Donor Specific Antibody and Donor-derived cell-free DNA in kidney Allograft Rejection

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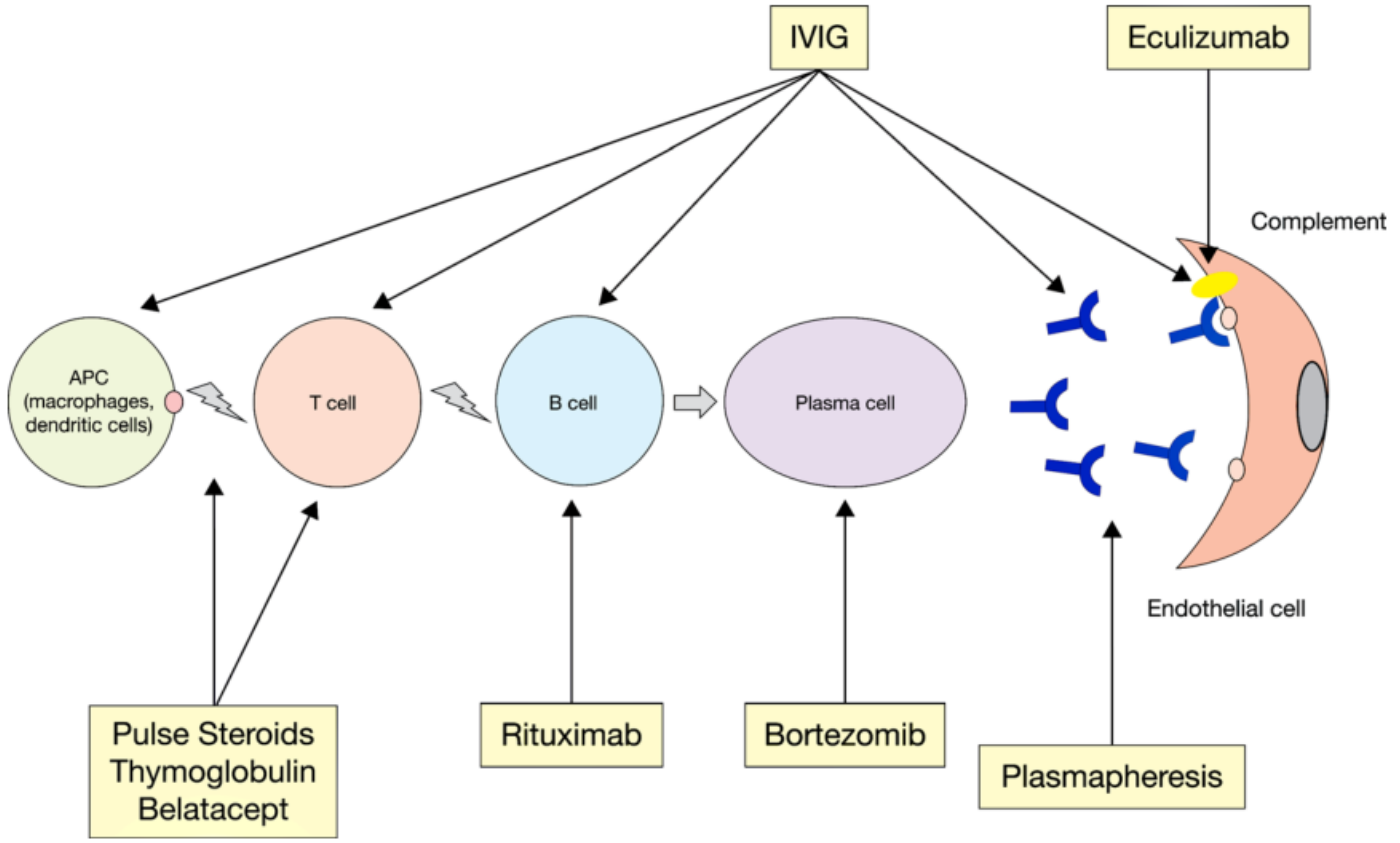
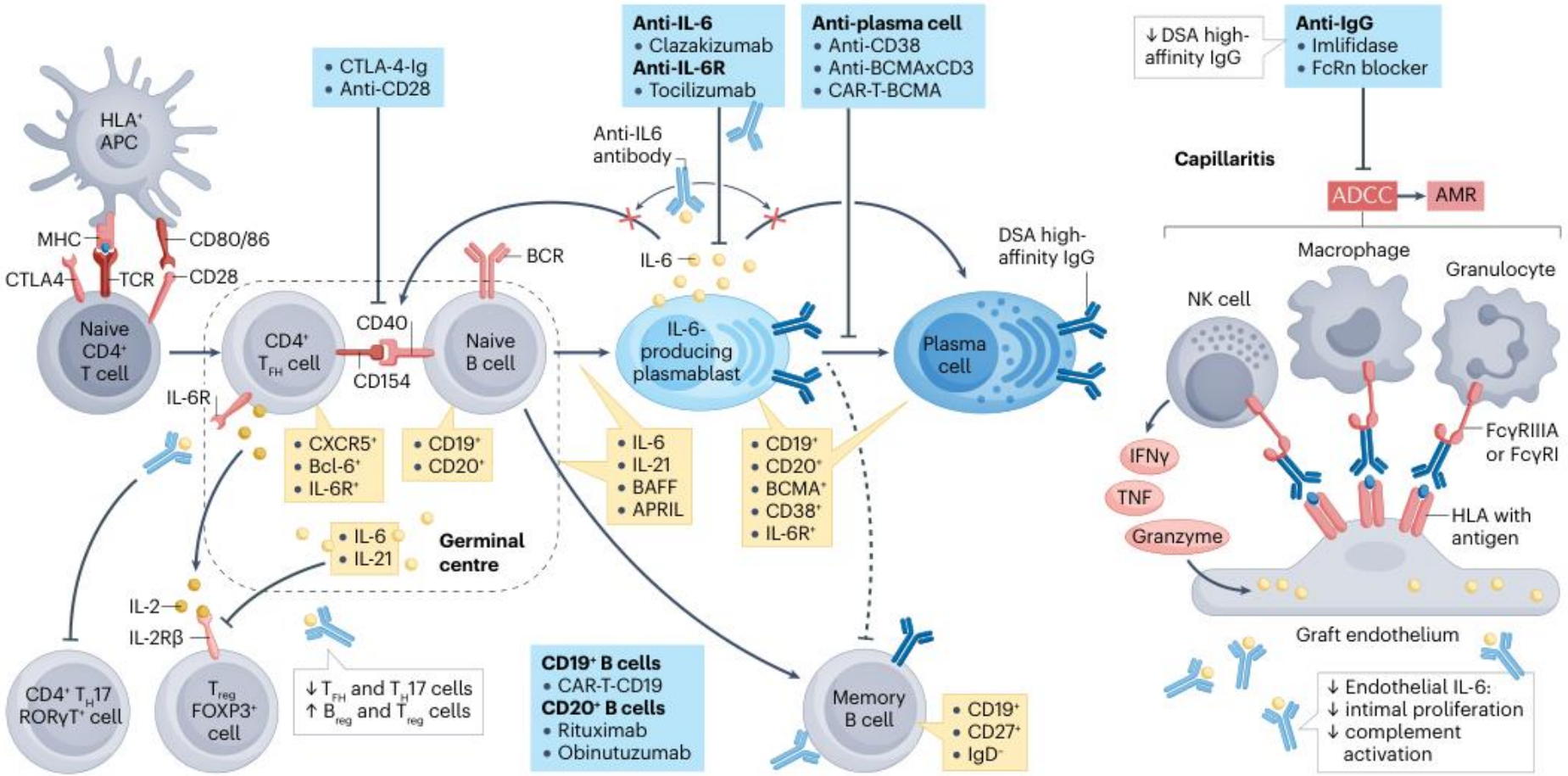
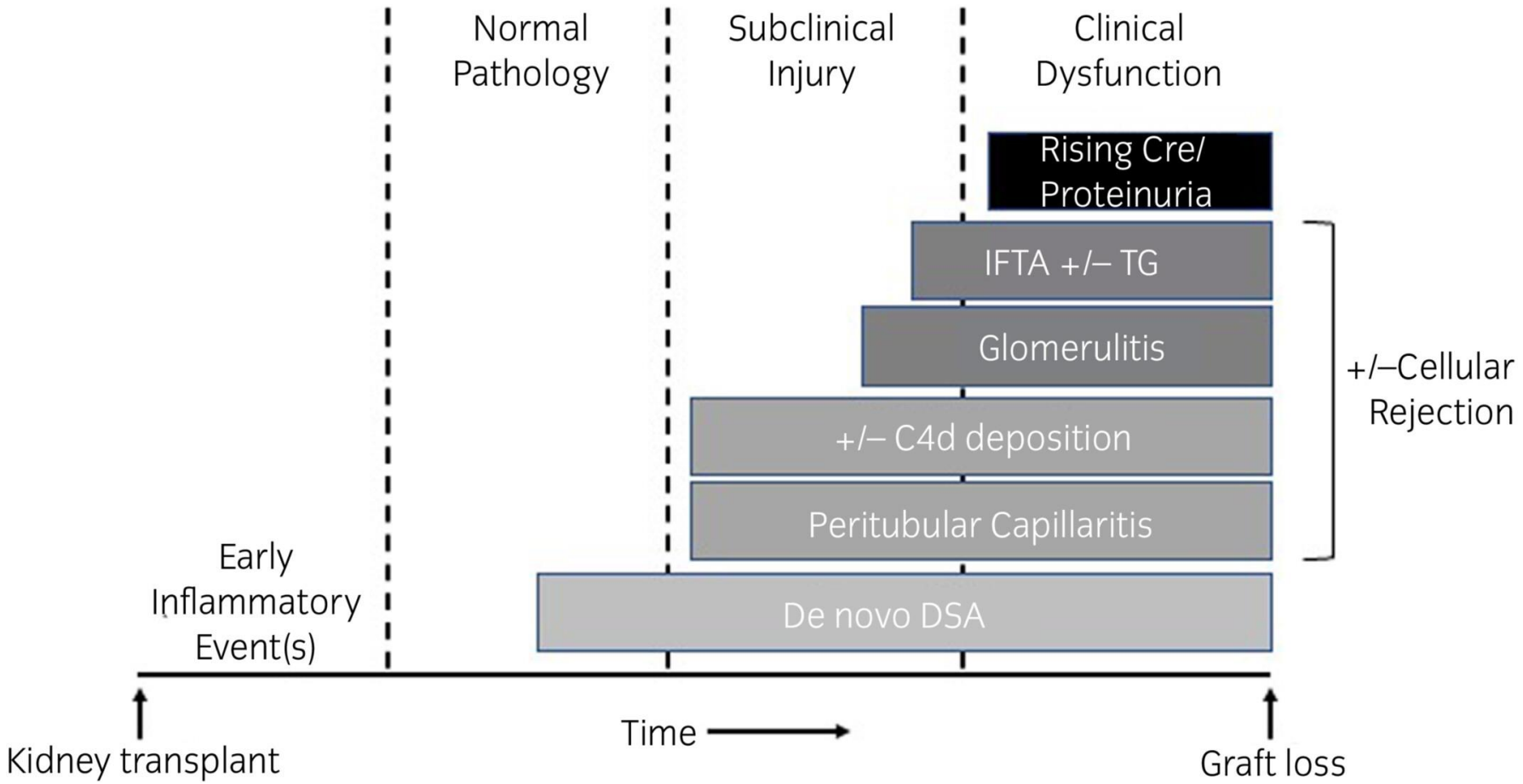
Why early rejection matters?



Why Detecting early Rejection matters?



Phases of Rejection



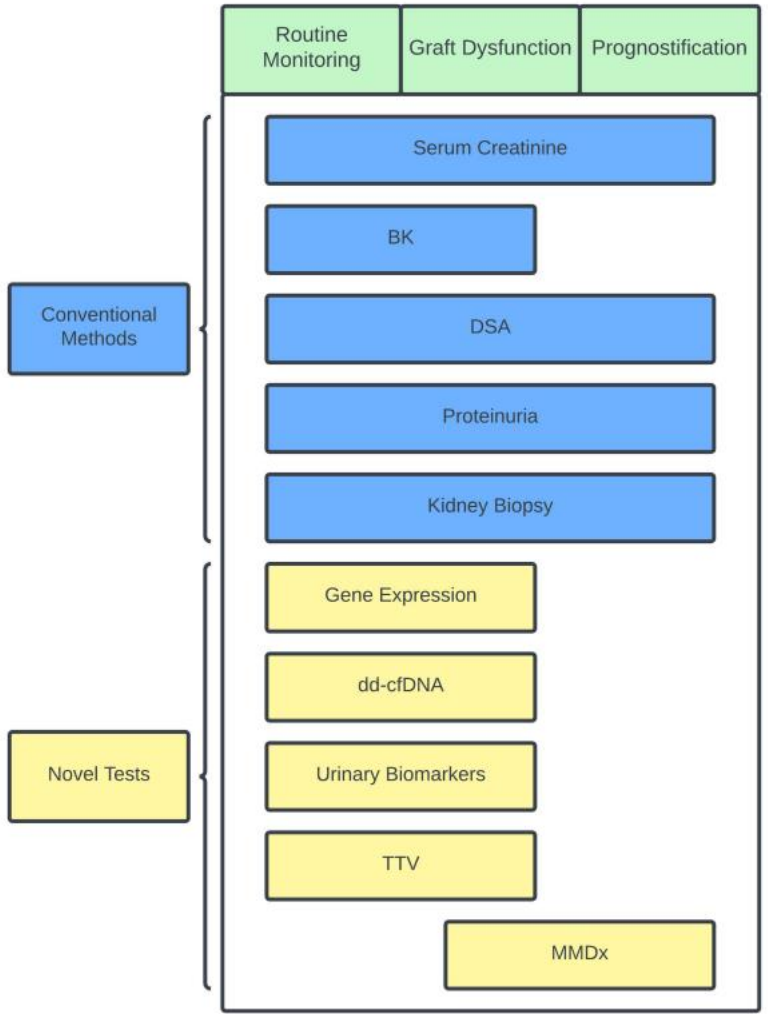
DSA: donor-specific antibodies, IFTA:interstitial fibrosis and tubular atrophy,
TG: transplant glomerulopathy

Rejection biomarkers

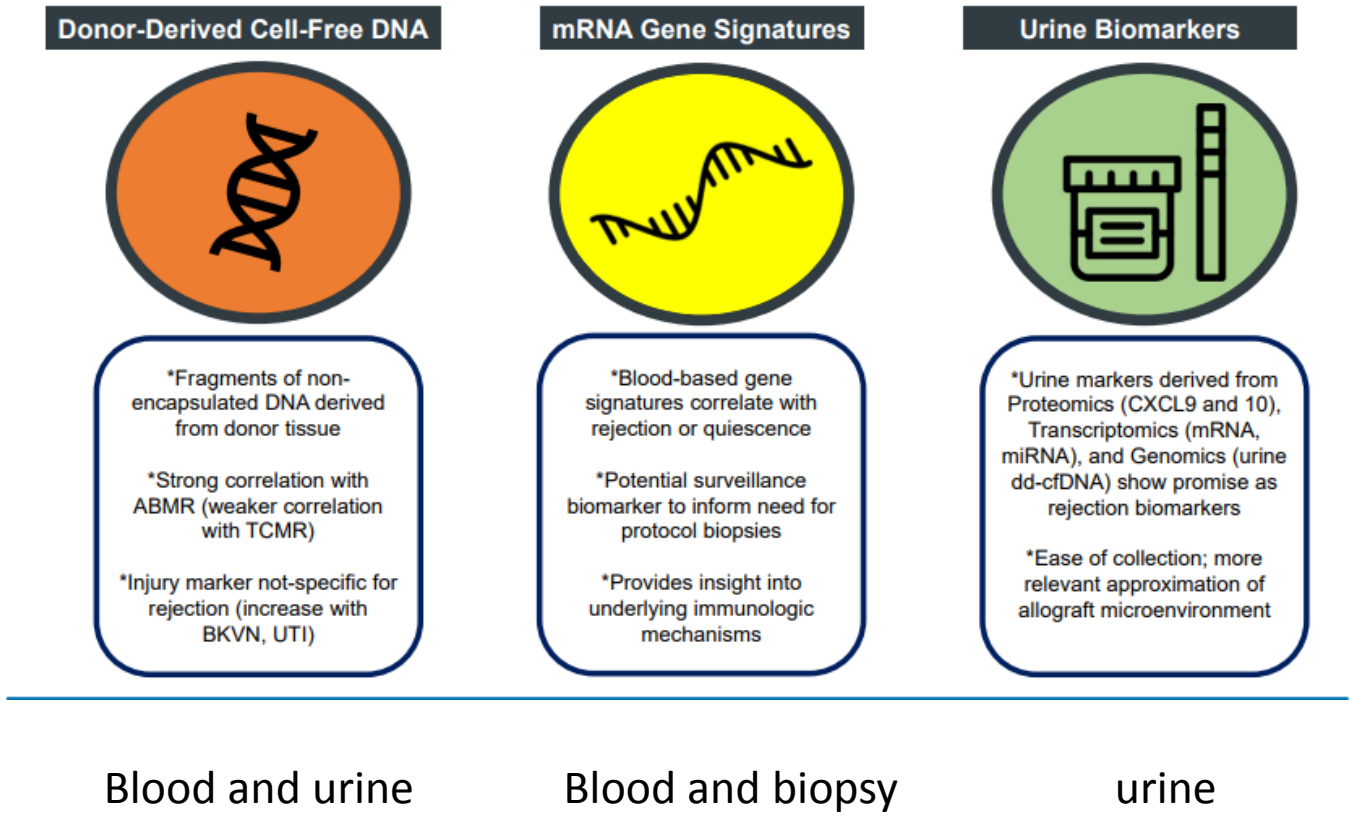
- Conventional:
- creatinine, proteinuria
- Gold standard: biopsy

Non-invasive approaches:
(early detection, avoid unnecessary biopsy!!!)

- DSA,
- Non-HLA Ab,
- dd-cf-DNA (blood or urine),
- Urine proteome,



Sample types for evaluation



Blood and urine

Blood and biopsy

urine

Post transplant DSA monitoring

TABLE 3.4 Suggested Protocol for DSA Monitoring Post-transplant

Status	Frequency of DSA Monitoring
DSA positive	Week 2, 4, and 8; 6 months; 1 year; and annually
Desensitized patients	Day 4, week 2, 4, and 8; 6 months; 1 year; and annually
DSA negative and low sensitized	6 months, 1 year, and annually
Highly sensitized patients	4 weeks, 6 months, 1 year, and annually

Indications:

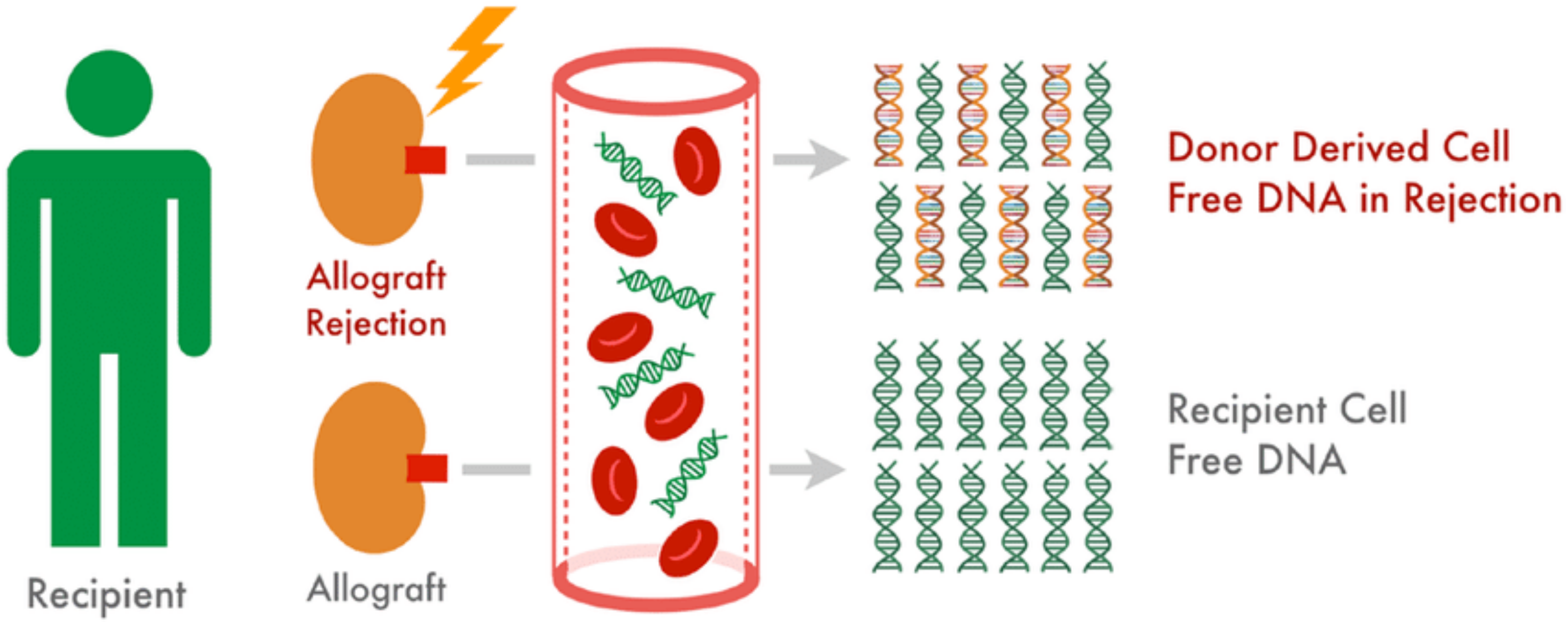
- Kidney disfunction in any time post transplant
- High risk recipients
- Multiple HLA mismatch
- Second transplant
- Desensitized patients
- Transplant with DSA
- Low adherence to therapy
- Decreasing immunosuppressive
- BKV and CMV nephropathy
- Post DGF

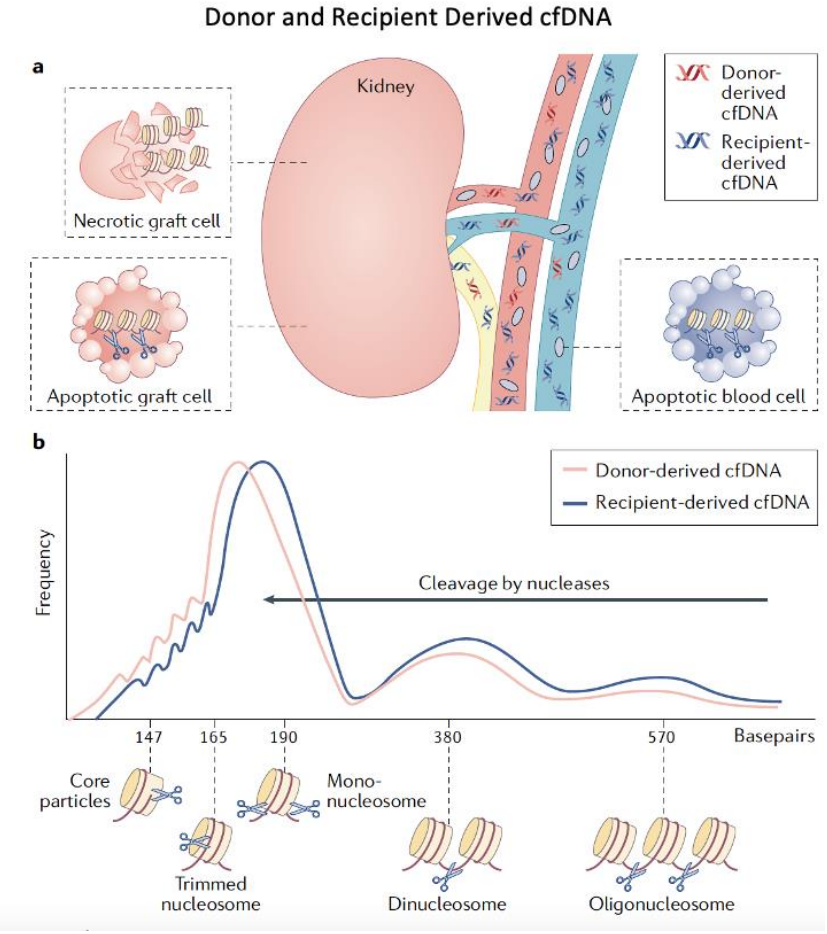
Indications in low risk recipients with stable function ?

- DSA and disfunction
- Sensitivity?

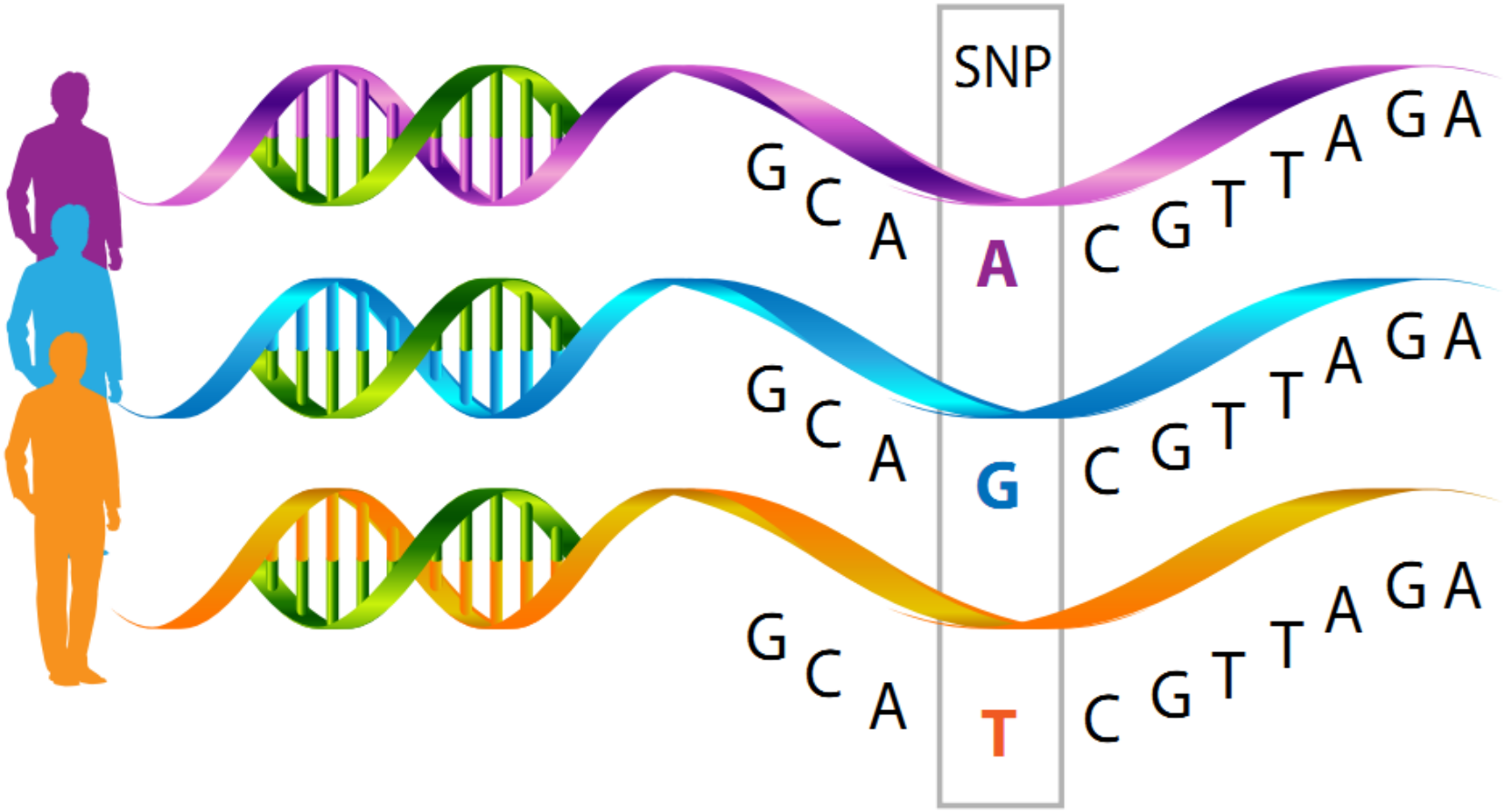
Study	Type of study	Total patients (n)	Total DSA+	Biopsied Patients with Subclinical DSA (n)	dnDSA/ preformed DSA	Time of biopsy	Subclinical aABMR (n) (%)*	Subclinical c.aABMR (n) (%)*	Subclinical cABMR (n) (%)*	Subclinical TCMR (n) (%)*	Mixed rejection (n) (%)*	No rejection (n) (%)*
Wiebe et al. (21)(52)	Retrospective Single center	508	64	45	dnDSA	6 months post-transplant At dnDSA detection Graft dysfunction	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified
Bertrand et al. (53)	Retrospective Multicenter	123	123	123	dnDSA	At dnDSA detection	32 (26%)	19 (15.5%)	Not specified	Not specified	Not specified	No ABMR: 72 (58.5%)
Loupy et al. (54)	Retrospective Single center + external validation	1001	?	?	dnDSA + Preformed DSA	1 year post-transplant	142 (14.2%)**			132 (13.2%)**	Not specified	727 (72.6%)**
Schinstock et al. (50)	Retrospective Single center	771	54	40 biopsied at detection of DSA 34 biopsied 1 year post detection of DSA Not all subclinical	dnDSA	4, 12, 24, 60 months post-transplant at dnDSA detection Graft dysfunction	At dnDSA detection: 10 (25%) 1 year post dnDSA detection 18 (53%)	Not specified	At dnDSA detection: 3 (7.5%) 1 year post dnDSA detection 13 (38.2%)	At dnDSA detection: 8 (20%) 1 year post dnDSA detection 5 (14.7%)	Not specified	Not specified
Yamamoto et al. (55)	Retrospective Single center	899	95	43	dnDSA	At dnDSA detection	18 (42%)			Not specified	Not specified	No ABMR: 25 (58%)
Parajuli et al. (56)	Retrospective Single center	45	45	29	dnDSA	At dnDSA detection "Other indications"	9 (31%)			3 (10%)	3 (10%)	14 (48%)
Waldecker et al. (57)	Retrospective Single center	865	132	34	dnDSA	At dnDSA detection Graft dysfunction	11 (26%)	3 (9%)	1 (3%)	5 (15%)	4 (12%)	5 (15%)

Donor-derived cell-free DNA (dd-cfDNA)

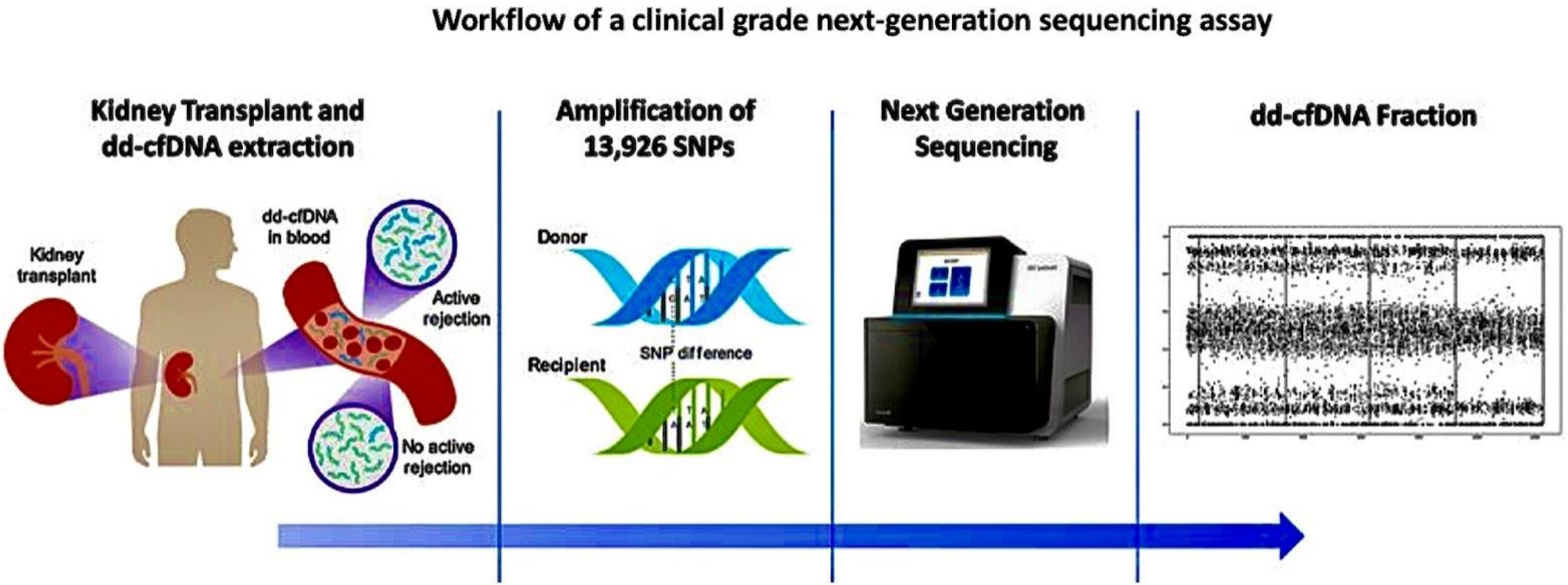




How to detect and discriminate donors DNA?



Work flow of dd-cfDNA



Dd-cfDNA is released from kidney allograft into circulation; blood is drawn and centrifuged, and plasma is isolated. cfDNA is extracted from plasma samples and used for library preparation followed by targeted PCR amplification of SNPs, performed using mmPCR. Amplicons are sequenced on a next-generation sequencer, and sequencing data are analyzed using a maximum likelihood estimate method to give a dd-cfDNA fraction value, which is reported to the physician.

Rejection threshold?

Selected seminal studies assessing dd-cfDNA in plasma for diagnosis of acute rejection

Study	Target for NGS	Study design	Enrolled patients	Acute rejection incidence	Rejection threshold	Sensitivity/ specificity	PPV/NPV	AUC
Kidney transplantation								
Bloom et al	266 SNPs	Prospective multicenter	384	24% (107 Bx)	1%	59/85	61/84	0.74
Sigdel et al	13 392 SNPs	Retrospective single center	300	18% (217 Bx)	1%	89/73	-	0.87
Liver transplantation								
Schutz et al	40 SNPs	Prospective multicenter	107	16%	10%	90/93	-	0.97
Heart transplantation								
Khush et al	266 SNPs	Prospective multicenter	740	4% (841 Bx)	0.20%	44/80	9/97	0.64
Lung transplantation								
De Vlaminck et al	53 423 SNPs	Prospective single center	51	7% (113 Bx)[a]	1%	100/73	-	0.9

[a]Only moderate-severe rejection episodes>2 mo post lung transplant analyzed.
AUC, area under the curve; Bx, allograft biopsy; dd-cfDNA, donor-derived cell-free DNA; NGS, next-generation sequencing; NPV, negative predictive value; SNPs, single-nucleotide polymorphisms.

Acute injury and dd-cfDNA

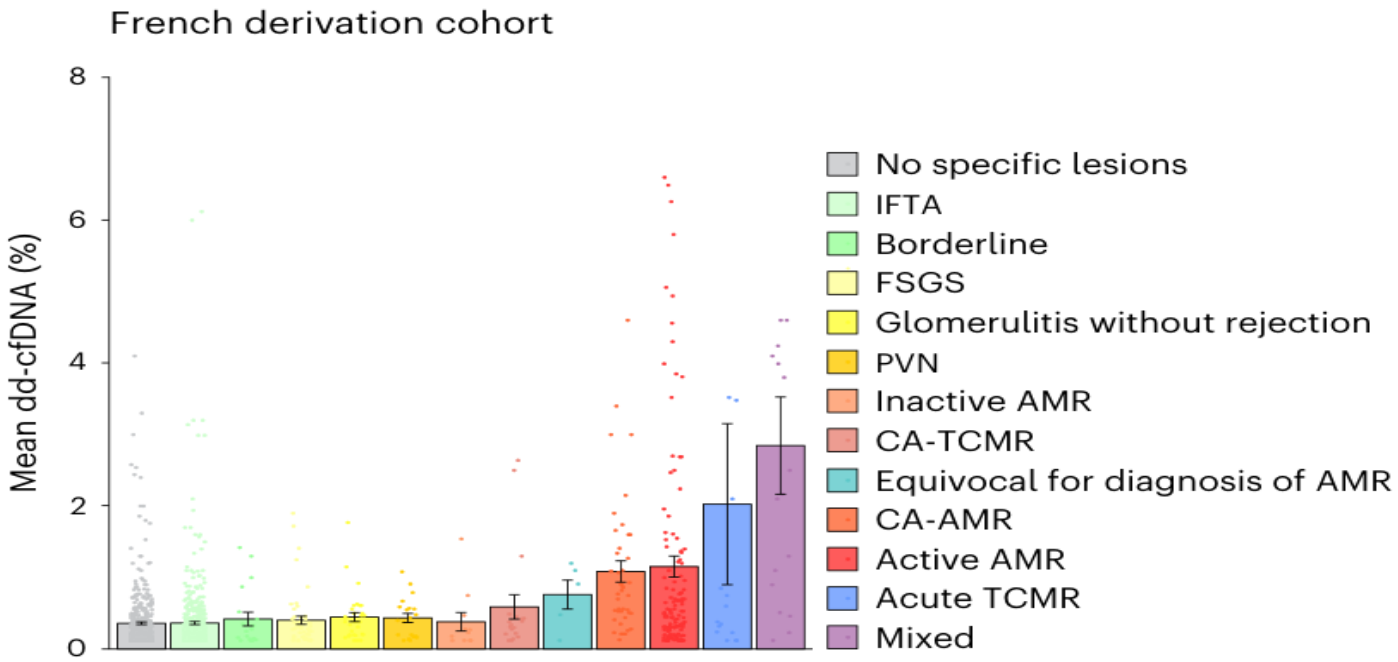
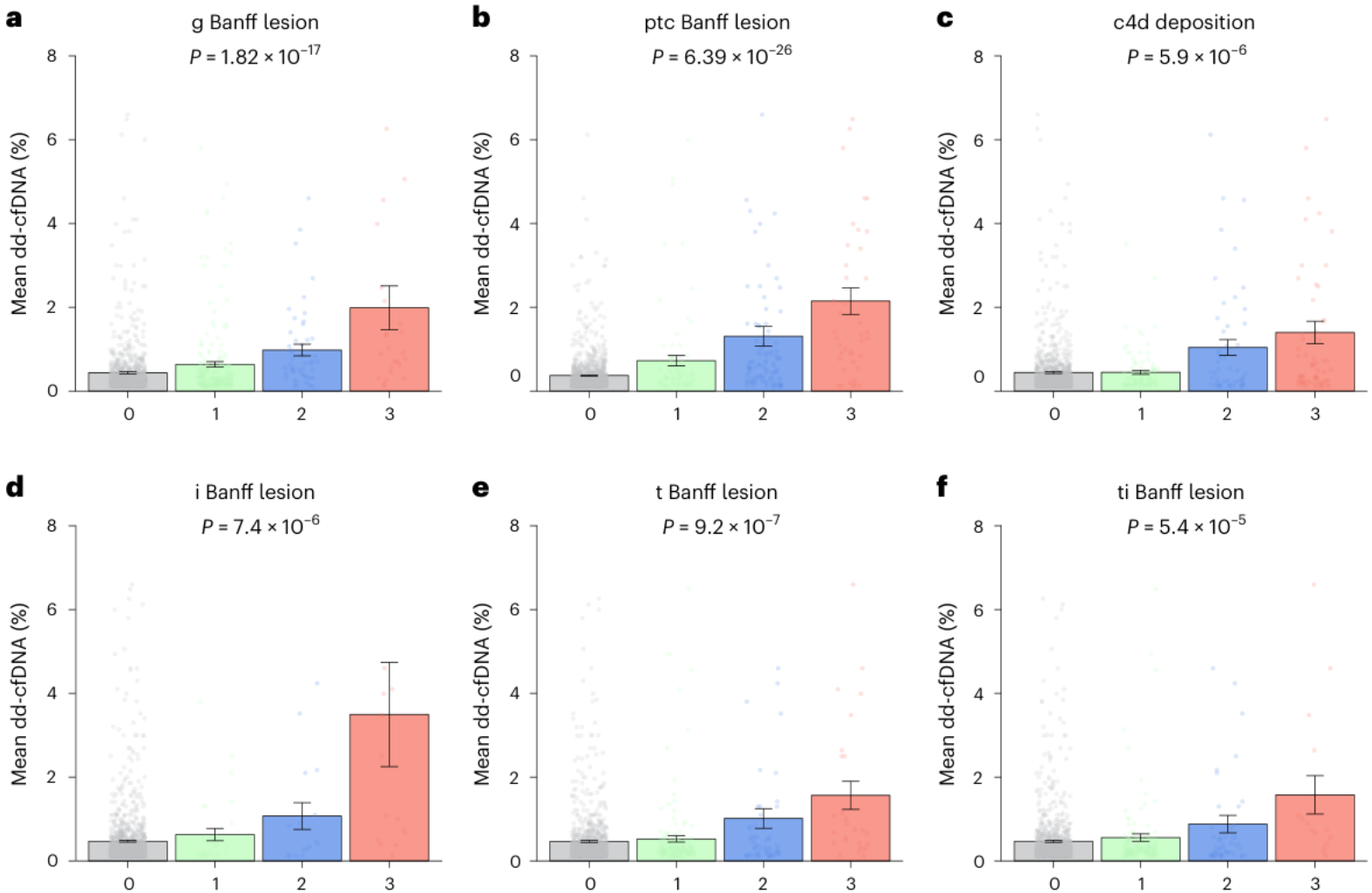


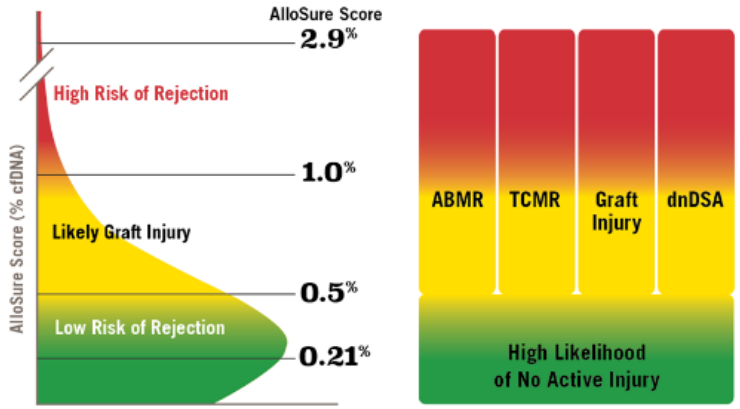
Fig. 1 | dd-cfDNA levels according to kidney allograft diagnoses. Mean level of dd-cfDNA according to the histological biopsy results. Each bar corresponds to one histological diagnosis with its mean dd-cfDNA value. Each dot corresponds to an individual dd-cfDNA value. Data are presented as mean ± s.e.m. The figure shows the increment of dd-cfDNA with active diseases (CA-TCMR, CA-AMR, active AMR, acute TCMR and mixed rejection (AMR + TCMR)). CA-TCMR, chronic active T cell-mediated rejection; CA-AMR, chronic active antibody-mediated rejection; FSGS, focal segmental glomerular sclerosis; PVN, polyomavirus-associated nephropathy.

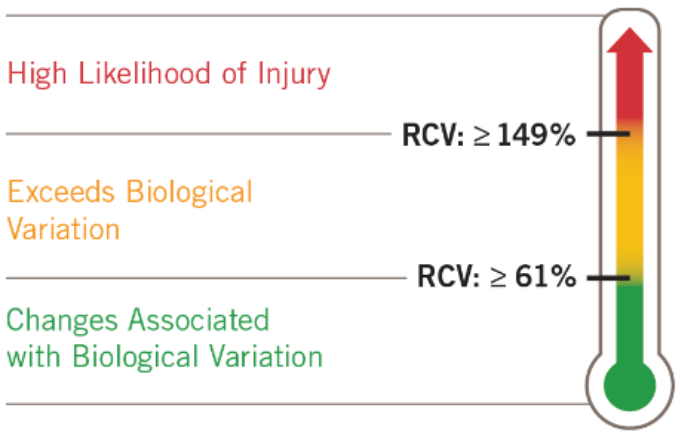

a
g Banff lesion
$P = 1.82 \times 10^{-17}$
b
ptc Banff lesion
$P = 6.39 \times 10^{-26}$
c
c4d deposition
$P = 5.9 \times 10^{-6}$
d
i Banff lesion
$P = 7.4 \times 10^{-6}$
e
t Banff lesion
$P = 9.2 \times 10^{-7}$
f
ti Banff lesion
$P = 5.4 \times 10^{-5}$
Mean dd-cfDNA (%)
0
2
4
6
8
0
1
2
3

AlloSure is validated to detect ABMR and TCMR, allograft injury, and dnDSA.[3,4,5,6]

Results over 2.9% with the presence of DSAs is highly predictive of antibody-mediated rejection with a PPV of 89%.

Results below 0.21% has a 95% NPV for active rejection.



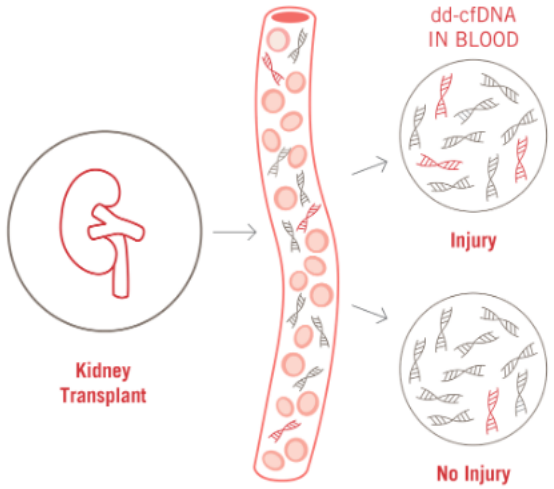


AlloSure is validated to inform clinical decision making based on RCV.[3,7]

Relative Change Value (RCV) is calculated between sequential AlloSure results above 0.20%.

In addition to the absolute AlloSure result, "relative change" between results is also important: increases over 149% between results may indicate a high likelihood of allograft injury.

Treatment efficacy



AlloSure is a simple blood test for kidney transplant organ health.

Developed specifically for transplant patients, AlloSure is a blood test that analyzes SNPs selected across all 22 somatic chromosomes to detect DNA released from a patient's kidney allograft, known as donor-derived cell-free DNA (dd-cfDNA).

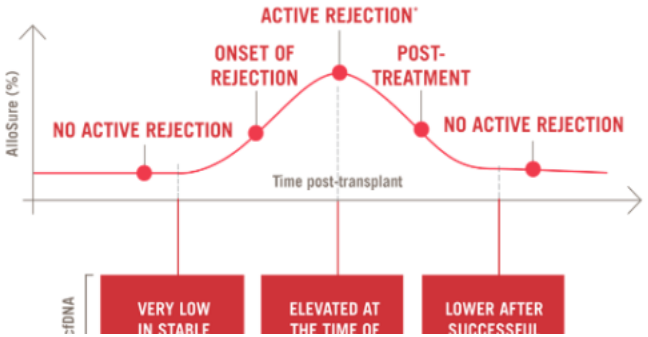
What is donor-derived cell-free DNA?

Cell-free DNA is fragments of DNA in the bloodstream that originate from cells undergoing injury and death. AlloSure can quantify increasing levels of dd-cfDNA, serving as a leading indicator of graft injury.

An early marker of injury and rejection for kidney transplant patients.

Broad utility in:

- High risk patients
- Patients with allograft rejection
- Repeat kidney transplant patients



ESOT recommendations 2024

Question 1. In kidney transplant patients with stable graft function, is plasma dd-cfDNA measurement a reliable diagnostic tool for subclinical acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or surveillance biopsy)?

Recommendation 1.1 - We suggest that clinicians consider measuring serial plasma dd-cfDNA in patients with stable graft function to exclude the presence of subclinical antibody mediated rejection.
Quality of Evidence - Moderate
Strength of Recommendation - Weak in Favor

Comment to Recommendation 1.1

Concomitant testing for donor-specific HLA and non-HLA antibodies along with plasma dd-cfDNA may further increase the ability to detect the presence of antibody-mediated rejection (ABMR).
Screening with dd-cfDNA alone does not appear to be a reliable tool for the detection of subclinical T-cell-mediated rejection (TCMR). Combining this test with other noninvasive biomarker technologies (gene expression profiling) may improve the detection of subclinical TCMR.

The optimal timing and frequency of screening have not been established.

Question 2. In kidney transplant patients with acute allograft dysfunction, is plasma dd-cfDNA measurement a reliable diagnostic tool for acute rejection monitoring when compared with standard of care (eGFR/creatinine monitoring or for cause biopsy)?

Recommendation 2.1 - We recommend that clinicians measure plasma dd-cfDNA in patients with acute graft dysfunction to exclude the presence of rejection, particularly antibody mediated rejection.

Quality of Evidence - Moderate.
Strength of Recommendation – Moderate in Favor.

Comment to Recommendation 2.1

Concomitant testing for donor specific HLA and non-HLA antibodies along with plasma dd-cfDNA may further increase the ability to detect the presence of ABMR. Low levels of ddcfDNA do not necessarily exclude the presence of TCMR in the graft.

Molecular Microscope Diagnostic System (MMDx)

Molecular Microscope® Diagnostic System (MMDx®) measures gene expression in biopsy samples of kidney and heart transplant patients. This whole-genome microarray chip technology uses machine-learning algorithm to calculate probability scores for particular rejection types, acute injury or fibrosis. MMDx is not intended to replace histology, but can be used for the objective assessment of challenging cases.

Depending on severity of transplanted organ rejection or injury, particular genes are activated and produce unique patterns of RNA.

Using chip technology, thousands of different mRNA are quantified simultaneously, compared to reference sets of 5087 measured kidney and 3000 heart biopsies and evaluated by machine-learning algorithm.

The result is graphical output, where examined biopsy is displayed against the background of biopsy reference set which are colored according to the type of rejection.

Patient Information

Clinical Interpretation

Summary of Molecular Changes (injury, rejection, scores)

Visualization Relationship of biopsy to others in reference set

Indicates this biopsy

Molecualr Microscope® Diagnostic Report for Kidney (MMDx-Kidney)

Patient and Institution Information

Patient Name or ID	PC191210 191203009MM	**Lab ID Number**	191210004MM
Patient DOB	Not Provided	**Ordering Physician**	Dr. S Jones
Patient MRN	Not Provided	**Ordering Institution**	Kashi Clinic TEST ACCT

Testing and Clinical Information

Test Date	12/10/2019	**Time from Transplant to Biopsy**	1 years
Report Date	12/11/2019	**DSA Status**	Not Provided
Transplant Date	05/26/2018	**Biopsy Indication**	Surveillance, compare to previous
Biopsy Date	12/02/2019	**Primary Disease**	HTN

Pure Molecular Interpretation (Results Summary)

	Percent cortex[1]
Abnormal kidney transplant biopsy. Moderate early-stage ABMR. No TCMR. Mild atrophy-fibrosis with minimal inflammation and AKI. Compared to the biopsy of June 26th, 2019, there has been resolution of inflammation and AKI features. Note: the Molecular Microscope® Diagnostic System cannot exclude primary glomerular diseases.	96%

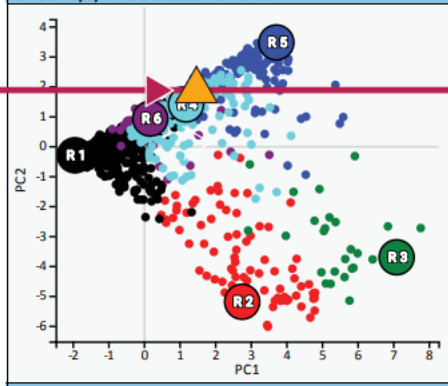
Result Details

Biopsy Rejection and Injury Scores

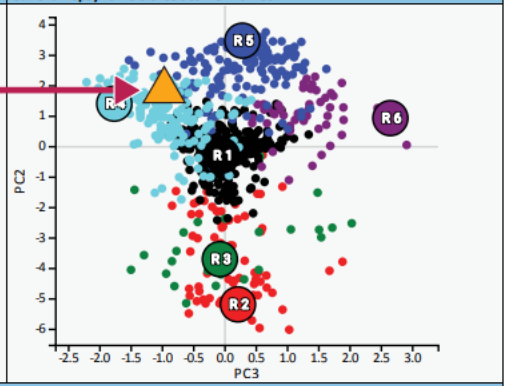
	Classifier / Gene Sets	Biopsy Score	Range of Values[2]	Upper Limit of Normal[3]	Interpretation
Injury Scores	Global Disturbance Score	-1.19	-3.8 —5.8	0.03	Minimal
	Acute Kidney Injury (AKI) Score	-0.13	-0.6 —1.6	0.55	Mild
	Atrophy-Fibrosis Score	0.34	0 —1	0.52	Mild
Rejection Scores	Rejection Score	0.57	0 —1	0.30	Moderate
	T Cell-Mediated Rejection (TCMR) Score	0.01	0 —1	0.10	Normal
	Antibody-Mediated Rejection (ABMR) Score	0.56	0 —1	0.20	Moderate

Archetypal Analysis (please see Archetypal Analysis Description on Page 2 for details)

Current Biopsy vs Reference Set: PC2 vs PC1



Current Biopsy vs Reference Set: PC2 vs PC3



Rejection phenotype[4] (six scores, R1-R6, adding up to 1.0)			
R1 **Non-rejecting**	0.13	R4 **Early-Stage ABMR (EABMR)**	0.62
R2 **TCMR**	0.00	R5 **Fully-Developed ABMR (FABMR)**	0.25
R3 **Mixed Rejection**	0.00	R6 **Late-Stage ABMR (LABMR)**	0.00
Current Sample 191210004MM		**All ABMR (Sum of R4, R5, and R6)**	0.87

1. Percent cortex is a quality control measure.
2. The 2.5th to 97.5th percentiles in the entire Reference Set.
3. 90th percentile in relevant Reference Set biopsies.
4. Scores from archetypal analysis.

MMDx Kidney:

General information:

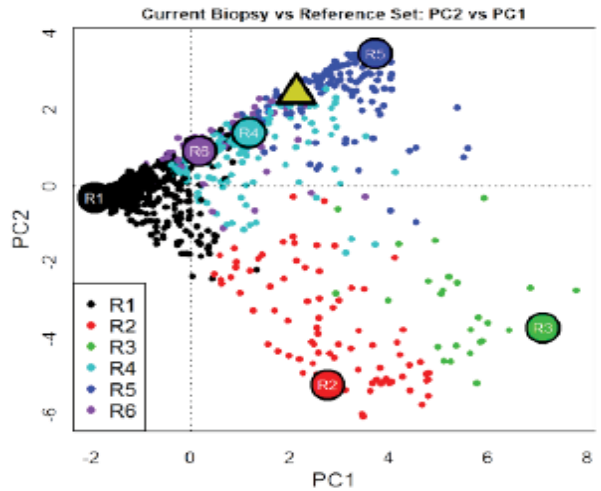
Surname		First Name		Physician	
Date of Birth		Sample ID			
Date Received (Y-M-D)		Time of Biopsy Post-Tx	9.6 years		
Date Reported (Y-M-D)		Transplant Type	---		
Date of Transplant (Y-M-D)		Biopsy Indication	---		
Date of Biopsy (Y-M-D)		Primary Disease	---		

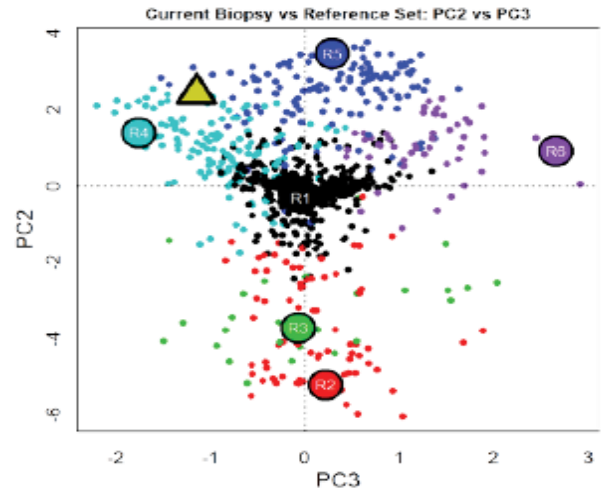
Pure molecular interpretation

Abnormal biopsy. Severe early-stage ABMR with g and ptc-related molecular features. No TCMR. Mild inflammation, AKI and atrophy-fibrosis. Note that MMDx cannot exclude primary renal diseases.

	Classifier/gene sets[1, 2]	Biopsy	Range of values[A]	Upper limit of normal[B]	Interpretation
Injury Scores	Inflammation Score[3]	-0.32	-3.8 – 5.8	0.03	Mild
	Acute Kidney Injury (AKI) Score[4]	0.16	-0.6 – 1.6	0.39	Mild
	Atrophy-Fibrosis Score[5]	0.33	0.0 – 1.0	0.82	Mild
Rejection Scores	Rejection Score[6]	0.74	0.0 – 1.0	0.30	Severe
	T Cell-Mediated Rejection (TCMR) Score[7, C]	0.01	0.0 – 1.0	0.10	Normal
	Antibody-Mediated Rejection (ABMR) Score,[8, C]	0.81	0.0 – 1.0	0.20	Severe

Rejection phenotype[8, D] (six scores, R1-R6, adding up to 1.0)				
	R1 Non-rejecting	0.00	All ABMR (Sum of R4, R5, and R6)	1.00
	R2 TCMR	0.00	*R4 Early-Stage ABMR (EABMR)*	0.59
	R3 Mixed Rejection	0.00	*R5 Fully-Developed ABMR (FABMR)*	0.41
			R6 Late-Stage ABMR (LABMR)	0.00





Survival in patients with similar biopsies in the Reference Set		Percent cortex[10, E]
1-year: 92%	3-years: 76%	96%

Clinical Notes

A - The 2.5th to 97.5th percentiles in the entire Reference Set
B - 90th percentile in relevant Reference Set biopsies
C – Mean of scores from page 2
C - Scores from archetypal analysis
E - %cortex is a quality control measure

Table 1. Comparison of light microscopy and MMDx findings in biopsy of transplanted kidney.

Patient's Credentials	Type of the Biopsy	Light Microscopy Findings	MMDX	Concordances/ Discordances
J.B.	I	Chronic ABMR, transplant glomerulopathy, FSGS	Severe, fully developed ABMR, moderate IFTA, and mild AKI	Concordant in rejection Fibrosis discordant
J.Š.	I	No rejection, discrete CNI toxicity, IFTA1	No ABMR/TCMR, IFTA1, minimal AKI and minimal inflammation	Concordant in rejection Inflammation discordant
T.D.	I	No rejection, transplantation glomerulopathy with FSGS, IFTA 2	No ABMR/no TCMR, AKI, IFTA 2	Concordant in rejection AKI discordance
D.O.	I	Acute ABMR	Mild, early-stage ABMR, no TCMR, extensive atrophy-fibrosis, moderate AKI, and inflammation	Concordant in rejection IFTA, AKI, and inflammation discordant
M.P.	P	Borderline changes	No ABMR/TCMR, minor molecular signs of ABMR, moderate inflammation, and IFTA1	Rejection discordance Inflammatory and IFTA discordance
S.H.	I	Borderline, transplantation glomerulopathy, ATN-like, IFTA 1	Fully developed ABMR, IFTA 3	Rejection discordance IFTA discordance
J.M.	I	TCMR IIa, C4d +	No ABMR/no TCMR, mild AKI, and minimal IFTA	Rejection discordance AKI and IFTA discordance
P.B.	I	Possible TCMR	No ABMR/TCMR, minimal AKI, minimal IFTA	Rejection, inflammation, and fibrosis discordant
M.B.	I	Suspect subclinical TCMR, possible infection injury	No ABMR/no TCMR	
M.D.	I	No rejection, IFTA2, possible recurrence of FSGF	Moderate to severe TCMR, no ABMR, Extensive atrophy and fibrosis, AKI gr.2	Rejection discordance
V.G	I	Not examined in light microscopy	No ABMR/no TCMR, mild molecular signs of TCMR, mild atrophy-fibrosis signs	Not applicable
Ľ.K.	I	Not representative, possible C4d focal positivity	No ABMR/no TCMR, mild AKI, minimal IFTA	Rejection discordance

ABMR—antibody-mediated rejection; AKI—acute kidney injury; CNI—calcineurin inhibitors; FSGS—focal segmental glomerulosclerosis; I—indication biopsy; IFTA—interstitial fibrosis and tubular atrophy; MMDx—molecular microscope; P—protocolar biopsy; TCMR—T-cell-mediated rejection.