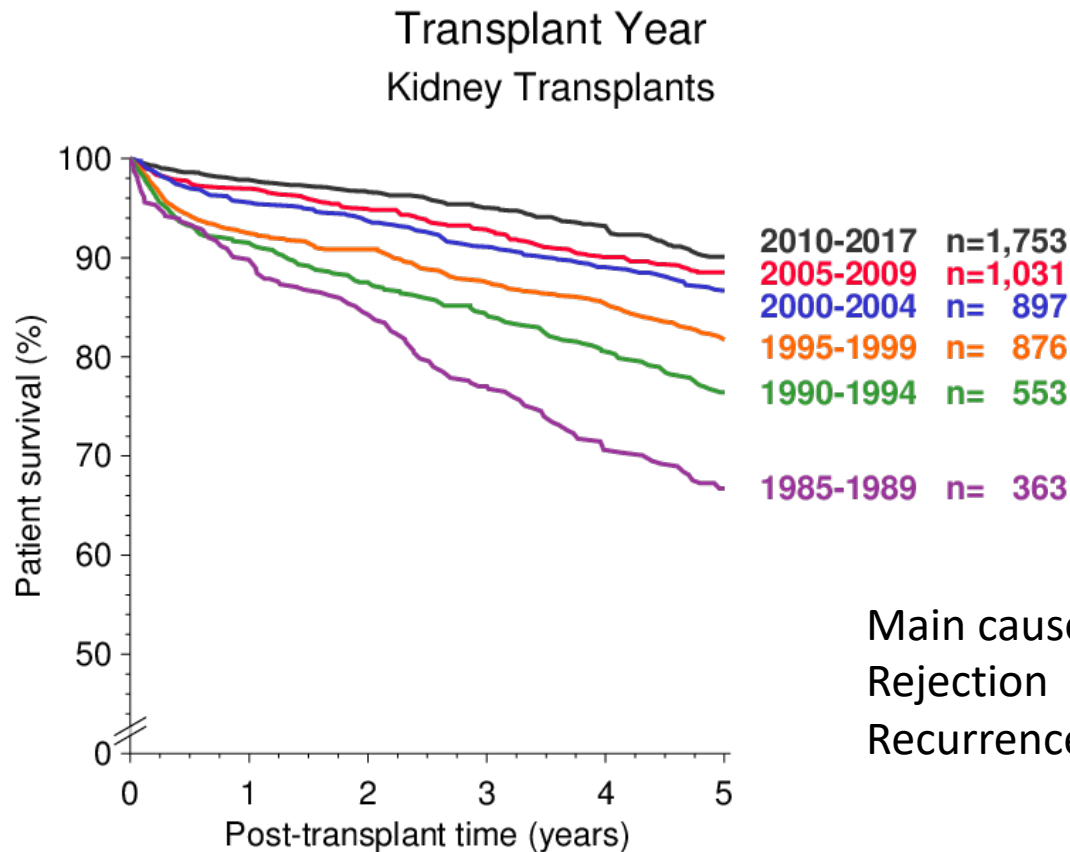


---

# dd-cf DNA as a tool to monitor antibody-mediated rejection in kidney transplantation

Petra Hrubá,  
Transplant laboratory  
Institute for Clinical and Experimental Medicine Prague

# Kidney graft survival is excellent in recent primary kidney transplants



Main causes of graft failure:  
Rejection  
Recurrence of primary disease

# Types of rejection

---

## 1. T – cell mediated rejection (TCMR)

- a. Acute
- b. Chronic

## 2. Antibody – mediated rejection (ABMR)

- a. Acute
- b. Chronic

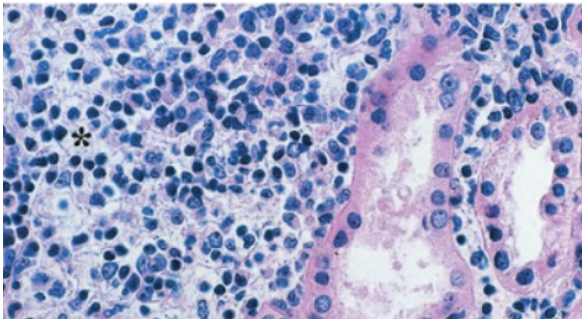
## 3. Mixed

Different types of rejection need different treatments.

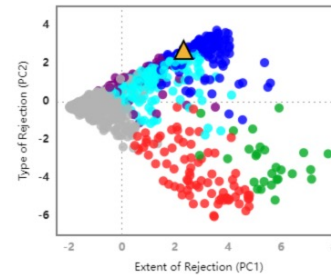
# Diagnostics of rejection

Kidney graft biopsy

Histology

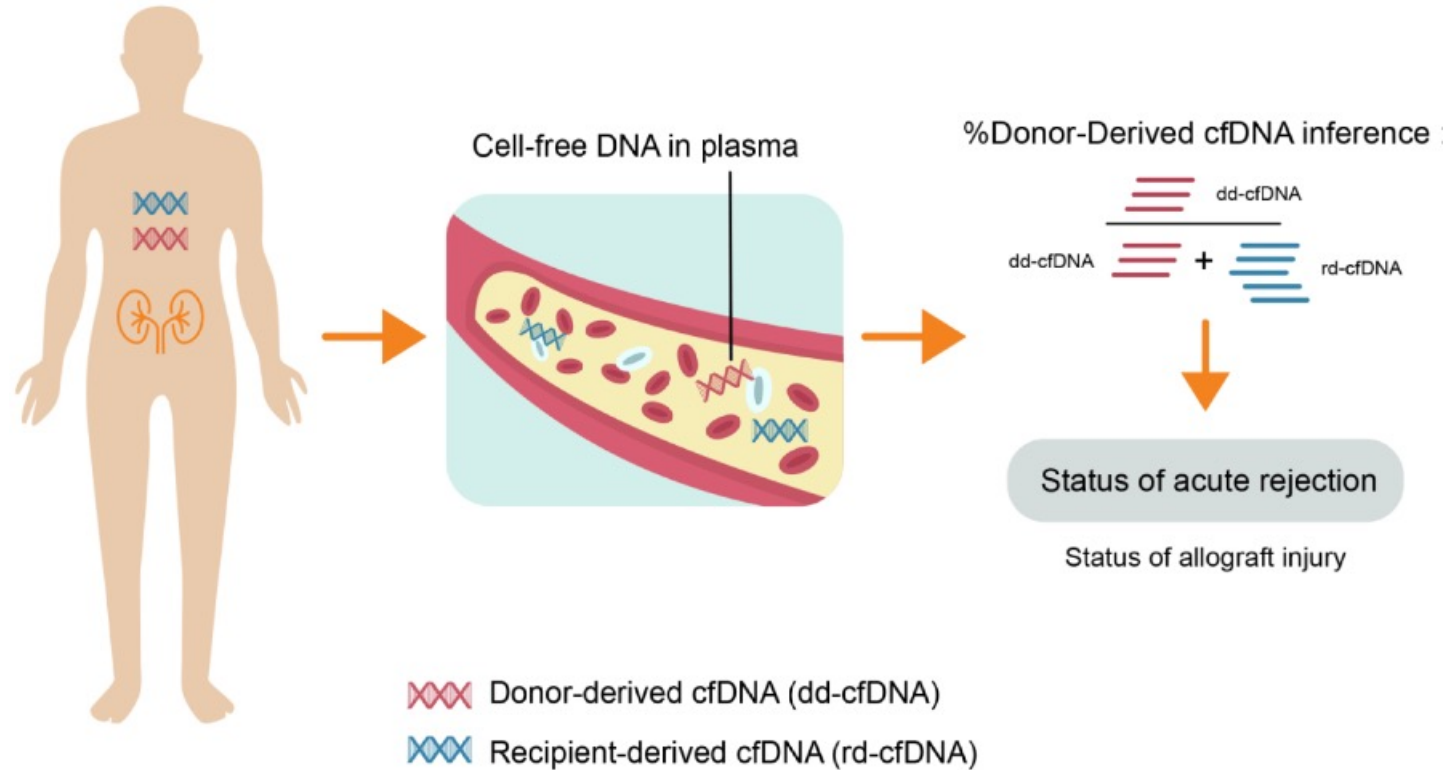


Transcripts profiling  
(Molecular Microscope  
Diagnostic System)



Liquid biopsy  
(dd-cf DNA)

# dd-cf DNA in solid organ transplantation



# Commercial tests available

---

Based on targeted NGS

1. Allosure (CareDx)<sup>1</sup>: 266 SNPs, cutoff>1%
2. Prospera (Natera)<sup>2</sup>: 13 392 SNPs, cutoff>1%
3. Trac (Viracor Eurofins)<sup>3</sup> : >100 000 SNPs, cutoff>0.69%

US\$2200–2800/test

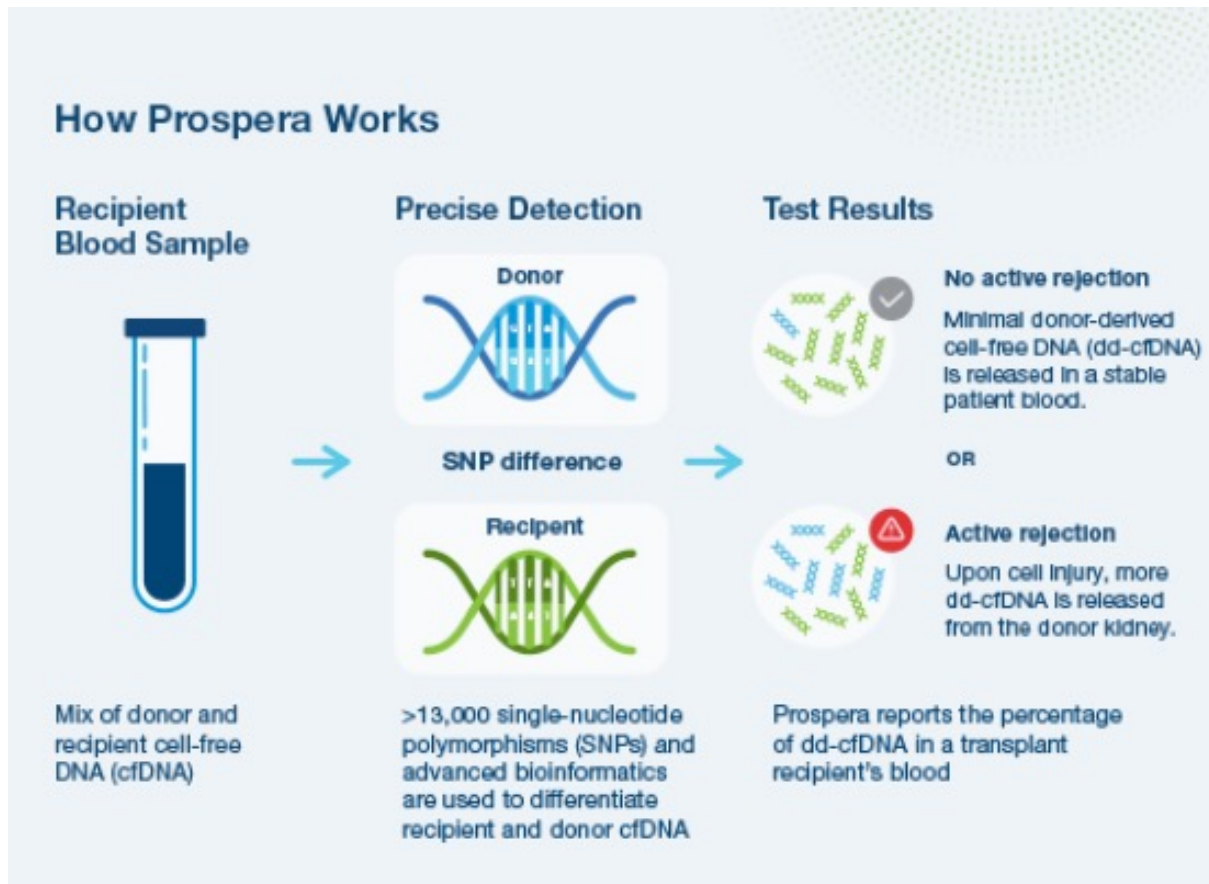
Cost effective detection of subclinical rejection...US\$700

<sup>1</sup>Bloom, R.D. et al. *J. Am. Soc. Nephrol.* **2017**

<sup>2</sup>Sigdel, T.K. et al. *J. Clin. Med.* **2019**

<sup>3</sup> Sharon E et al. *PLoS Comput Biol* 2017

# Prospera test principle



# Conclusions from published studies

---

Allosure test, n=102, 27 rejections

	AUC	Sensitivity	Specificity	NPV	PPV
Any rejection	0.74	59%	85%	84%	61%
ABMR	0.87	81%	83%	96.4%	44.4%

Prospera test, n=277, 38 rejections

	AUC	Sensitivity	Specificity	NPV	PPV
Any rejection	0.87	88.7%	72.6%	95.1%	52%

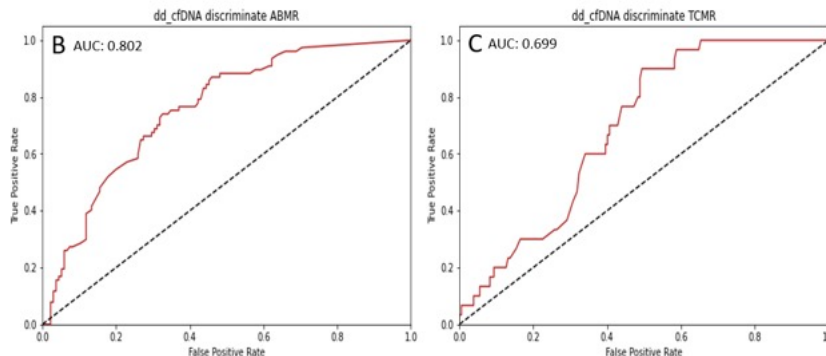
low dd-cfDNA predicted allograft quiescence, which can help to avoid the need of protocol biopsies

*Bloom, R.D. et al. J. Am. Soc. Nephrol. 2017, 28; Sigdel, T.K. et al. J. Clin. Med. 2019, 8(1)*



# Conclusions from published studies

- dd-cf DNA are higher in antibody-mediated (ABMR) compared to T-cell mediated rejection (TCMR)
- The best C-statistics of dd-cfDNA test is for ABMR

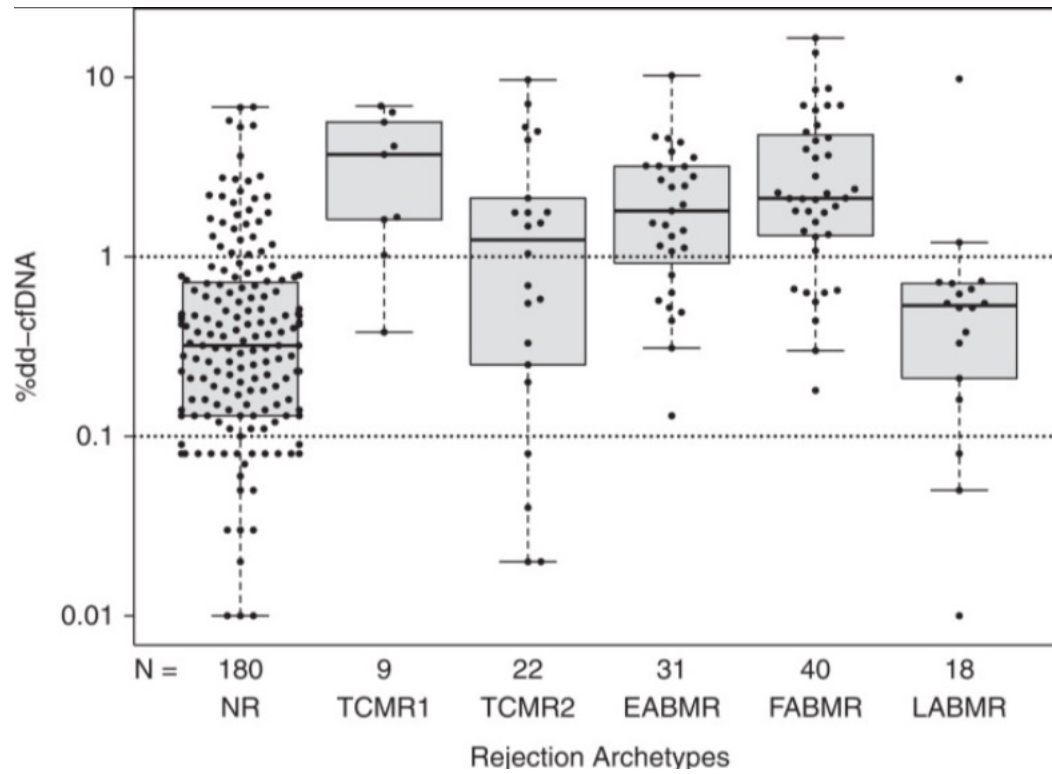


- dd-cfDNA  $\geq 0.5\%$  were associated with the risk of *de novo* donor-specific antibodies (HR 2.71) and were elevated ahead of donor specific antibody identification (a median of 91 days) or ahead of rejection.
- Elevation of dd-cf DNA during infections

*Bu et al. Kidney Int 2021 (ADMIRAL study)*

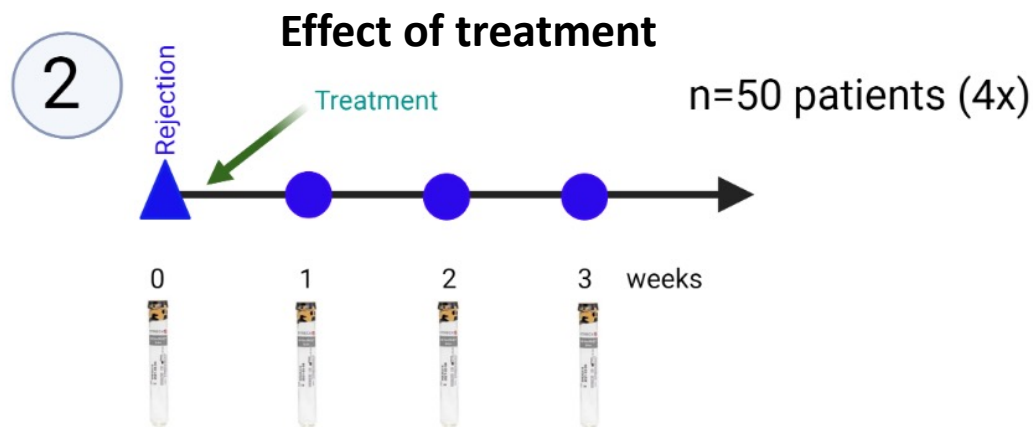
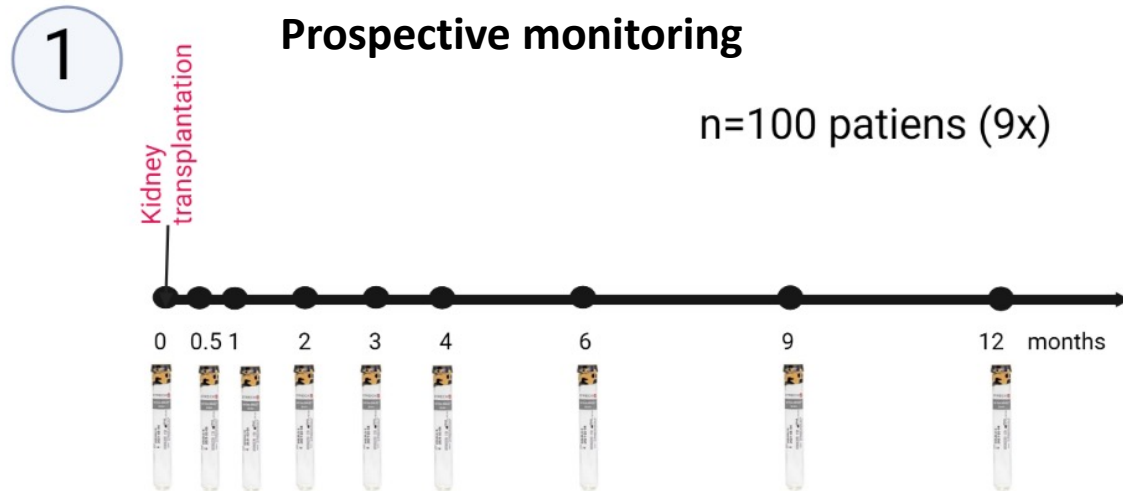
# Trifecta study

300 biopsies examined by MMDx plus dd-cf DNA at time of biopsy  
(53 biopsies from IKEM)

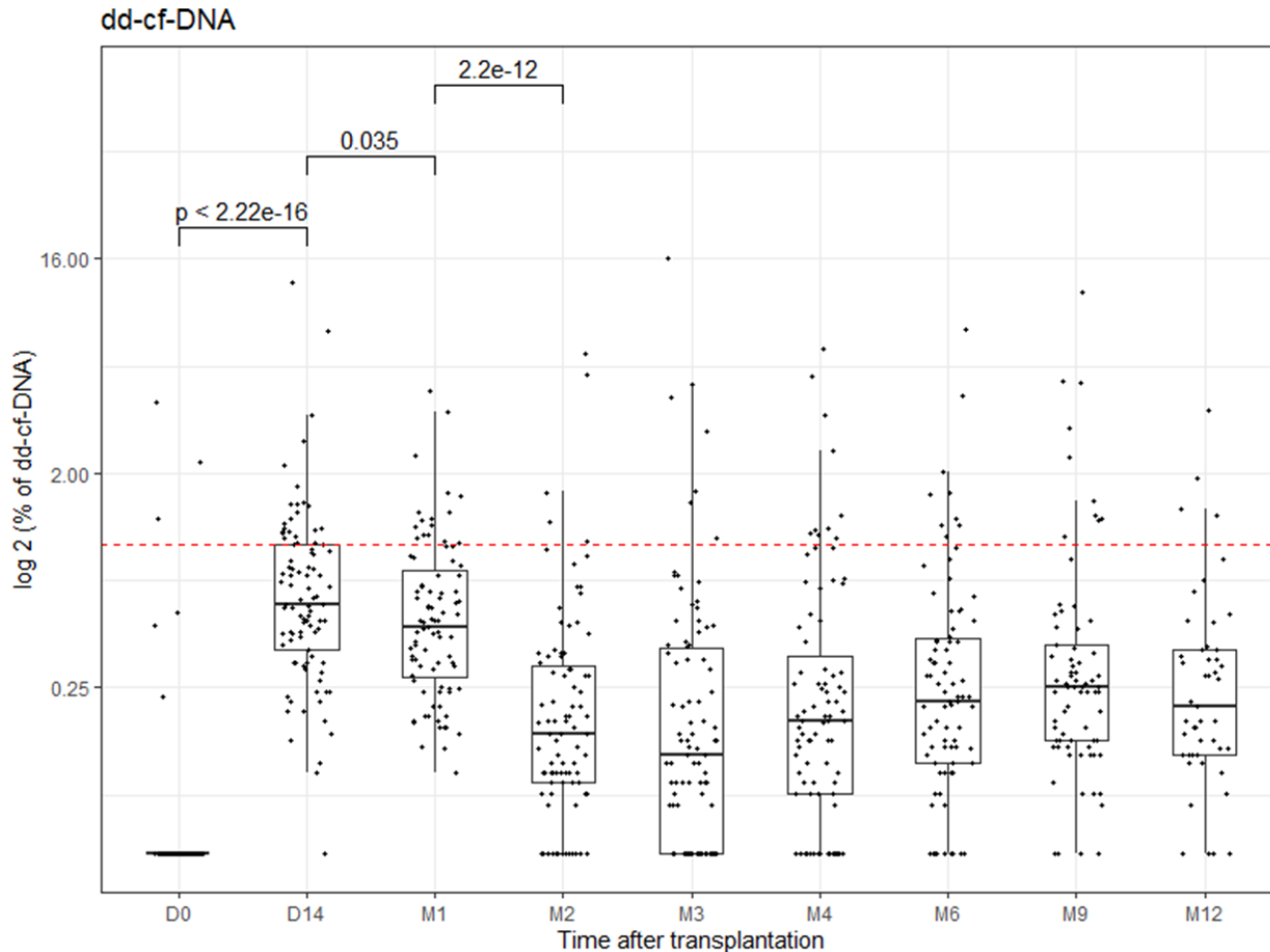


Halloran, P.F. et al. *J Am Soc Nephrol.* 2022 Feb;33(2):387-400.

# Single-center, prospective study monitoring dd-cf DNA in kidney transplantation

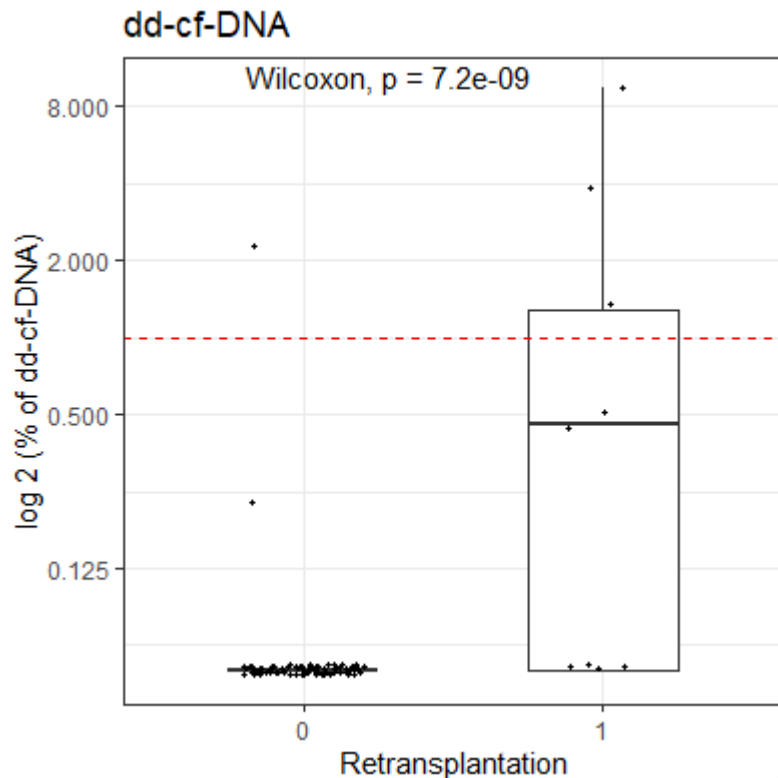


# Dynamics of dd-cf DNA in the first year after transplantation

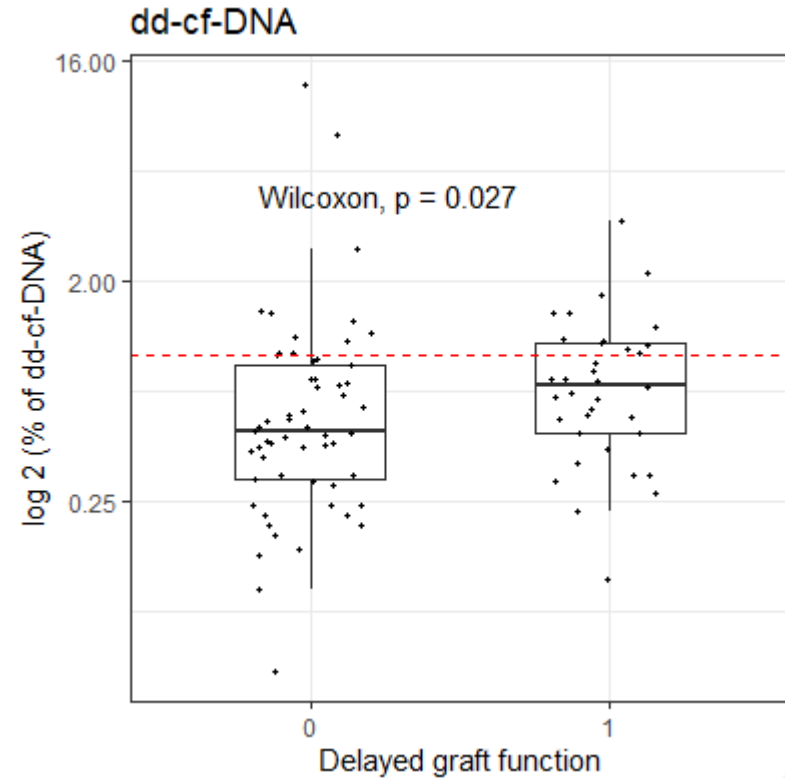


# dd cf-DNA higher in biopsies with delayed graft function

Before transplantation



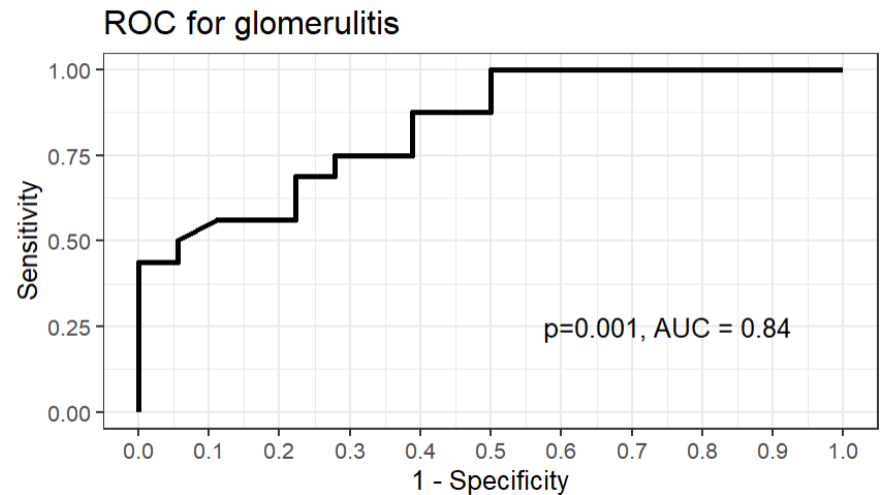
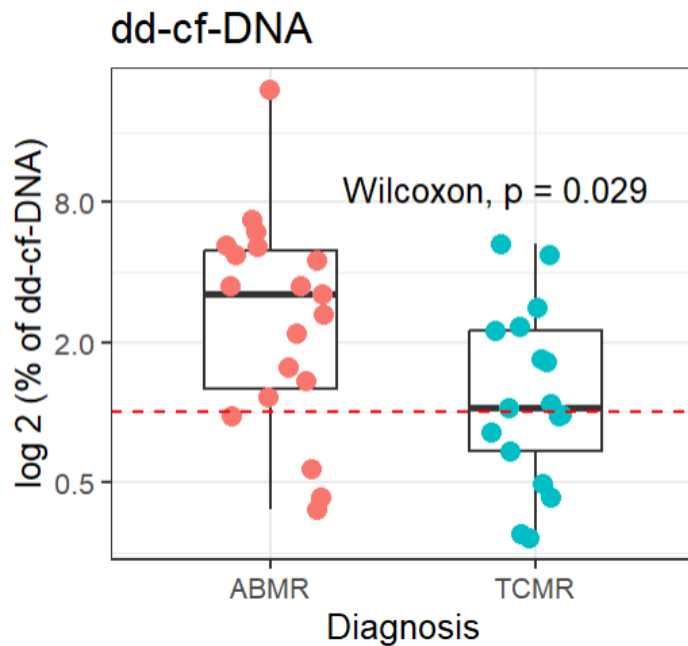
14 days post-transplantation



# Biopsy cohort demographic (n=36)

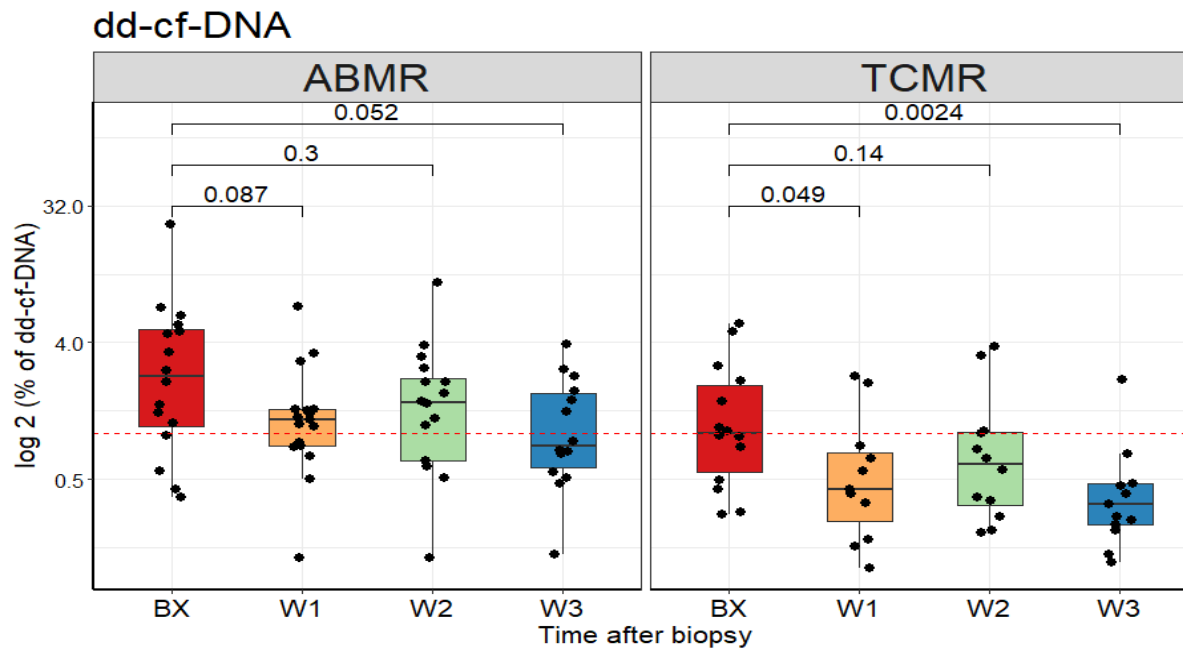
	dd cf-DNA<1% 12	dd cf-DNA>1% 24	p value
Recipient age, years	50 [34;68]	51 [19;71]	0.615
Donor age, years	53 [23;73]	49[31;80]	0.477
HLA mismatch	4 [2;5]	3 [1;6]	0.882
PRA	3 [0;47]	8 [0;97]	0.214
Dialysis vintage, years	1.3 [0;4.7]	1.5 [0;10.1]	0.404
<b>Biopsy POD</b>	13 [6;120]	370[7;11029]	<b>0.003</b>
Renal function, eGFR (ml/s/1.73m <sup>2</sup> )	0.5 [0.1;0.9]	0.48 [0.1;1.03]	0.638
dd-cf DNA (%)	0.53 [0.29;0.97]	3.02 [1.04; 24.4]	<0.001
Absolute number of dd-cf DNA	3179 [738;8880]	11150 [1472;123324]	0.001
<b>Type of Rejection</b>			
acute TCMR	8	6	
chronic TCMR	0	<b>3</b>	
acute ABMR	4	6	
chronic ABMR	0	<b>9</b>	

# dd cf-DNA higher in biopsies with antibody-mediated (ABMR) vs. T-cell mediated (TCMR) rejection



g, glomerulitis  
Histologic feature of ABMR

# The treatment of TCMR significantly decreased dd-cf DNA



**Figure 1.** The effect of treatment on the level of dd-cf DNA (in %) at week 1, 2 and 3 after indication biopsy (BX) with ABMR and TCMR. ABMR, antibody-mediated rejection. TCMR, T-cell mediated rejection.



# Conclusions from our study

---

- Increased levels of dd-cf DNA in the 1st month after transplantation limits its usage at this time as a marker of rejection
- Patients after retransplantation had often increased dd-cf DNA due to previous transplant
- dd-cf DNA predicts better antibody-mediated rejection (compared to T-cell mediated rejection)
- dd-cf DNA can be used to monitor non-invasively the effectivity of rejection treatment

# Acknowledgements

---

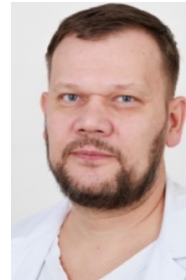
## IKEM, Prague



Ondrej Viklicky



Michal Schmalz



Vladimír Hanzal

## Natera, CA



Adam Prewett

Christopher Sotto



Lab team



Nurses, coordinators and patients