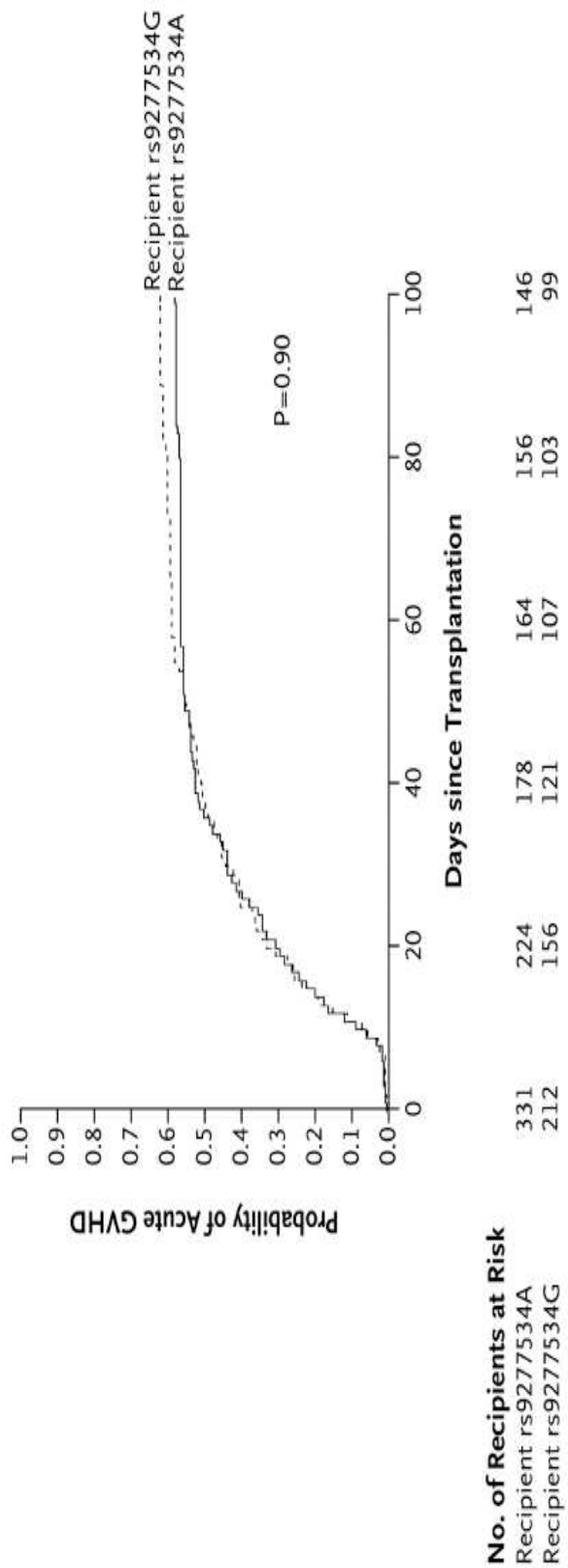


Figure 3. Correlation of HLA-DPB1 Expression with the rs9277534 Allele in the 3' Untranslated Region of HLA-DPB1
HLA-DPB1 messenger RNA (mRNA) expression was determined by means of a quantitative polymerase-chain-reaction assay (TaqMan Gene Expression assay) in 81 persons: 49 with the rs9277534AA genotype and 32 with the rs9277534GG genotype. The data are presented as normalized individual data points (2^{-deltaCt} [delta Ct = Ct gene of interest - Ct endogenous control]) in box-and-whisker plots. The horizontal line within each box indicates the median expression value; vertical lines indicate the smallest and the largest non-outliers in these data.

A Donor rs9277534A



B Donor rs9277534G



Conclusions and outlook

- HLA-DP mismatches: **structural and expression** level models highly overlapping
- Differences in **expression up to 8x**: no impact on in vitro alloreactive response levels
- **Peptide repertoire deregulation** significantly increases alloreactive responses
- Structurally-defined peptide repertoires: main force driving **permissiveness** in in vitro model
- **In vivo models** should address role of HLA-DP expression levels
 - Differences in expression abrogated by IFN- γ

IdeS (Imlifidase): A Novel Agent That Cleaves Human IgG and Permits Successful Kidney Transplantation Across High-strength Donor-specific Antibody

Objectives: The presence of a donor-specific positive crossmatch has been considered to be a contraindication to kidney transplantation because of the risk of hyperacute rejection. Desensitization is the process of removing hazardous preformed donor-specific antibody (DSA) in order to safely proceed with transplant. Traditionally, this involves plasmapheresis and intravenous immune globulin treatments that occur over days to weeks, and has been feasible when there is a living donor and the date of the transplant is known, allowing time for pre-emptive treatments. For sensitized patients without a living donor, transplantation has been historically difficult.

Summary of Background Data: IdeS (imlifidase) is an endopeptidase derived from *Streptococcus pyogenes* which has specificity for human IgG, and when infused intravenously results in rapid cleavage of IgG.

Methods: Here we present our single-center's experience with 7 highly sensitized (cPRA98–100%) kidney transplant candidates who had DSA resulting in positive crossmatches with their donors (5 deceased, 2 living) who received IdeS within 24 hours prior to transplant.

Results: All pre-IdeS crossmatches were positive and would have been prohibitive for transplantation. All crossmatches became negative post-IdeS and the patients underwent successful transplantation. Three patients had DSA rebound and antibody-mediated rejection, which responded to standard of care therapies. Three patients had delayed graft function, which ultimately resolved. No serious adverse events were associated with IdeS. All patients have functioning renal allografts at a median follow-up of 235 days.

Conclusion: IdeS may represent a groundbreaking new method of desensitization for patients who otherwise might have no hope for receiving a lifesaving transplant.



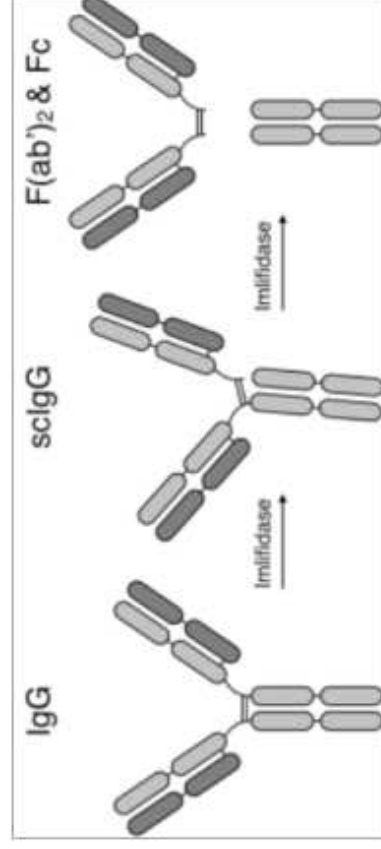
Imlifidase: IgG-degrading enzyme of *S. pyogenes* (IdeS)



- IgG-specific (not IgM, IgA, IgD or IgE), human and rabbit IgG
- Cleaves all forms of IgG: free, bound to antigen and B-cell receptor (IgG-type)
- A two-step cleavage reaction
 - 1st heavy chain cleaved: single cleaved IgG (scIgG) – limited by ADA
 - 2nd heavy chain cleaved: one F(ab')₂ and one dimeric Fc fragment – limited by concentration

- Imlifidase treatment inhibits Fc-mediated activities

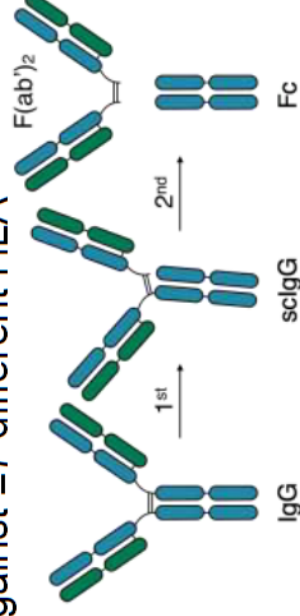
- IgG mediated CDC
- IgG mediated ADCC
- IgG mediated phagocytosis



Phase IIa study

A phase II, single arm, ascending dose, single center study to evaluate the *safety, tolerability, pharmacokinetics* and *efficacy* of intravenous *IdeS* after administration of ascending doses in chronic kidney disease

- Site: Uppsala University Hospital, Sweden
- Patients: 8 chronic kidney disease patients on dialysis, one patient received a kidney transplant
- Patients had antibodies with MFI >3000 against ≥ 7 different HLA



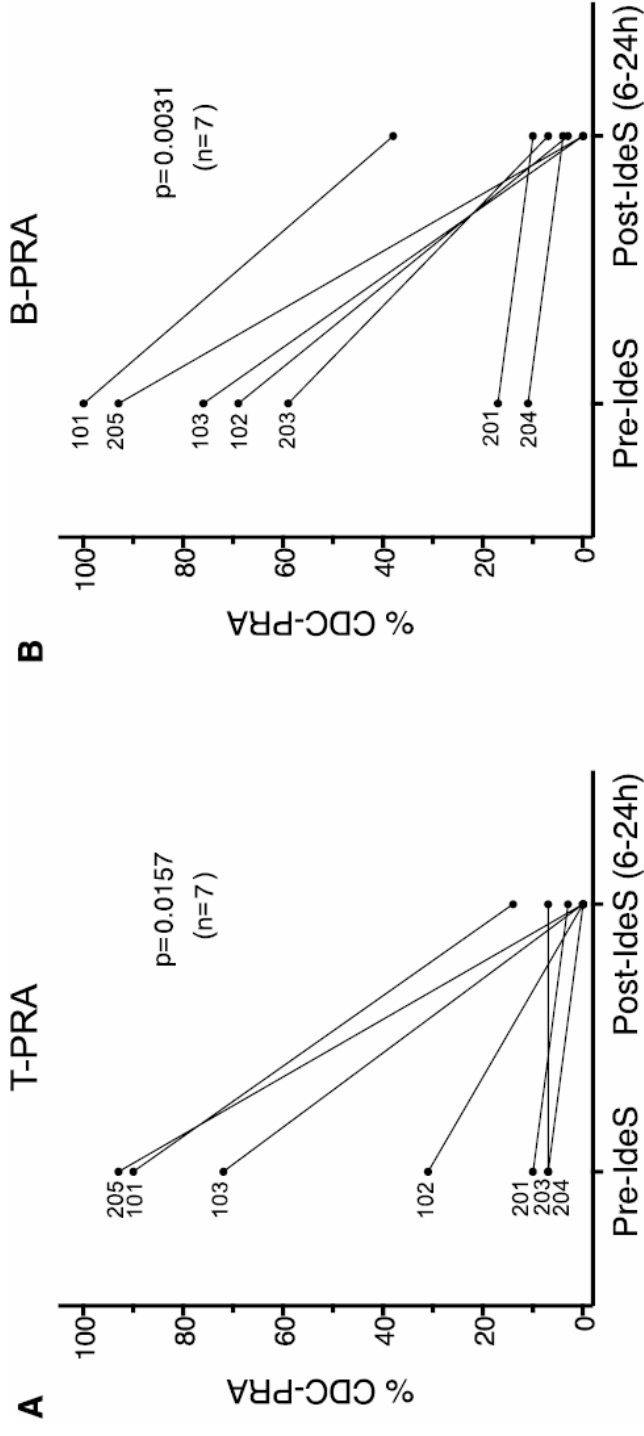


UPPSALA
UNIVERSITET

cPRA after imlifidase treatment



AKADEMISKA
SJUKHUSET



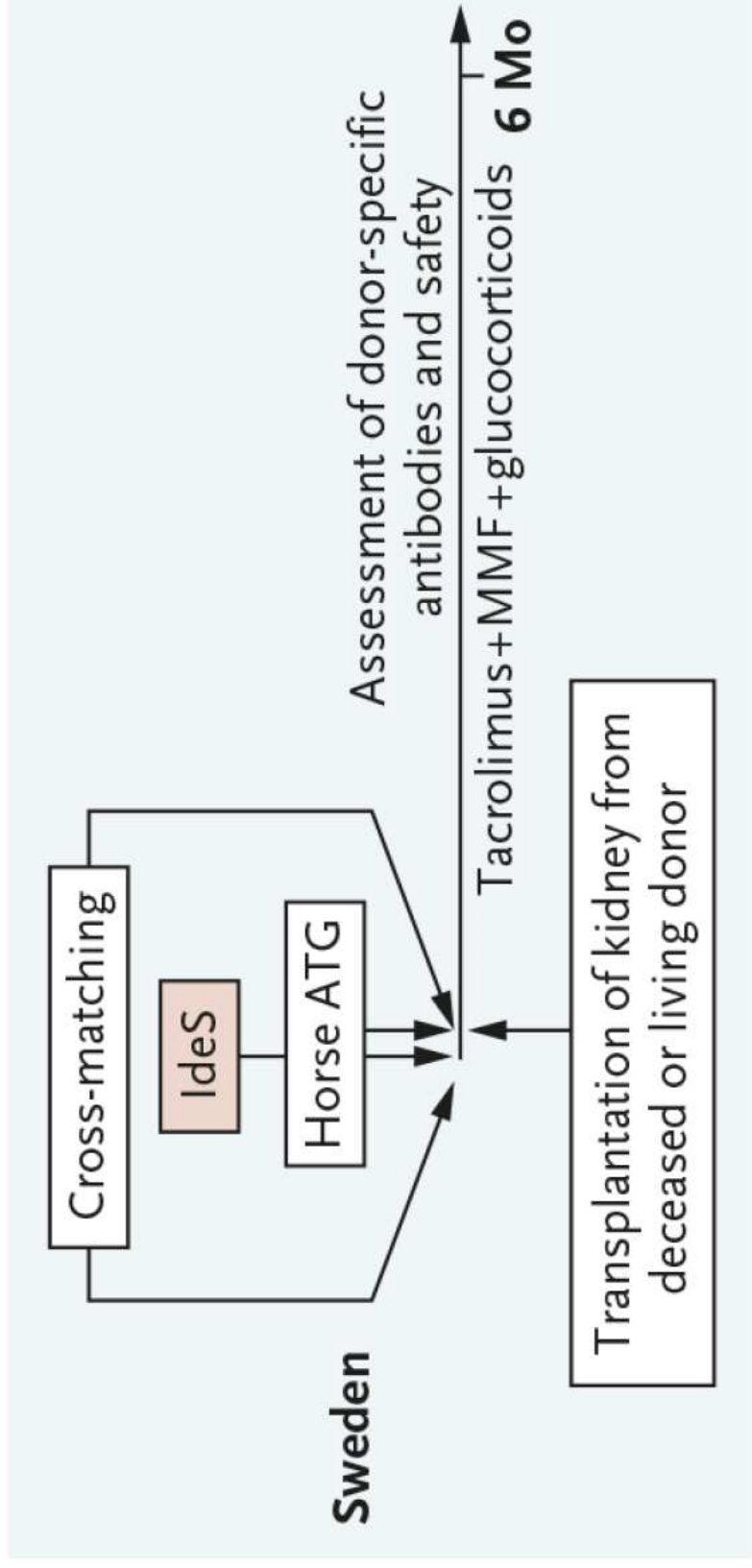
Lorant et al. AJT. 2018.

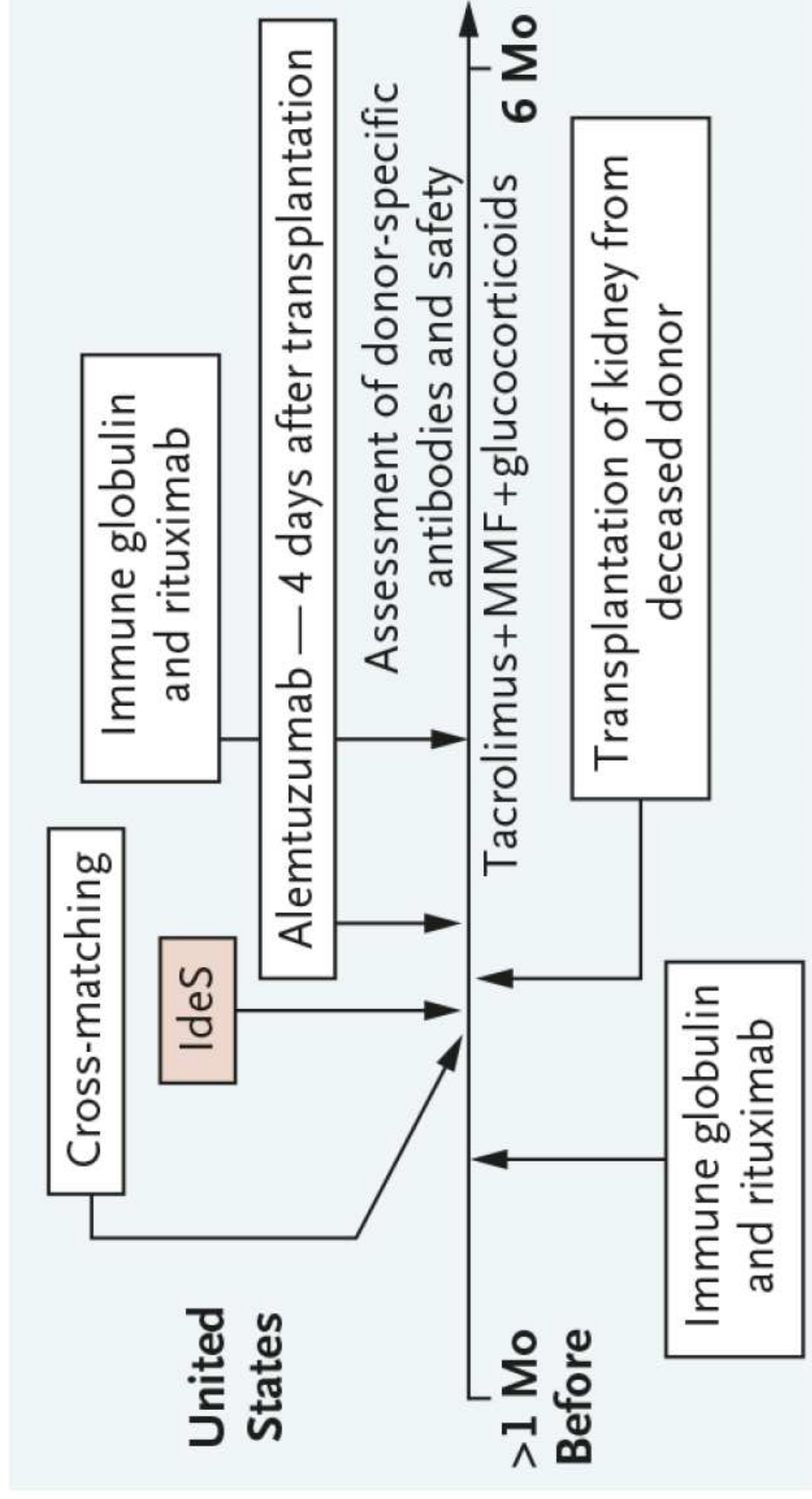


IgG Endopeptidase in Highly Sensitized Patients Undergoing Transplantation

S.C. Jordan, T. Lorant, J. Choi, C. Kjellman, L. Winstedt, M. Bengtsson, X. Zhang,
T. Eich, M. Toyoda, B.-M. Eriksson, S. Ge, A. Peng, S. Järnum, K.J. Wood,
T. Lundgren, L. Wennberg, L. Bäckman, E. Larsson, R. Villicana, J. Kahwaji,
S. Louie, A. Kang, M. Haas, C. Nast, A. Vo, and G. Tufveson

Immunosuppressive Regimens





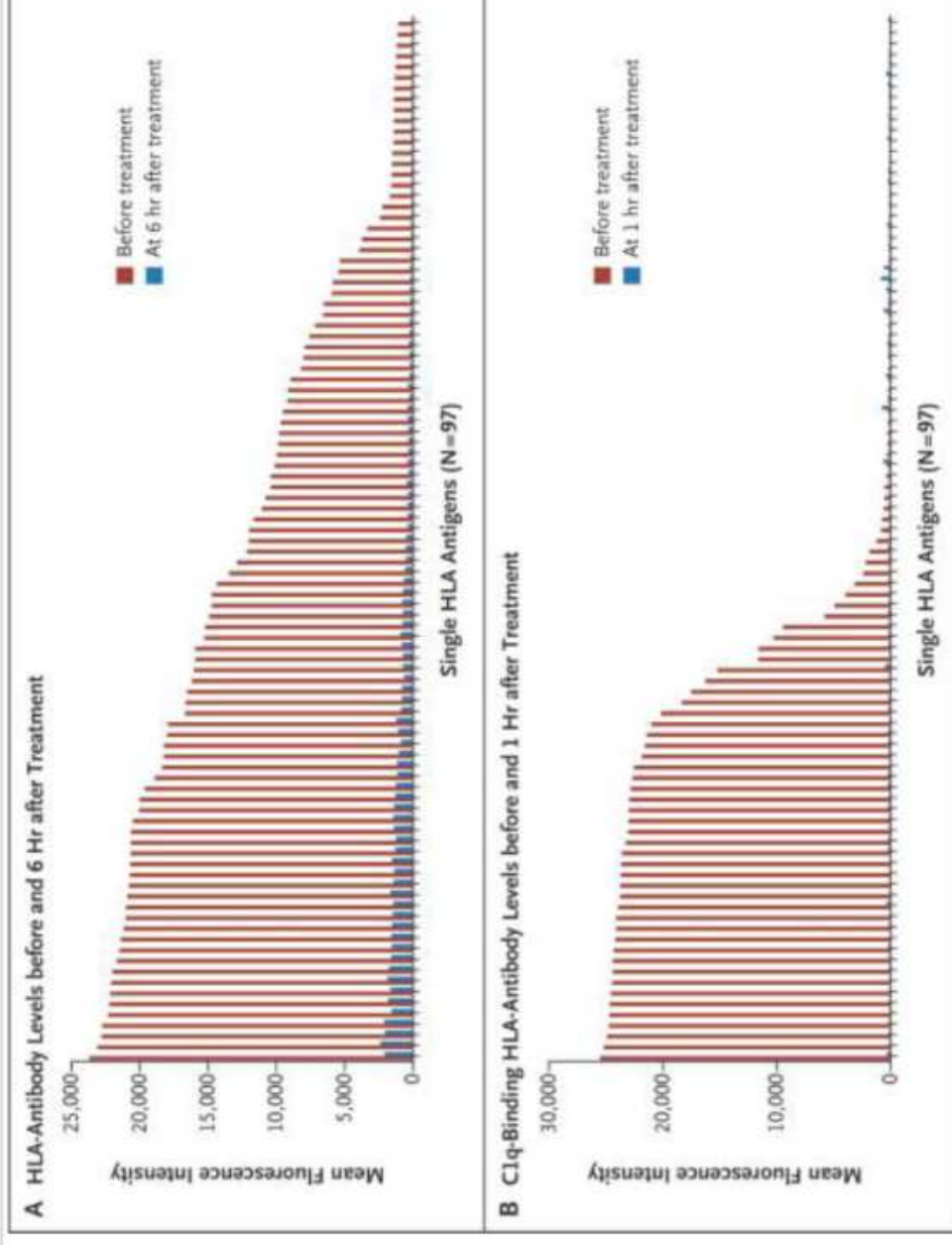


Effect of IdeS on cross match in highly sensitized patients

- 20 of 25 patients had a positive cross match prior to transplantation
- All cross matches turned negative within 2 hours.
- 24 patients were successfully transplanted
- 1 immediate graft loss due to IgM-mediated rejection

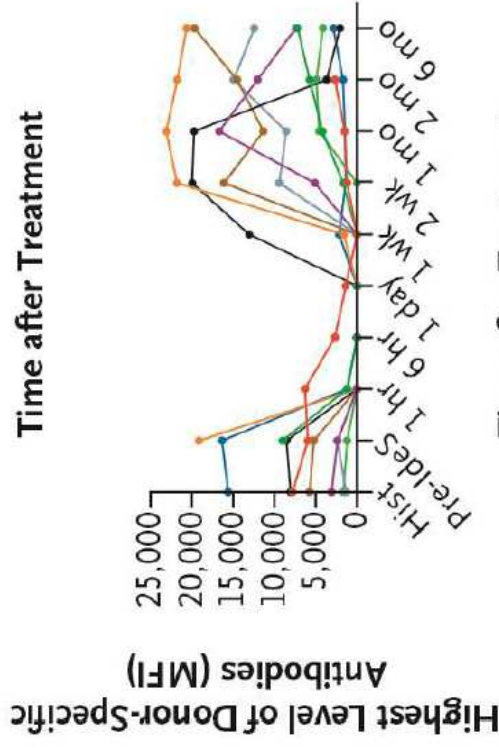
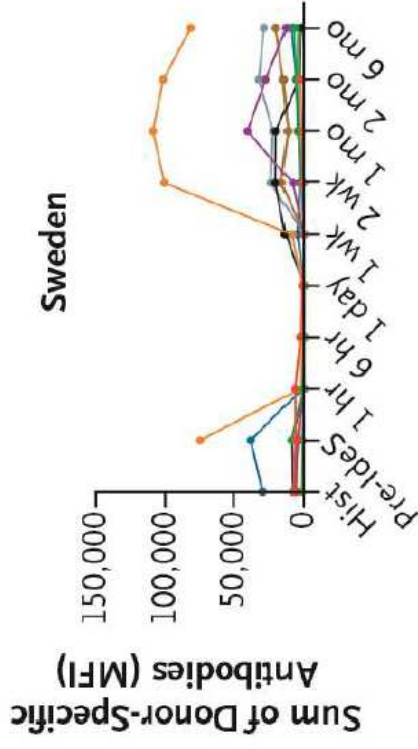


Levels of HLA Antibodies and C1q-Binding HLA Antibodies after IdeS Administration





Donor-specific antibody levels in individual patients



B	C	D	E
Section	Standard version 7	Draft standard	Draft Standard v8
A12.2.1	Must be accredited by EFI or by ASHI, if the testing is covered by EFI standards		
A12.2.2	Should have documented expertise and/or accreditation in genetic systems not covered by EFI Standards		
		A13	Partial Testing In Other Laboratories
		A13.1	An accredited laboratory may engage another laboratory to perform part of the testing i.e. core
		A13.2	When core facilities are used:
		A13.2.1	They must fulfill relevant standards
		A13.2.2	Facilities must be available for inspections
- PERSONNEL QUALIFICATIONS			
B1	For the purposes of this document, EFI defines the Director as the person who is responsible for the H&I laboratory		For the purposes of this document, EFI defines the Director as the person who is responsible for the H&I laboratory activities for which accreditation is applied for
B2	The laboratory must employ one or more individuals who meet the qualifications and fulfil the responsibilities of:		
B3	The Director and/or Co-Director		
B3.1	A Director, that must:		
B3.1.1	Hold a qualification approved by EFI, such as an ESHI or national diploma, earned doctoral degree in a biological science, or be a physician, and		
B3.1.2	Have minimum qualifying experience equivalent to either of the following:		
B3.1.2.1	Four years* relevant experience two of which were devoted to full time training in human H&I testing, or		
B3.1.2.2	Four years of working experience at full time in human H&I testing		
B3.1.2.3	Additional qualifications required according to national legislation also apply		
		B3.1.2.4	For chimaerism, KIR, HPA, HNA two years of working experience at full time
B3.1.3	Have documentation of professional competence in the appropriate activities in which the laboratory is engaged. This should be based on sound knowledge of the fundamentals of immunology, genetics and histocompatibility testing		Have documentation of professional competence in the appropriate activities in which the laboratory is engaged. This should be based on sound knowledge of the fundamentals of immunology, genetics and histocompatibility testing as appropriate to the areas in which accreditation is sought
B3.1.4	If a Co-Director is appointed, this person must also fulfil Standards B1.1.1 - B1.1.3		If a Co-Director is appointed, this person must also fulfil Standards B3.1.1 - B3.1.3
B3.1.5	The Director and/or Co-Director must:		

Section	Standard version 7	Draft standard	Draft Standard v8
B3.1.5.4	Be held responsible for the proper performance, interpretation and reporting of all laboratory procedures		
B3.1.5.5	Ensure the laboratory's successful participation in proficiency testing		
B3.1.5.6	Be informed of the relevant national legislation and professional standards		
B3.1.5.7	Comply with the relevant national legislation and professional standards		
B3.1.5.8	Demonstrate active participation in H&I related clinically relevant professional development, such as national or international conferences or workshops		Demonstrate active participation in clinically relevant professional development, such as national or international conferences or workshops
B3.1.6	The Director or Co-Director should:		
B3.1.6.1	Have publications in peer-reviewed journals		
B4	Technical Staff		
B4.1	A Technical Supervisor, that must:		
B4.1.1	Have minimum qualifying experience equivalent to either of the following:		
B4.1.1.1	Hold a bachelor's degree or equivalent and have three years' relevant experience in human histocompatibility and immunogenetics testing under the supervision of a qualified Director or Co-Director		Hold a bachelor's degree or equivalent and have three years' relevant experience in the areas for which accreditation is sought under the supervision of a qualified Director or Co-Director
B4.1.1.2	Five years of supervised experience if a bachelor's degree has not been earned		
		B4.1.1.3	One year of supervised experience for chimaerism, KIR, HPA, HNA
B4.2	A Quality Manager, who must establish and maintain a comprehensive quality management programme covering all aspects for the accredited facility, addressed by		

Section	Standard version 7	standard	Draft Standard v8
B4	Technical Staff		
B4.1	A Technical Supervisor, that must:		
B4.1.1	Have minimum qualifying experience equivalent to either of the following:		
B4.1.1.1	Hold a bachelor's degree or equivalent and have three years' relevant experience in human histocompatibility and immunogenetics testing under the supervision of a qualified Director or Co-Director		Hold a bachelor's degree or equivalent and have three years' relevant experience in the areas for which accreditation is sought under the supervision of a qualified Director or Co-Director
B4.1.1.2	Five years of supervised experience if a bachelor's degree has not been earned		
		B4.1.1.3	One year of supervised experience for chimaerism, KIR, HPA, HNA
B4.2	A Quality Manager, who must establish and maintain a comprehensive quality management programme covering all aspects for the accredited facility addressed by these standards		
B4.3	The resources of the laboratory must be sufficient to accommodate the workload		
B5	Competency Evaluation and Continuous Education		
B5.1	The Director/Co-Director or designee must:		
B5.1.1	Evaluate the competence of each technologist to accurately perform tests. This must be done at least yearly for each technique the technologist performs and must be based on a defined process		
B5.1.2	Maintain records of these evaluations for each individual		
B5.2	The Laboratory Director and the technical staff must participate in continuing education relating to each category for which FFI accreditation is sought		

Section	Standard version 7	Draft standard	Draft Standard v8
E2.8.2.1.3	Cleaning must be documented		
E2.8.2.2	Maintenance		
E2.8.2.2.1	The instrument must be maintained according to manufacturer's instructions, but at least once a year		
E2.9	Sequencer		
E2.9.1	Sequencing platform and the version of associated software, reagents and accessories must be specified		
E2.9.2	Automated systems and sequencing devices must be cleaned, calibrated and maintained according to manufacturer's instructions		
		E2.10	Real Time PCR systems
		E2.10.1	Accuracy of the instruments:
		E2.10.1.1	Must be verified by annual thermal verification of the block using a calibrated device designed specifically for this purpose
		E2.10.1.2	Calibration of the fluorescence detection must be performed at least annually
E3	COMPUTER ASSISTED ANALYSES		
E3.1	The Laboratory Director and/or the Supervisor must		

Section	Standard version 7	Draft standard	Draft Standard v8
E4.12	Analysis of Other Immunologic Markers		
E4.12.1	HPA Typing		
E4.12.1.1	HPA typing must be performed using a validated HPA typing technique. If typing is performed using DNA based methods, standards in sections C2.1.3 to C2.1.6 apply		
E4.12.1.2	Clinically significant HPA specificities must be defined and documented		
E4.12.2	Investigation of HPA antibodies		
E4.12.2.1	For bead array techniques, relevant standards from section E2.8 also apply		
E4.12.2.2	For ELISA based assays, standards in sections E2.2, E2.3 and E4.3 also apply		
E4.12.2.3	Laboratories must make all reasonable efforts to include HPA antigens in their antibody screening protocol which will aid the identification of clinically significant HPA alloantibodies		Laboratories must make all reasonable efforts to include HPA antigens in their antibody testing protocol which will aid the identification of clinically significant HPA alloantibodies
E4.12.2.4	The antibody screening technique must		The antibody testing technique must
E4.12.2.4.1	Be validated before use		
E4.12.2.4.2	Include positive and negative controls in each assay		
E4.12.2.4.3	In glycoprotein specific assays, a positive control for each glycoprotein used		

Section	Standard version 7	Draft standard	Draft Standard v8
E5.3.1.1.4	Level of resolution		
E5.3.1.1.5	Which party takes responsibility of the histocompatibility component of the transplant		
E5.3.1.1.6	The transplant agreement must be signed by all parties		
E5.3.2	Histocompatibility testing for related transplants		
E5.3.2.1	HLA-A, B or DR typing must be carried out on available members of the immediate family		
E5.3.2.2	Must include adequate testing:		
E5.3.2.2.1	To definitively establish HLA genotype identity (F1.3.2 applies), or		
E5.3.2.2.2	To type at high resolution for the relevant loci defined in the transplant protocol, if only phenotype identity has been established, or		
E5.3.2.2.3	To include high resolution typing for recipient and potential intra-familial donors who are not HLA identical siblings		
		E5.3.2.2.4	HLA typing for haploidentical donors
		E5.3.2.2.4.1	Identity of the shared haplotype must be defined by descent
		E5.3.2.2.4.2	If the identity of the shared haplotype can not be proven a high resolution typing must be performed
E5.3.2.3	HLA-A, B and DR typing as a minimum requirement must be repeated on both the recipient and the potential donor prior to transplantation using a new typing sample from each, so that each individual's typing is confirmed		
E5.3.3	HLA typing for Donors (related and blood only)		

Section	Standard version 7	Draft standard	Draft Standard v8
5.3.4.5.1	Prior to the conditioning regimen of the recipient, a verification typing must be performed:		
5.3.4.5.1.1	At a minimum level of low resolution for HLA-A, -B, and -DRB1		
5.3.4.5.1.2	Upon reception of the shipped unit		
5.3.4.5.1.3	On a segment of the tubing integrally attached to the unit, if available; otherwise a satellite vial shipped with the unit may be used		
5.3.4.5.2	If no segment is available, this step can be performed after transplantation and must be initiated as soon as possible after thawing the unit		
		E5.3.5	Antibody testing
		E5.3.5.1	Antibody testing must be performed
		E5.3.5.1.1	in case of mismatched transplant as specified by the transplant protocol
5.3.5	Crossmatching	E5.3.6	
5.3.5.1	Crossmatching must be performed	E5.3.6.1	
5.3.5.1.1	Prior to related and unrelated transplantation if required by the local transplant protocol	E5.3.6.1.1	
5.3.5.1.2	According to standards E4.2.5 (Crossmatching)	E5.3.6.1.2	
5.3.6	MICA allelic resolution must be performed if it is requested by the transplant protocol	E5.3.7	
5.3.7	Investigation of MICA antibodies	E5.3.8	
5.3.7.1	For bead array techniques, standards from section E2.8 (Bead Array) also apply	E5.3.8.1	

	or a designated individual				
	Information regarding the condition and disposition of specimen that did not meet the laboratory's criteria for acceptability				
		F3.5.8	The identity of the subcontracting laboratory and that portion of the testing for which it bears responsibility must be noted in the reports issued		
	The laboratory must have adequate systems in place to report results in a timely, accurate and reliable manner				
	Laboratories must have a procedure in place for resolving any discrepancies that may occur between laboratories				
	Reporting Antibody results				
	Terminology of HLA antibody specificities reported must conform to the latest report of the WHO Committee on Nomenclature				
	If solid phase screening methods are performed then it is acceptable to report assigned HLA serological equivalents		If solid phase antibody testing methods are performed then it is acceptable to report assigned HLA serological equivalents		
	If no serological equivalent has been assigned then the DNA nomenclature must be employed				
	the presence of HLA antibodies should be expressed as the % PRA or calculated %PRA value				
	Reporting Crossmatching results				
	Report must include:				
	Technique used to perform the test				

Grazie per l'attenzione