Antibodies and Their Interactions with the Kidney Allograft Tissue in Antibody Mediated Rejection

Presented by Dr. Ana Konvalinka

Moderated by Dr. Lakshman Gunaratnam

The webinar will begin shortly

Canadian Society of Nephrology/
Société canadienne de néphrologie
CSN/SCN

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LAND ACKNOWLEDGMENT

The Canadian Society of Nephrology Board of Directors has a commitment to diversity and inclusion.

We acknowledge that we are meeting on ancestral land that has been inhabited by Indigenous peoples from time immemorial. We are meeting virtually, so I would like to acknowledge that the Indigenous peoples are the traditional stewards of the lands and waters where each of us attends this meeting.

Ana Konvalinka
PhD, MD, FRCPC
Toronto, ON

Dr. Ana Konvalinka was recruited in 2015, as a transplant nephrologist and a Clinician Scientist at Toronto General Hospital, University Health Network. She is an Assistant Professor at the University of Toronto. Dr. Konvalinka completed medical studies at the University of Ottawa in 2003. She then completed internal medicine and nephrology training in Toronto in 2008. She subsequently embarked on a PhD in basic science at the University of Toronto. Her PhD thesis addressed the effect of angiotensin II on the proteome of primary human proximal tubular cells, and the relevance of this effect in vivo. Following completion of her PhD in 2013, she went on to complete the clinical kidney transplant fellowship at Toronto General Hospital. Her main clinical and research interests are in antibody-mediated rejection and kidney allograft fibrosis. She utilizes systems biology approaches and proteomics to enhance the understanding of the mechanisms, derive novel markers and to repurpose drugs for treatment of kidney disease. Dr. Konvalinka is the director of the Multi-Organ Transplant biobank for kidney, pancreas, and liver transplant programs. She is also the co-director of the Drug Discovery research group. She has received international research awards (the Human Proteome Project (2016), the American Society of Transplantation Faculty-Development Research Grant (2016) and the Advances in Organ Transplantation Award (2015)) and national research awards (Canadian Society of Nephrology New Investigator Lectureship (2017) and the KRESCENT New Investigator Award (2016)).

UHN Ajmera Transplant Centre

CSN Webinar

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Dr. Ana Konvalinka
Transplant Nephrologist, Clinician Scientist, University Health Network
Assistant Professor, University of Toronto
Senior Scientist, Toronto General Hospital Research Institute
Director, Ajmera Transplant Centre Multi-organ Transplant Biobank

Dec 13, 2022

OBJECTIVES

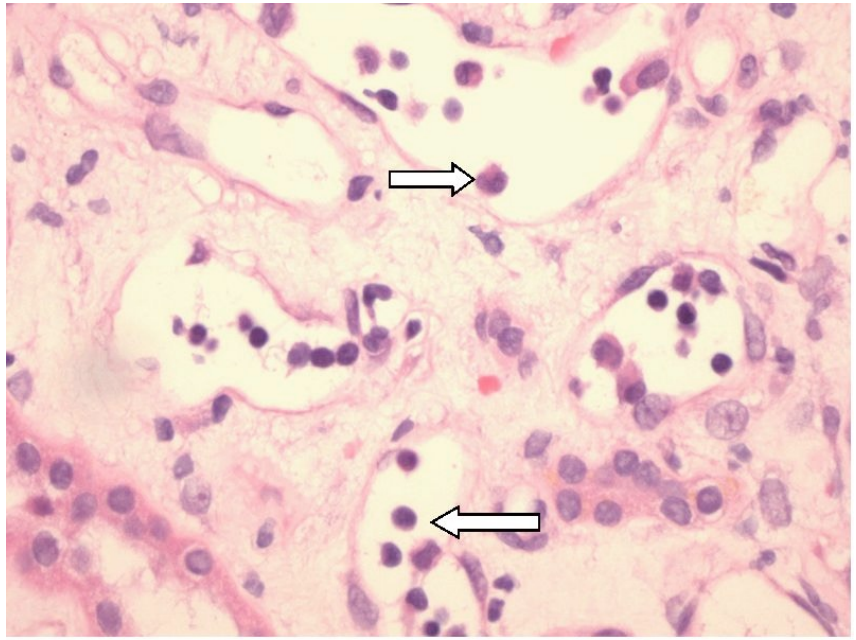
1. Provide an overview of antibody-mediated rejection in the kidney allograft
2. Describe the proteome changes in kidney glomeruli and tubulointerstitium in early antibody-mediated rejection
3. Review the importance of studying the extracellular matrix in the kidney
4. Describe novel approaches to delineate the biochemical features and roles of donor-specific antibodies in antibody-mediated rejection

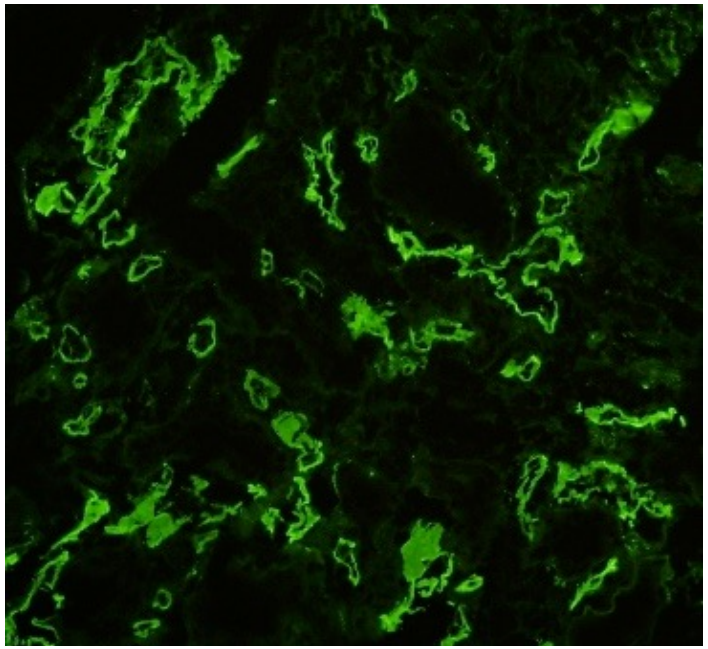
DISCLOSURES

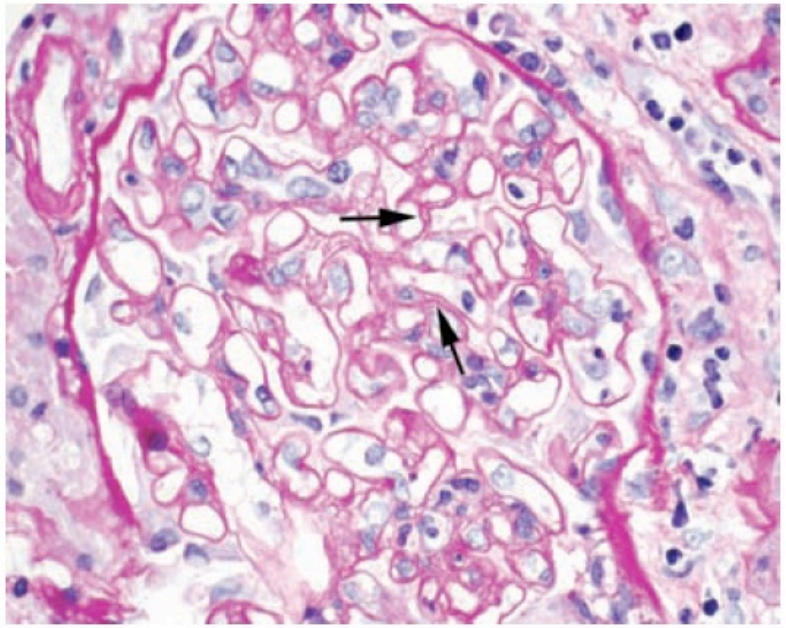
- Baxter (2022) - honoraria
- Promega - Academic access program

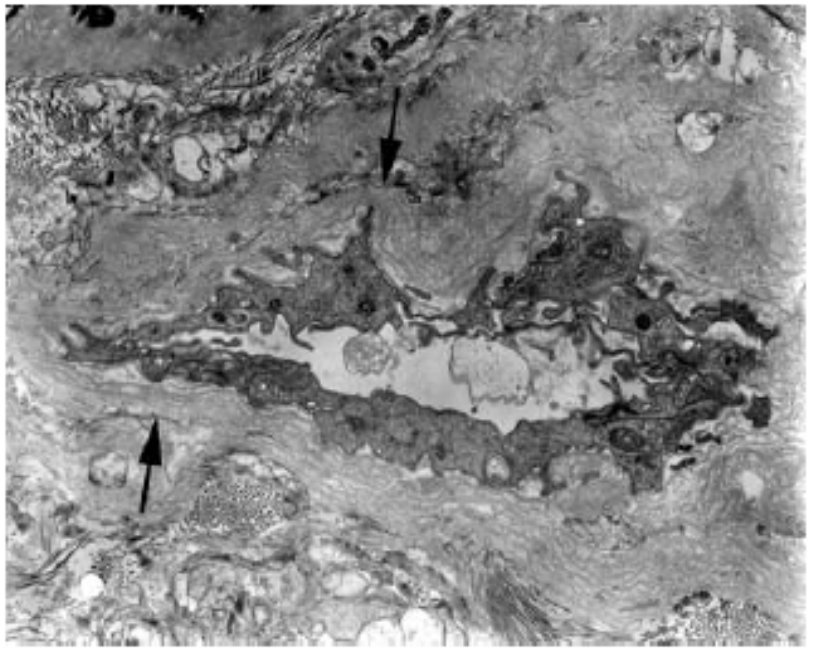
CASE 1

- 33F with ESKD due to IgA nephropathy
- Received a living donor kidney transplant 2 years previously
- Admits to non-adherence in the last few months; otherwise well
- Blood work reveals SCr of 210µmol/L from a base line of 120µmol/L
- US is normal
- New DSA against DQ6 is detected







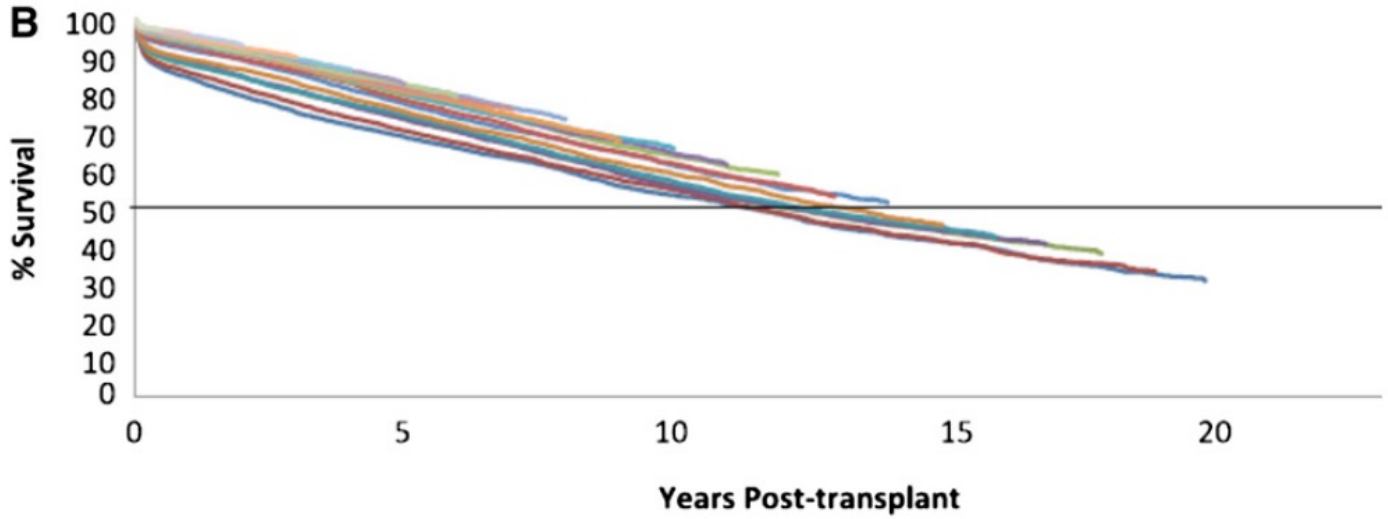


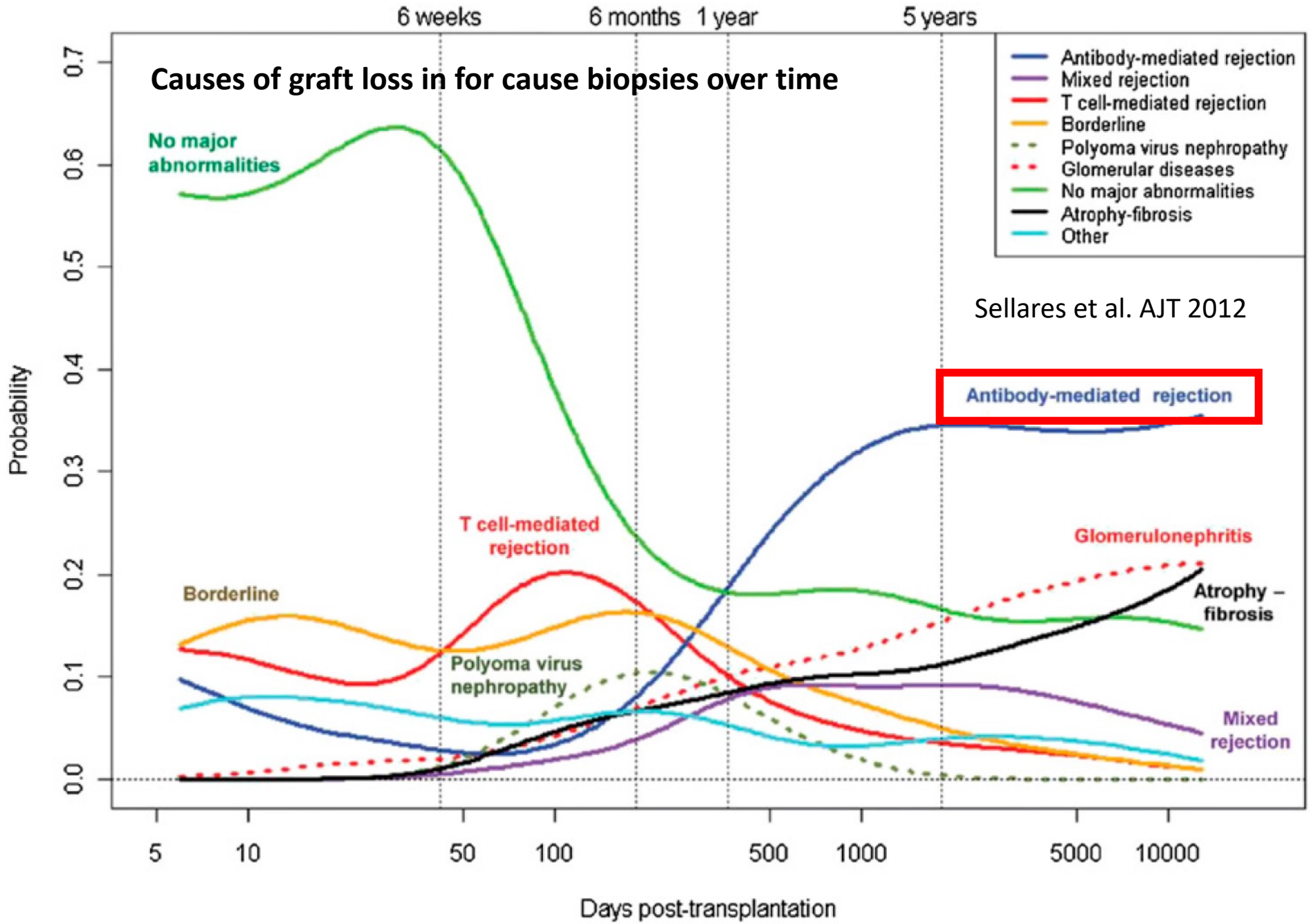
Overview

- Background
- Story #1 – Kidney glomerular and tubulointerstitial proteome in AMR
- Story #2 – Novel models to study AMR
- Story #3 – Assess circulating non-HLA antibodies in patients with AMR
- Future directions

BACKGROUND

- Kidney transplantation is the best treatment for end stage kidney disease
- The rates of early kidney allograft rejection have diminished
- However, long-term allograft survival has not changed significantly (Lamb et al. AJT 2011)




Causes of graft loss in for cause biopsies over time
6 weeks
6 months
1 year
5 years
Antibody-mediated rejection
Mixed rejection
T cell-mediated rejection
Borderline
Polyoma virus nephropathy
Glomerular diseases
No major abnormalities
Atrophy-fibrosis
Other
Sellares et al. AJT 2012
No major abnormalities
Antibody-mediated rejection
T cell-mediated rejection
Glomerulonephritis
Borderline
Atrophy – fibrosis
Polyoma virus nephropathy
Mixed rejection
Probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
5
10
50
100
500
1000
5000
10000
Days post-transplantation

Pathophysiology of AMR

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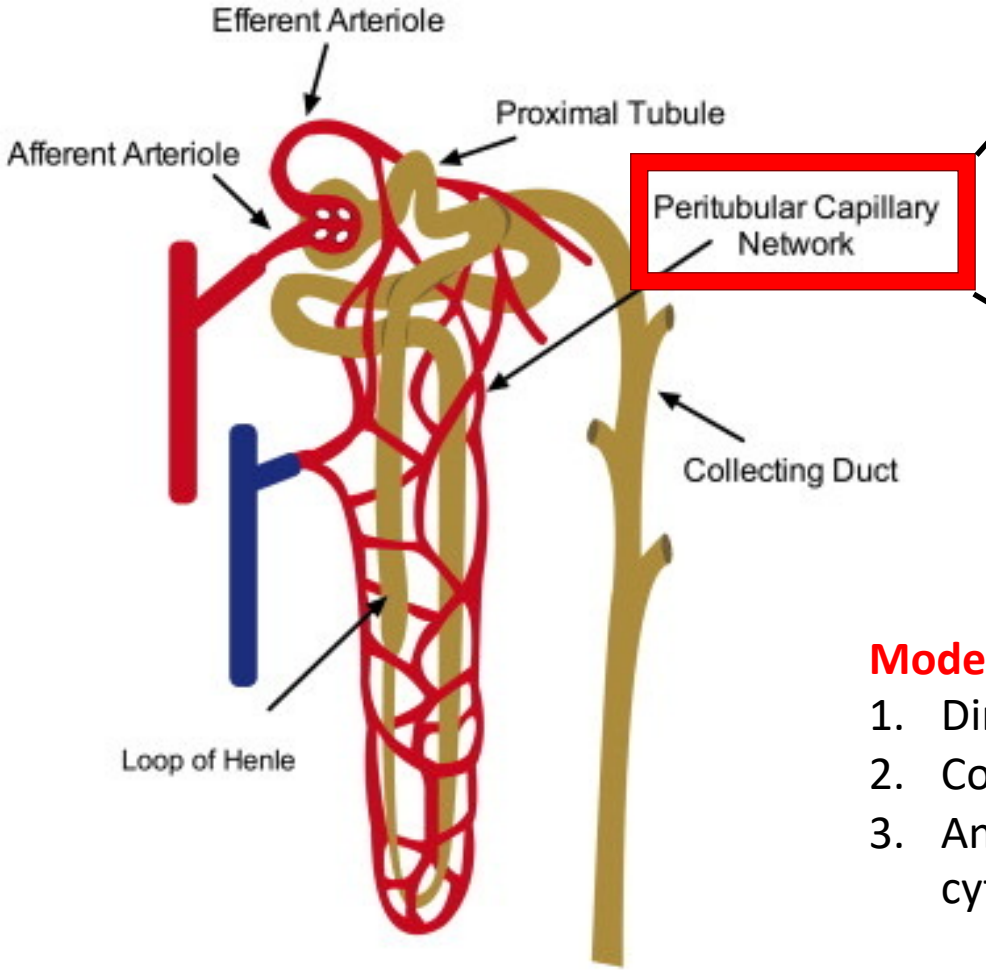

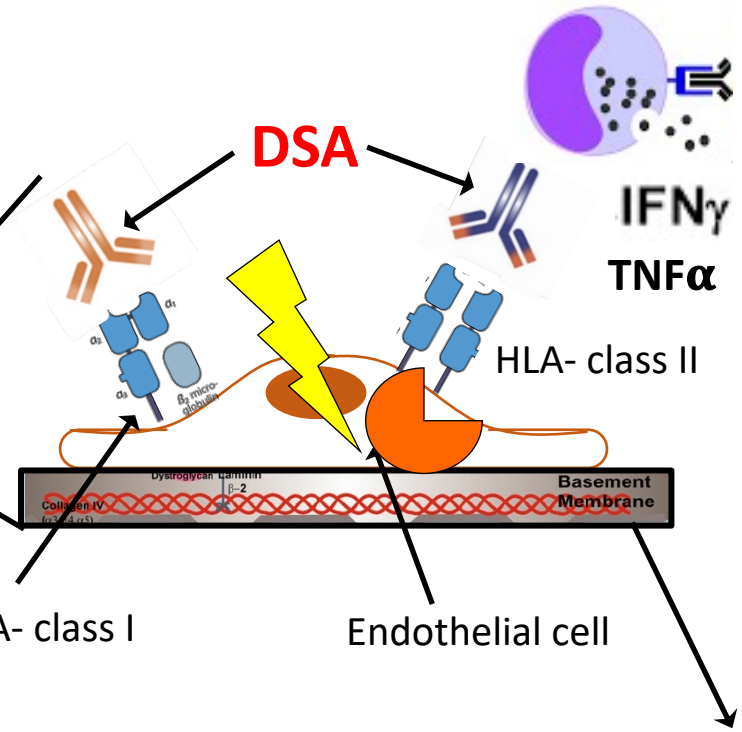

FcR interaction

Transplant glomerulopathy

Modes of injury by DSA:

1. Direct injury
2. Complement activation
3. Antibody-dependent cell-mediated cytotoxicity

Donor Specific Antibodies (DSA) – as a causal agent in AMR

- Class II HLA DSA appear more commonly after transplant and are associated with worse outcomes
- However, even in the presence of these antibodies, 30-60% of patients will do well
  - Yamamoto et al. *Transplantation* 2016
  - Matignon et al. *PLoS ONE* 2017
  - Heilman et al. *Transplantation* 2014
  - Lefacheur et al. *J Am Soc Nephrol* 2016
- Other DSA+ patients will experience AMR, TG and graft loss
  - Everly et al. *Am J Transplant* 2009, *Transplantation* 2010
  - DeVos et al. *Kidney Int* 2012
  - Willicombe et al. *Transplantation* 2012
  - Yabu et al. *Transplantation* 2011
- 40-60% of all AMR cases have no detectable DSA
  - Senev A, et al. Am J Transplant (2019) 19(3):763–80.
  - Bestard O, Grinyó J. Am J Transplant (2019) 19(3):952–3.
  - Koenig A, et al. Nat Commun (2019) 10(1):5350.

Gaps in knowledge

- The role of kidney tissue in antibody mediated rejection is poorly understood
- The most important non-HLA antibodies to be included in routine clinical monitoring are unknown
- Determination of pathogenic DSA is elusive
- Effective therapies are lacking

Gaps in knowledge

- The role of kidney tissue in antibody mediated rejection is poorly understood
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Story # 1

Assess proteome of glomerular and tubular compartments of kidney biopsies with AMR compared to other forms of graft injury

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Patient Characteristics

Group	Antibody-Mediated Rejection	Acute Cellular Rejection	Acute Tubular Necrosis
Number of patients	7	11	12
Sex - number of males (%)	4 (57.1)	9 (81.8)	9 (75)
Patient age at biopsy (years) median (IQR)	48 (43, 57.5)	42 (34, 48)	63.5 (57.5, 67.25)
Graft age (days post-transplant) median (IQR)	10 (8, 11)	16 (14.5, 57)	10 (8, 14)
Cause of ESRD (number)			
Diabetic Nephropathy	3	1	4
IgA Nephropathy	2	2	1
PCKD	0	2	3
FSGS	0	1	1
Vasculitis	0	2	0
Unknown	1	0	1
Other	1	3	2
Pre-existing autoimmune condition (number of patients)	1	1	0
Donor Type			
Deceased Donor	2	4	10
Living Donor	5	7	2
DSA pre-transplant (No of Patients)	4	0	4
Class 1	3	0	4
Class 2	3	0	0
Prior desensitization- number (%)	2 (28.5)	0	0
Induction Agent - number (%)			
ATG	5 (71.4)	6 (54.54)	12 (100)
Basiliximab	2 (28.57)	5 (45.45)	0
Maintenance immunosuppression – number (%)			
Calcineurin Inhibitor	7 (100)	11 (100)	12 (100)
Anti-proliferative	7 (100)	11(100)	12 (100)
Prednisone	7 (100)	11(100)	12 (100)

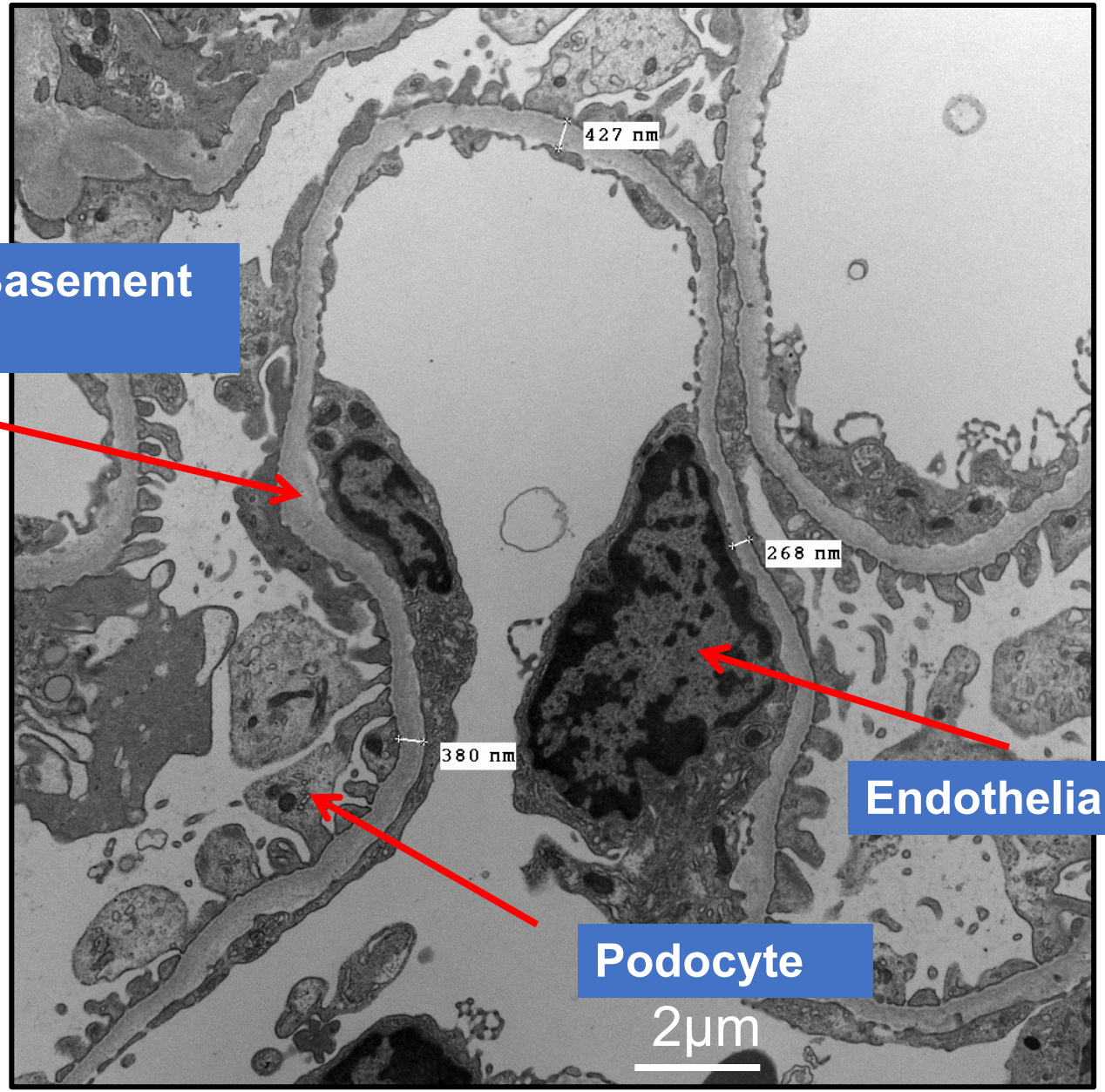
Dr. Joseph Kim

Patient Characteristics

Group	Antibody-Mediated Rejection	Acute Cellular Rejection	Acute Tubular Necrosis
Biopsy Findings:			
Acute tubular necrosis (ATN)	5/7	3/11	12/12
Antibody-mediated rejection (AMR)	7	0	0
Acute cellular rejection (ACR)	0	11	0
Grade: 1A	NA	1	NA
1B	NA	7	NA
2A	NA	3	NA
Banff Scoring- median (IQR)			
% globally sclerosed glomeruli (gsg)	0 (0, 4.38)	3 (0, 5)	1 (0, 5.43)
Interstitial Inflammation (i)	1 (0, 1)	2 (2, 2)	0 (0, 0)
Tubulitis (t)	0 (0, 0)	3 (2.5, 3)	0 (0, 0)
Total inflammation (ti)	1 (0, 1)	2 (2, 3)	0 (0, 0)
Glomerulitis (g)	1 (0.5, 1.5)	0 (0, 0)	0 (0, 0)
Peritubular capillaritis (ptc)	1 (0, 2)	1 (0, 1)	0 (0, 1)
Intimal arteritis (v)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Chronic Glomerulopathy (cg)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Interstitial Fibrosis (ci)	0 (0, 0)	0 (0, 1)	0 (0, 0)
Tubular atrophy (ct)	0 (0, 0)	1 (0.5, 1)	1 (0, 1)
Arteriolar hyalinosis (ah)	0 (0, 0)	0 (0, 0.5)	0 (0, 0)
Vascular Fibrous Intimal Thickening (cv)	0 (0, 0)	1 (0, 1)	1 (0, 1.25)
C4D Staining	2 (1.5, 3)	0 (0, 0)	0 (0, 0)

Dr. Rohan John

Histopathology



Glomerular Basement Membrane

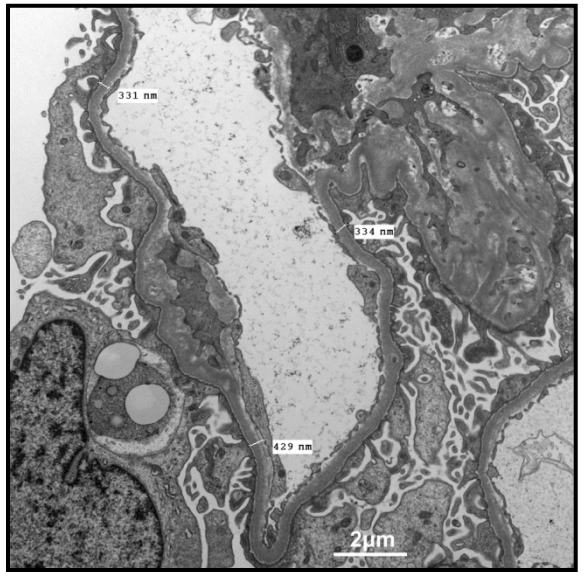
Endothelial cell

Podocyte

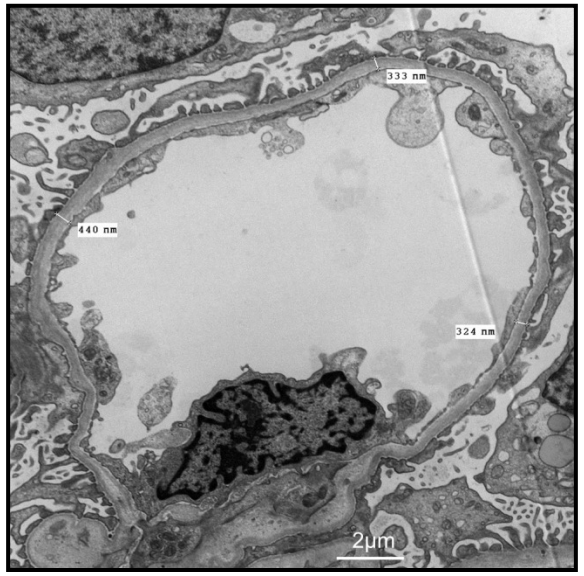
Histopathology

- No changes in <u>Glomerular Basement Membrane Thickness</u>

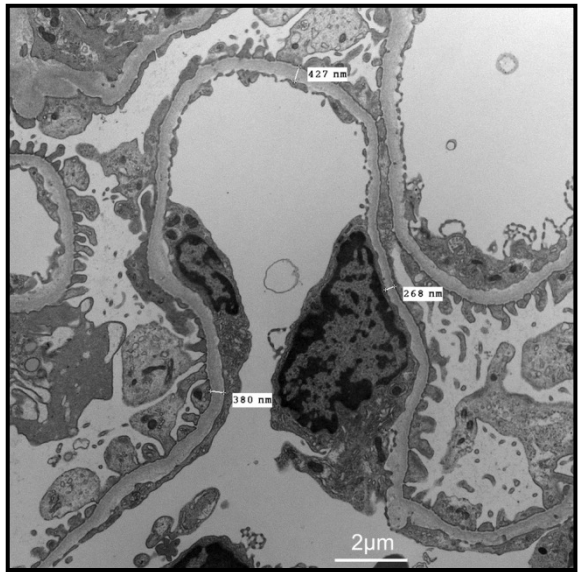
AMR



ACR

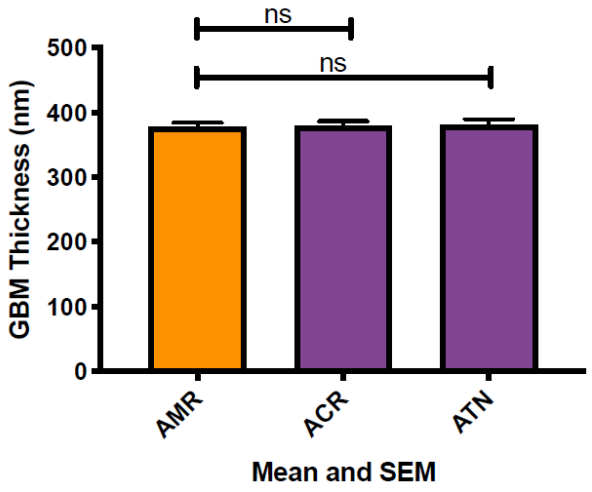


ATN

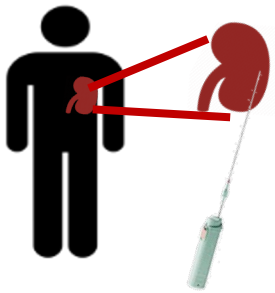


Dr. Rohan John

Dr. Caitriona McEvoy



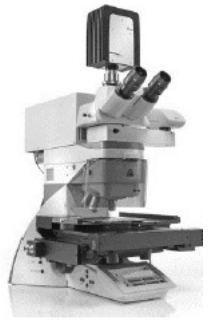
METHODS



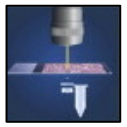
AMR (n = 7)
ACR (n = 11)
ATN (n = 12)

FFPE Kidney Biopsy

8µm sections



Laser capture microdissection

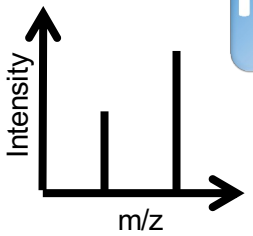


Tubulointerstitium

Glomeruli

Sample preparation

Dr. Sergi Clotet-Freixas



Q-Exactive

Mass spectrometry

Differentially expressed proteins

Gene ontology and pathway analysis

pathDIP

- Clinical verification
- IHC/IF of human biopsies
- *In vitro* studies

Clotet-Freixas S, *et al.*, *JASN*, 2020

RESULTS

GLOMERULAR compartment

2026 proteins identified

↓

1299 proteins quantified in at least 50% samples/group

TUBULOINTERSTITIAL compartment

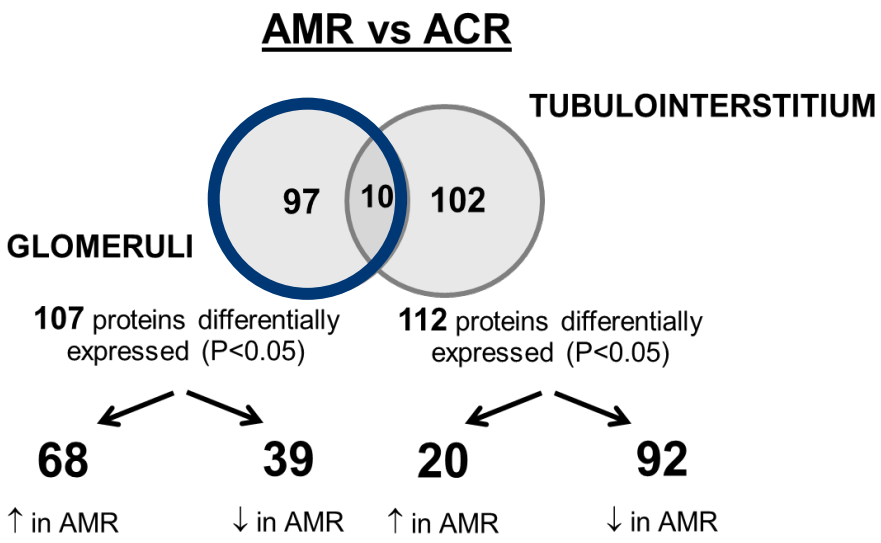
2399 proteins identified

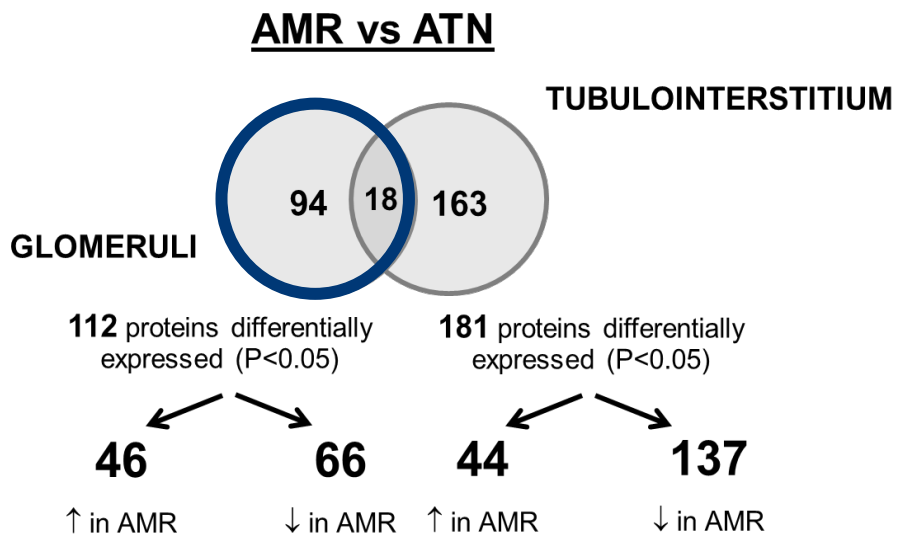
↓

1842 proteins quantified in at least 50% samples/group

Clotet-Freixas S, *et al*., *JASN*, 2020

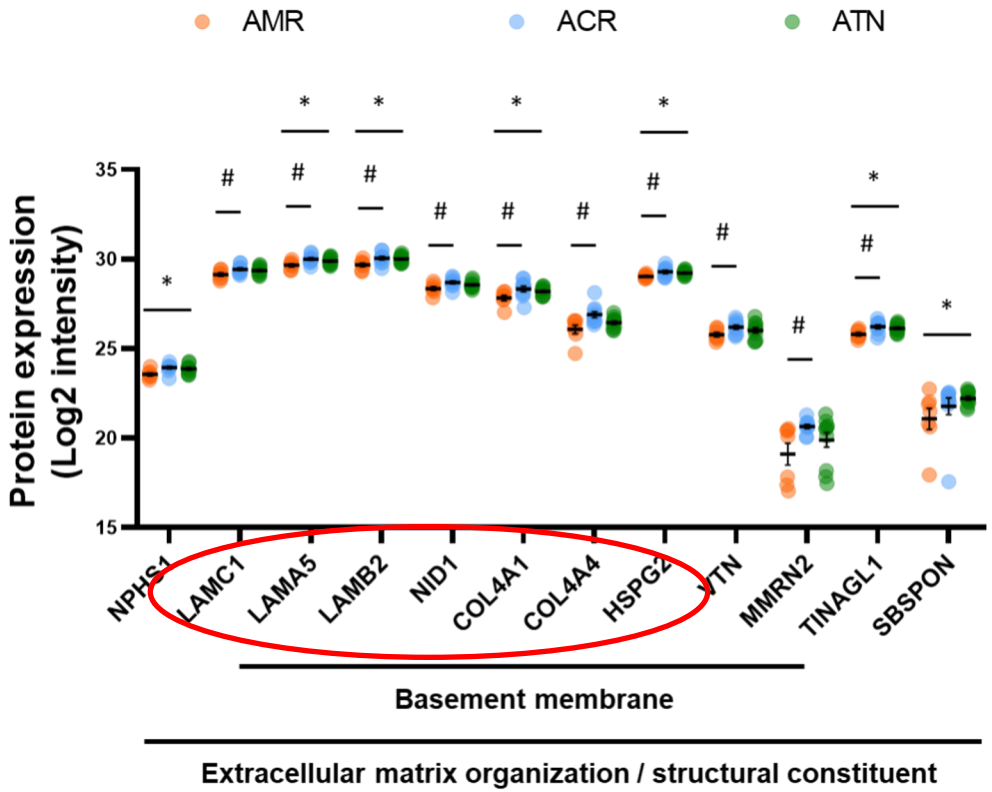
DIFFERENTIALLY EXPRESSED PROTEINS





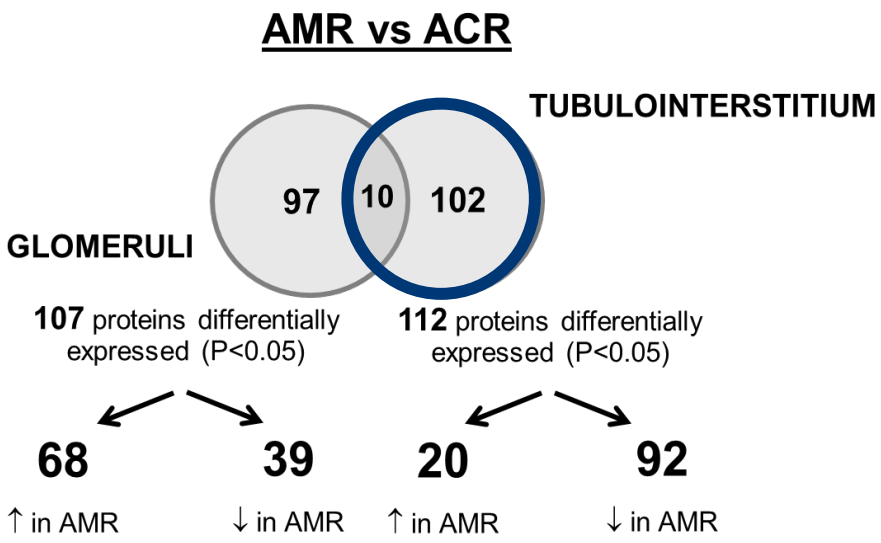
Clotet-Freixas S, *et al.*, *JASN*, 2020

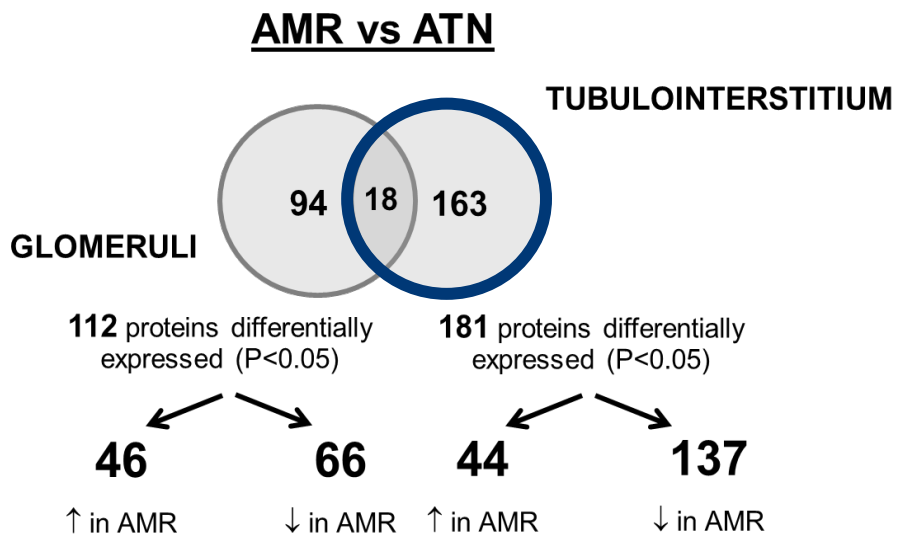
Glomerular AMR: Down-regulated proteins



Clotet-Freixas S, *et al.*, *JASN*, 2020

DIFFERENTIALLY EXPRESSED PROTEINS

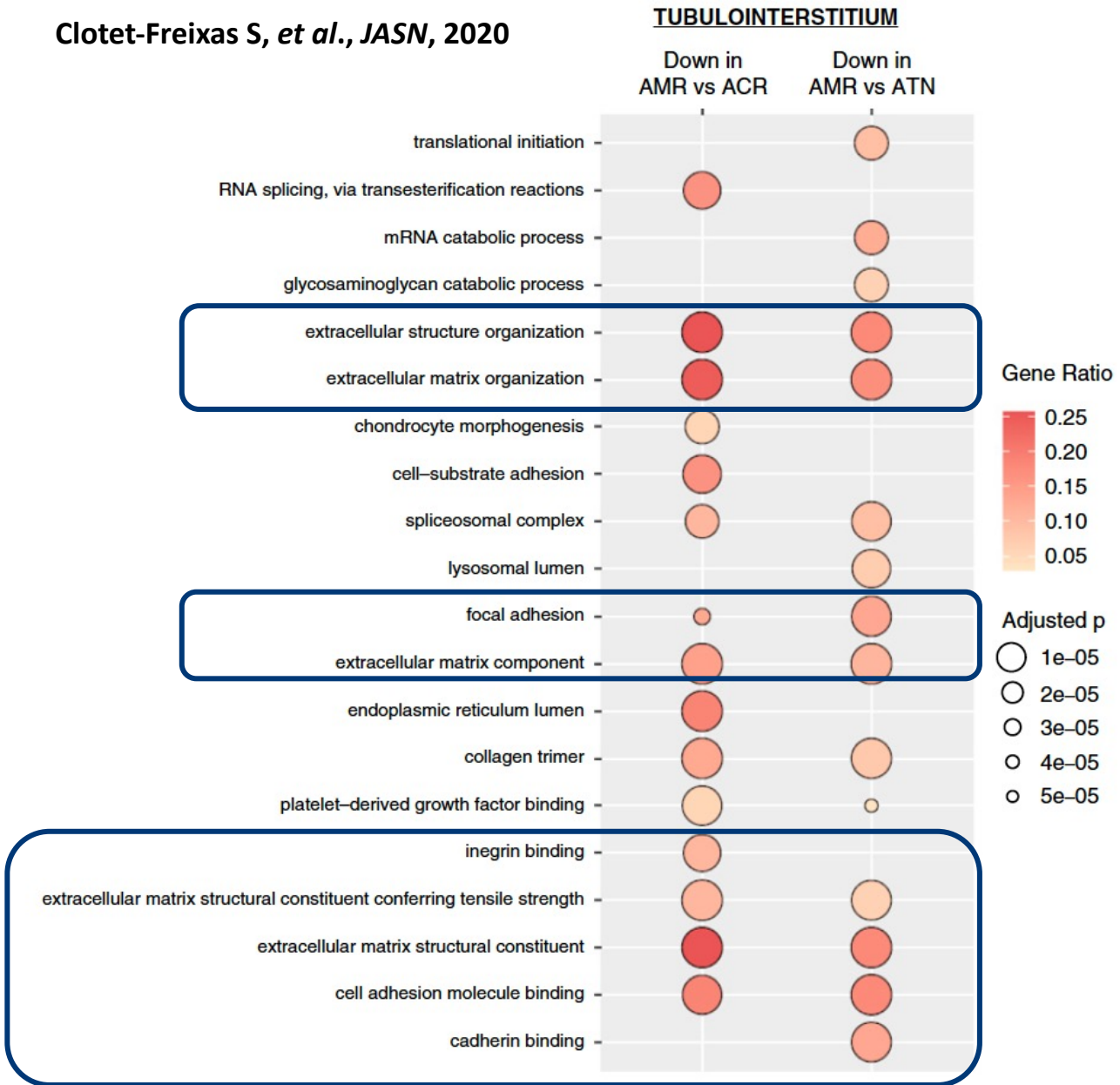


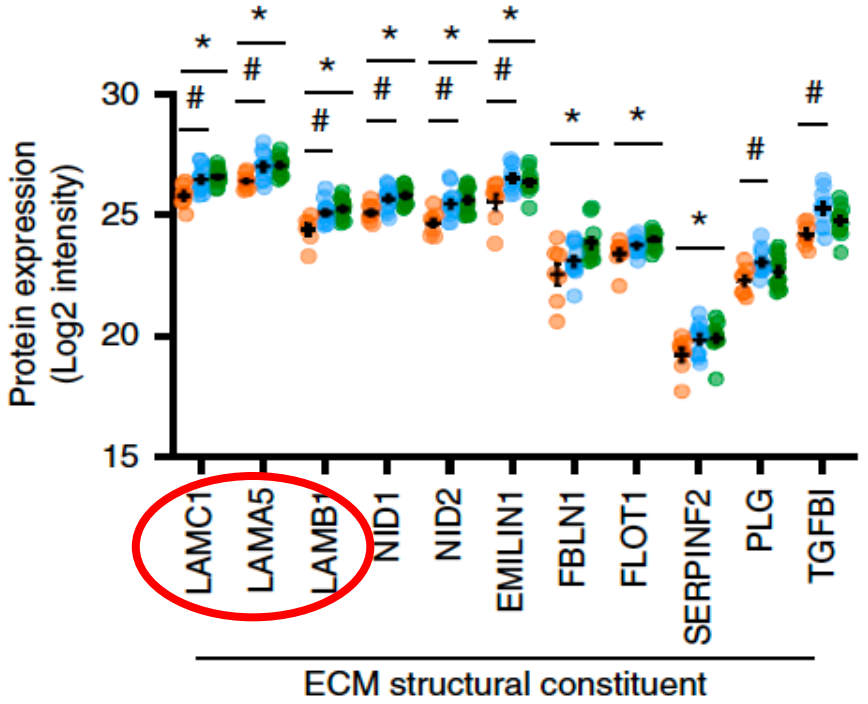


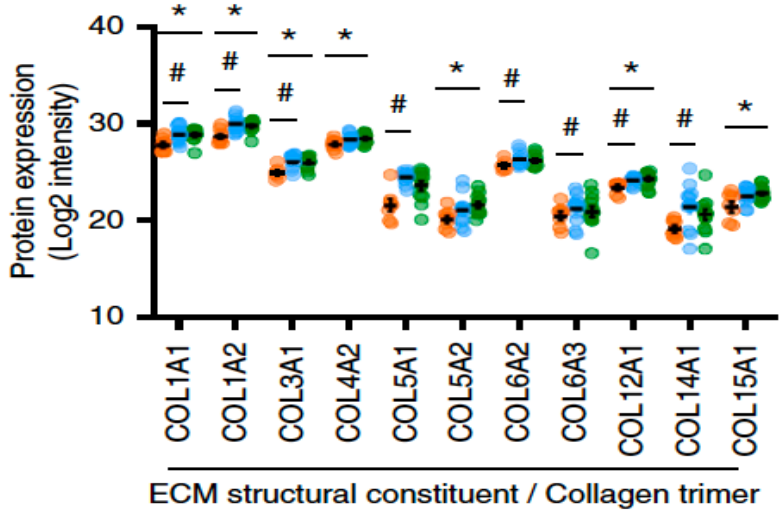
Clotet-Freixas S, *et al.*, *JASN*, 2020

Tubulointerstitial AMR: Down-regulated proteins

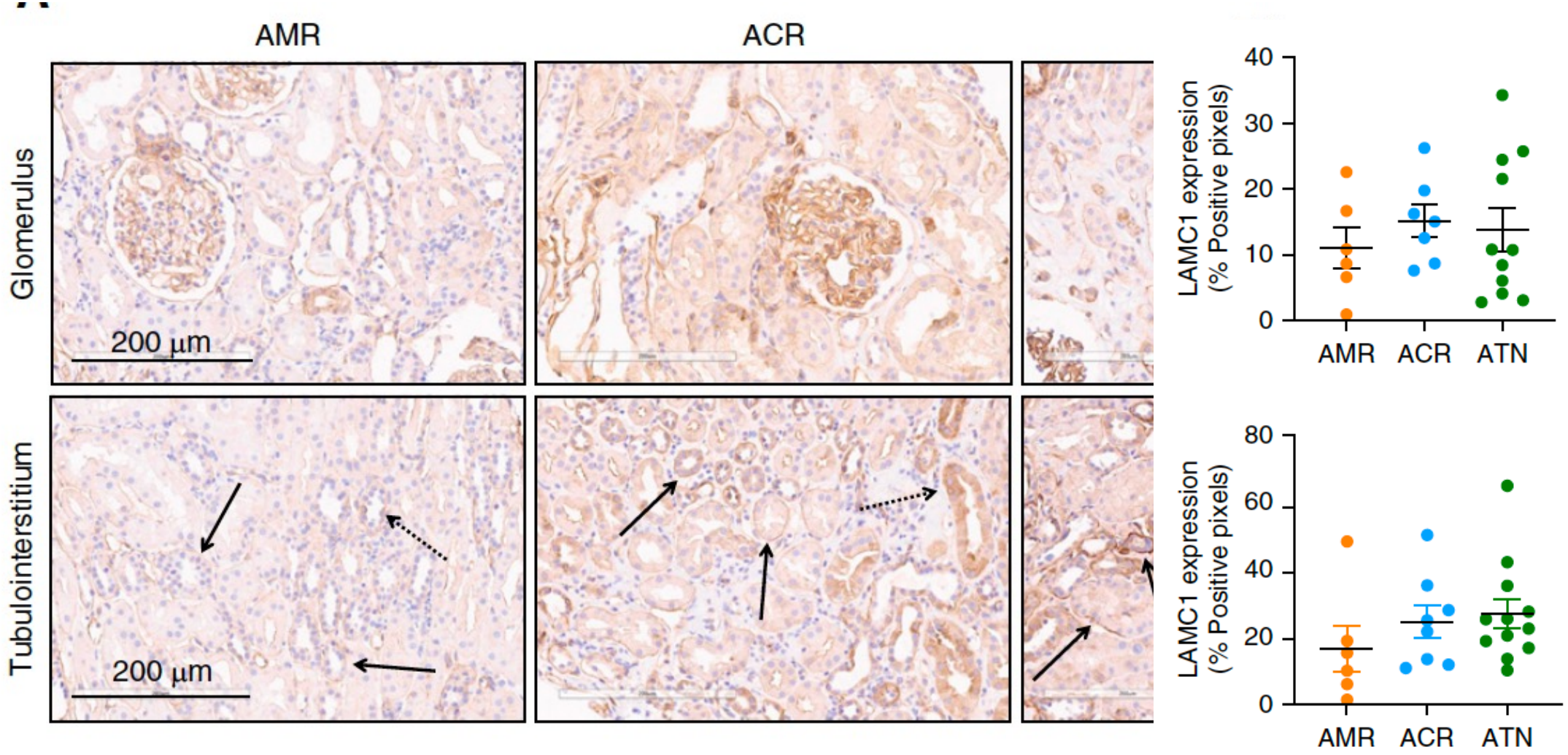
Clotet-Freixas S, *et al.*, *JASN*, 2020







LAMC1 Immunohistochemistry



Clotet-Freixas S, *et al.*, *JASN*, 2020

Glomerular and tubulointerstitial proteome in AMR

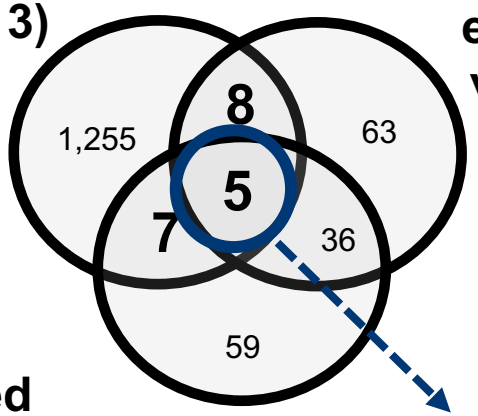
- Basement membrane and extracellular matrix proteins were significantly decreased in both compartments, even in the absence of histological lesions consistent with extracellular matrix remodeling

- Some proteins (e.g. LAMC1) were common to both compartments, while others were compartment specific

Glomerular AMR: External data set analysis

1,275 genes differentially expressed in AMR biopsies (Sellares et al. *Am J Transplant* 2013)

112 proteins differentially expressed in AMR vs ATN Glomeruli



107 proteins differentially expressed in AMR vs ACR Glomeruli

Galectin 1 (LGALS1) → UP in AMR

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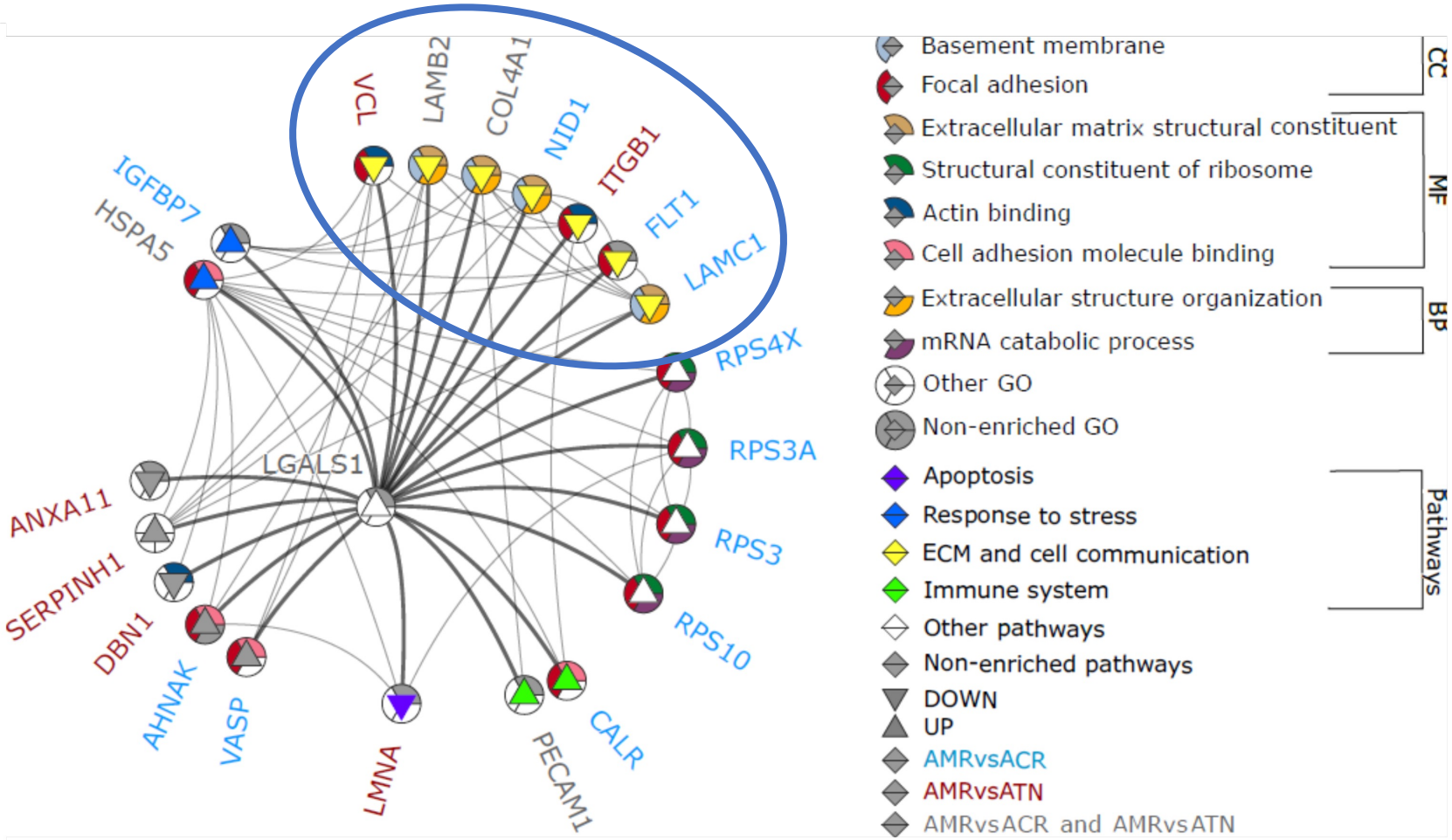
Galectin-1 (LGALS1) in glomerular AMR

Dr. Igor Jurisica



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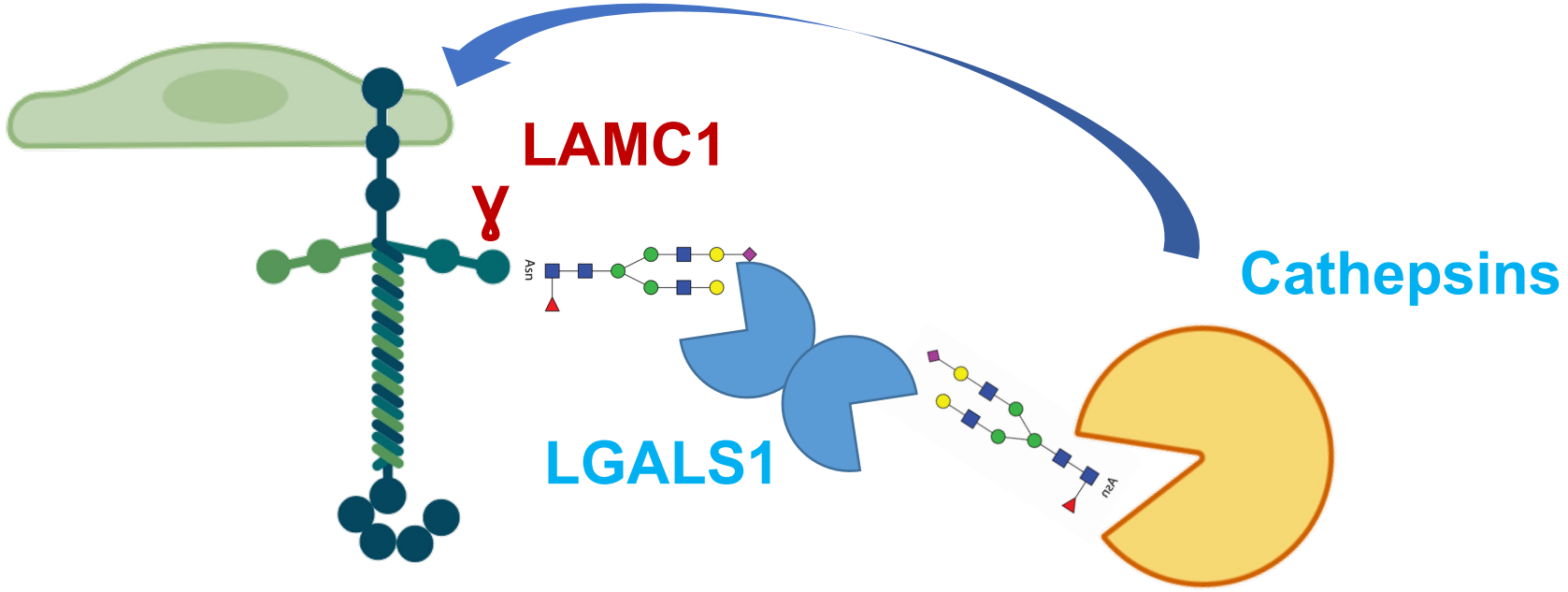
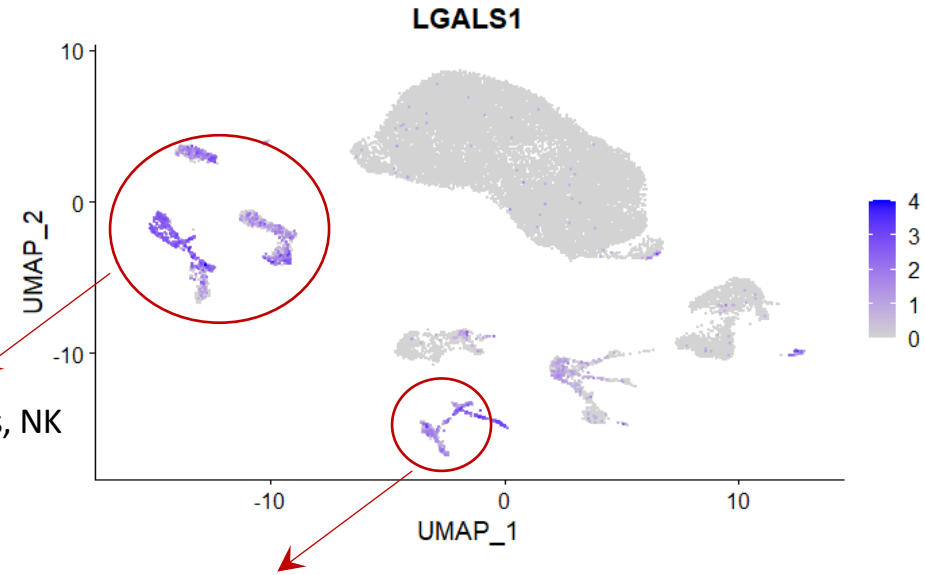
LGALS1: An interesting protein

- **Interacts with the ECM** by recognizing β-galactose sugar moieties of proteins such as laminin and fibronectin (Ilarregui, *et al*., *Annals of the Rheumatic Diseases*, 2005).
- **Interacts with cathepsins**, which in turn induce its production in endothelial cells (Pranjol, *et al*., *J Transl Med*, 2019).



- **Remodels endothelium** to prevent migration of T cells into the tumor microenvironment (Nambiar, *et al*., *J Clin Invest*, 2019 and He et al. *Lab Invest* 2006).

Where is LGALS1 Expressed in the Healthy Human Kidney?



Immune cells:
Monocytes/macrophages, NK cells, and T-cells

Endothelial cells

Dr. Caitriona McEvoy

Julia Murphy

Dr. Sarah Crome

Data from:
McEvoy, Murphy,….., Konvalinka*, Crome*, ***Nature Communications 2022***

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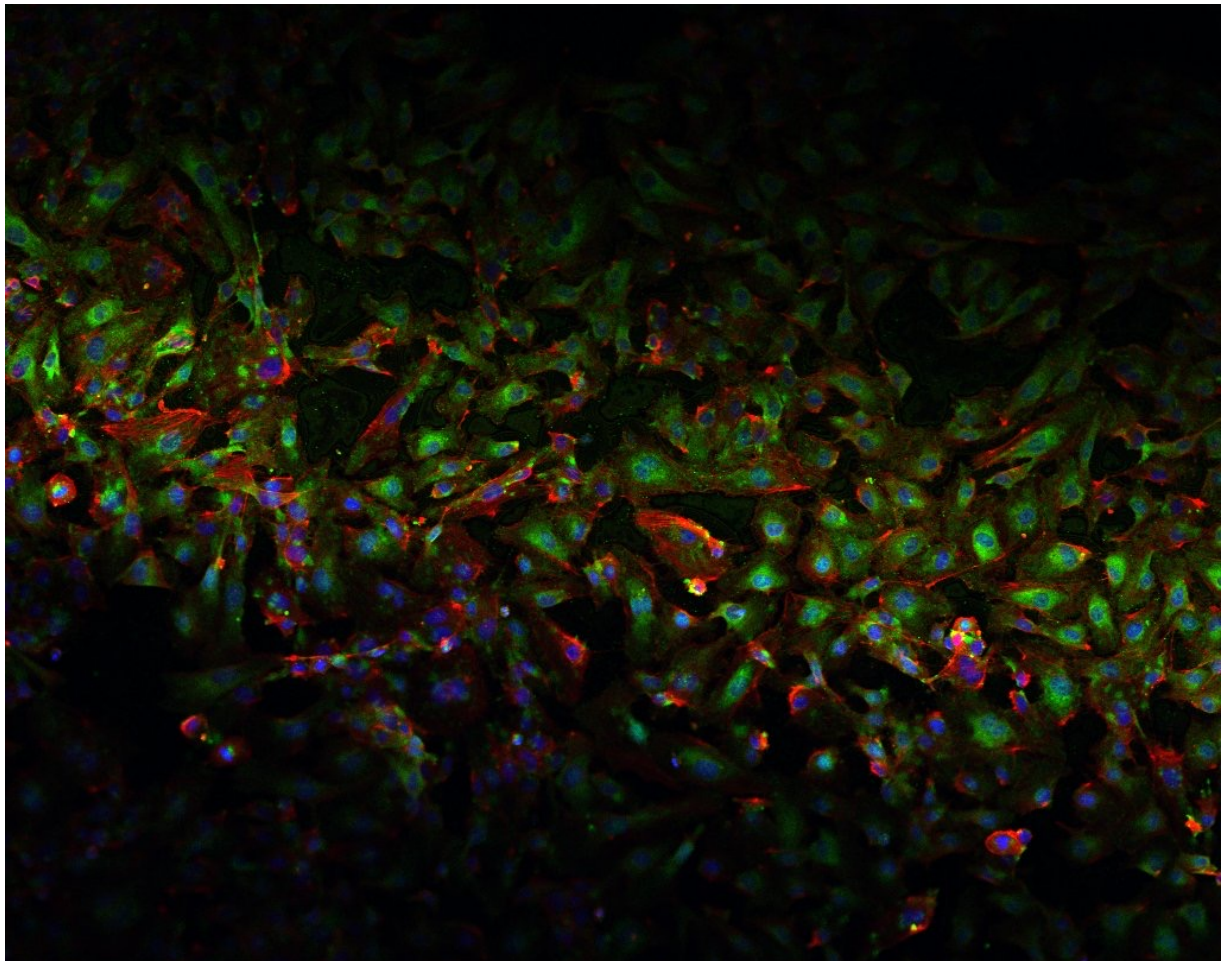
LGALS1 protein expression in kidney endothelial cells

Alex Boshart
PhD student

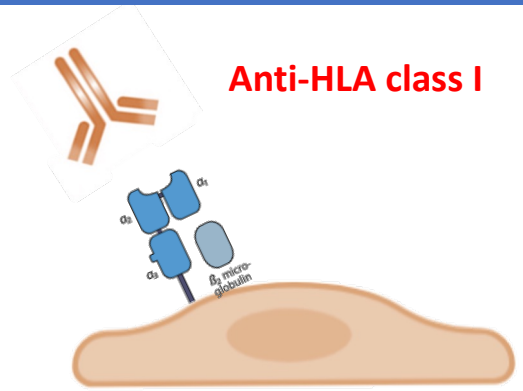
Legend:
- DAPI
- Actin
- LGALS1



UHN Ajmera Transplant Centre

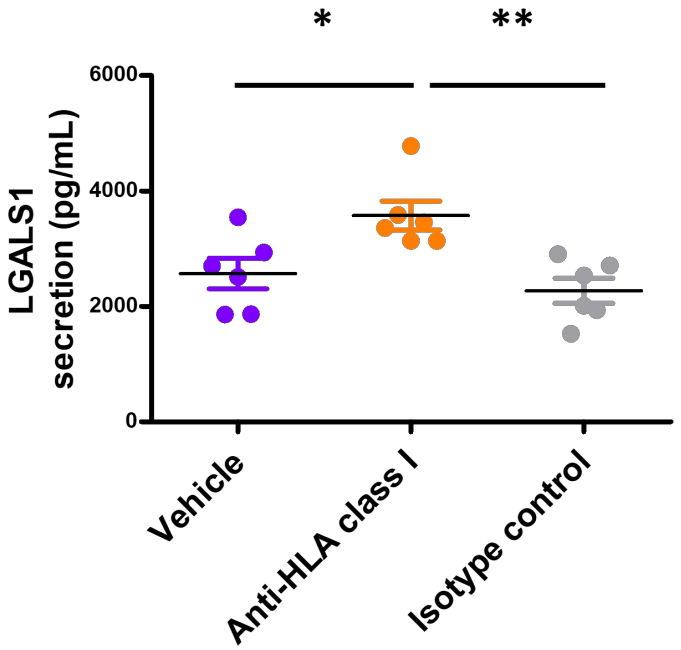
LGALS1 in glomerular AMR

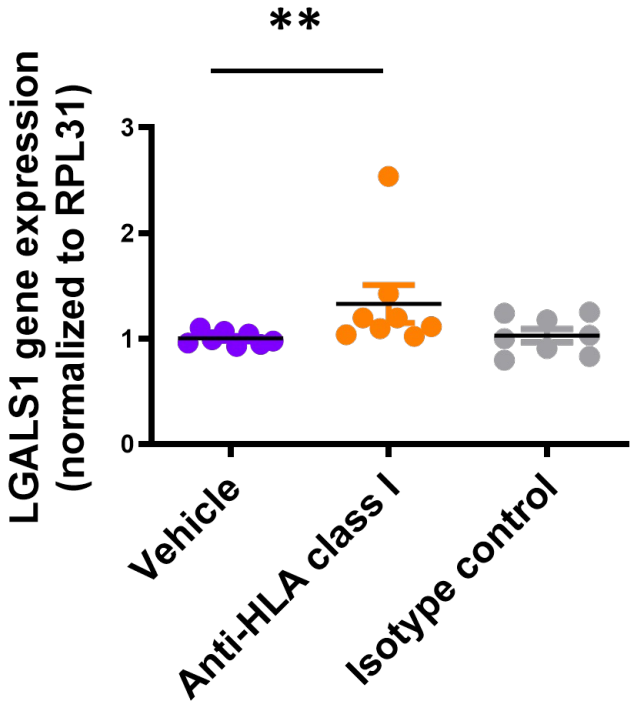
Anti-HLA class I



Primary Human Glomerular Microvascular Endothelial Cells (HGMECs)

Alex is now knocking down LGALS1 in these cells and studying their phenotype





*p < 0.05 **p < 0.01

Clotet-Freixas S, *et al.*, *JASN*, 2020

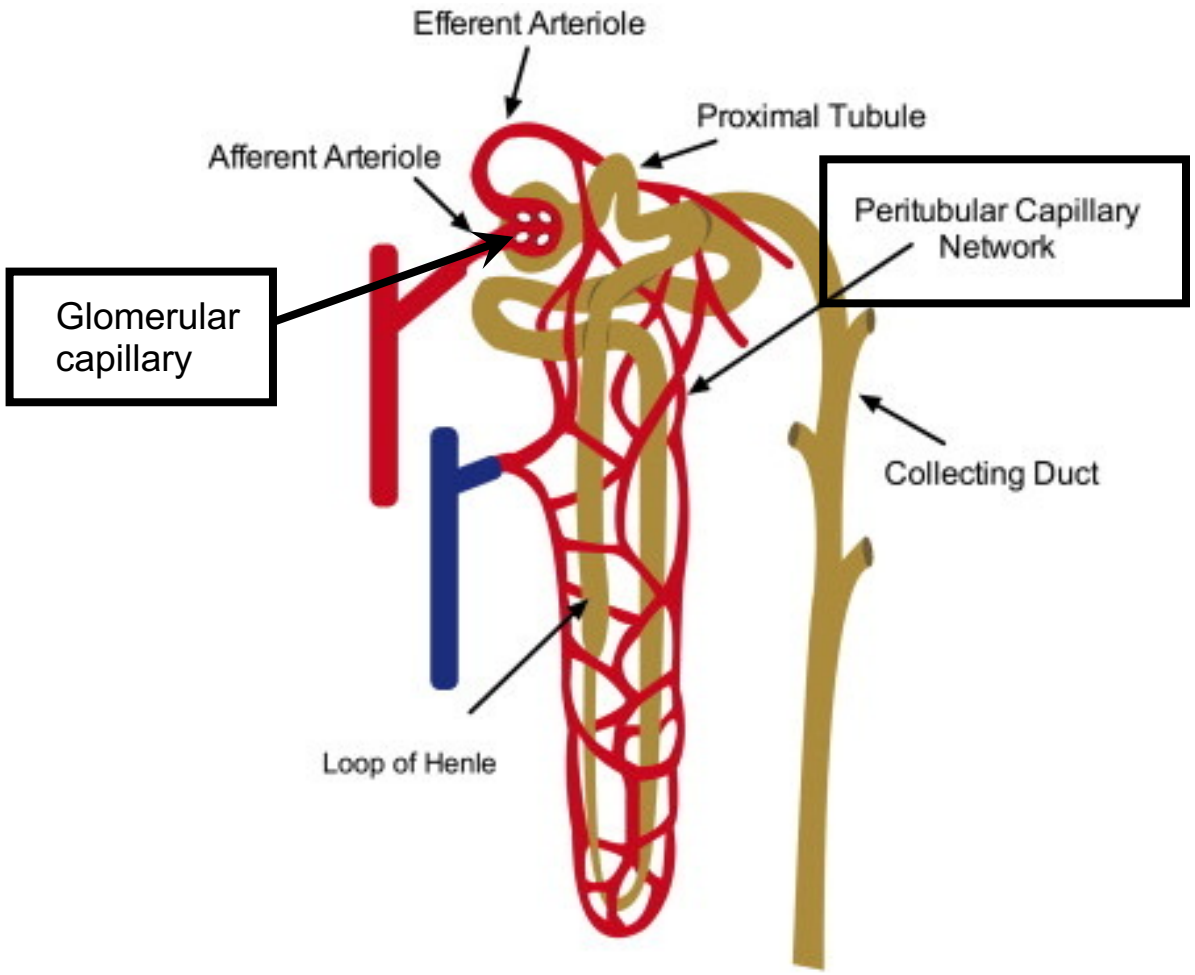
STORY #2

Novel Models to Study AMR in the Kidney Allograft

UHN Ajmera Transplant Centre

Dr. Boyang Zhang

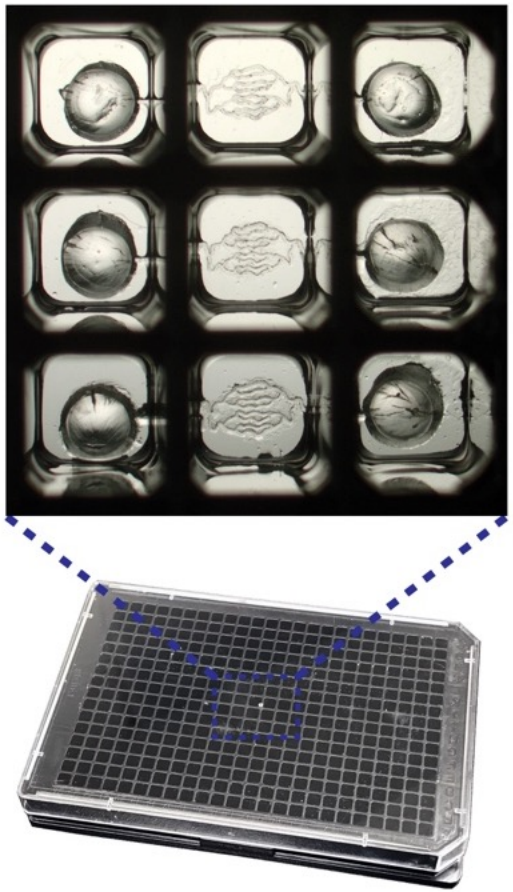
Dr. Milica Radisic

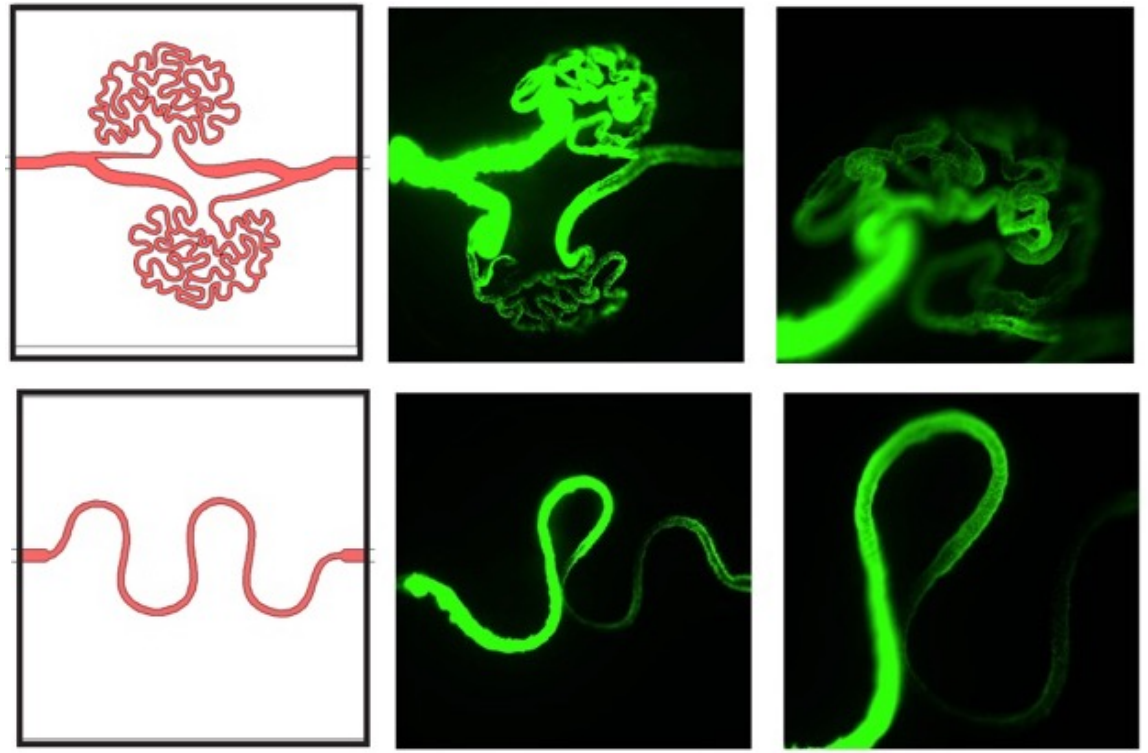


DEVELOPMENT OF NEW MODELS TO STUDY AMR

Dr. Boyang Zhang

Kidney-on-a-chip





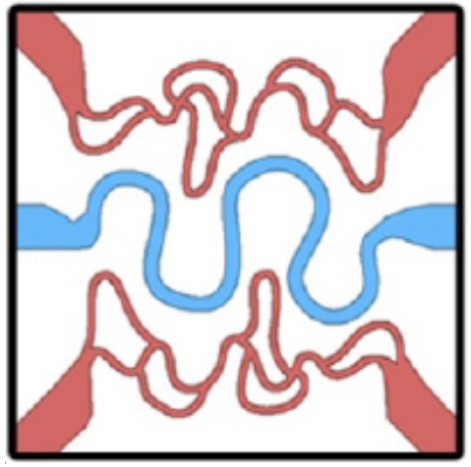
Rajasekar, *et al.*, *Lab on a Chip*, 2022

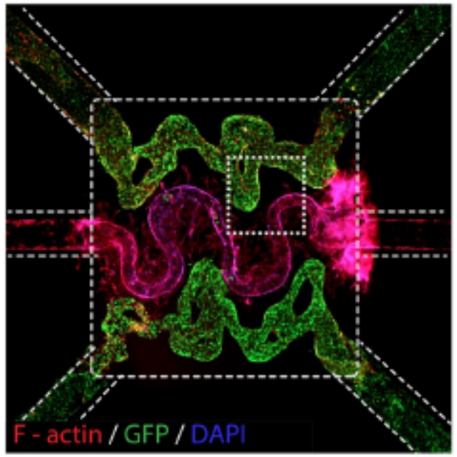
UHN Ajmera Transplant Centre

PERITUBULAR CAPIILLARY WITH PROXIMAL TUBULE

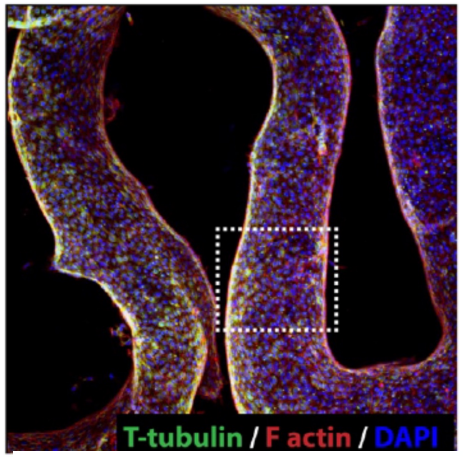
Dr. Boyang Zhang

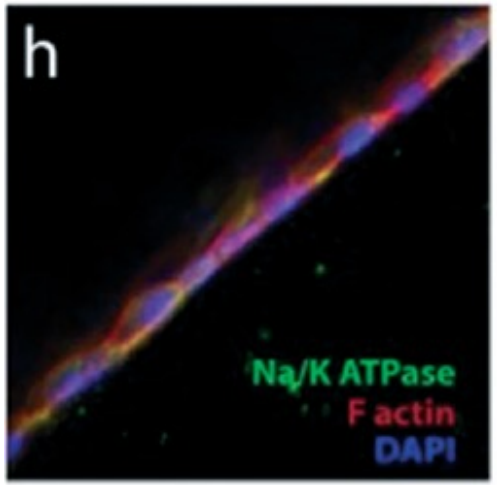
Kidney micro-capillary model

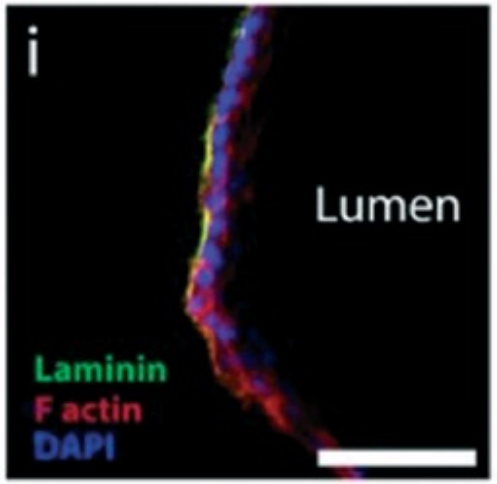


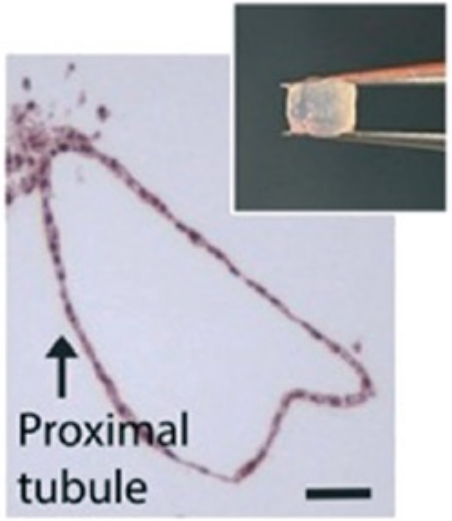


Rajasekar, *et al.*, *Lab on a Chip*, 2022

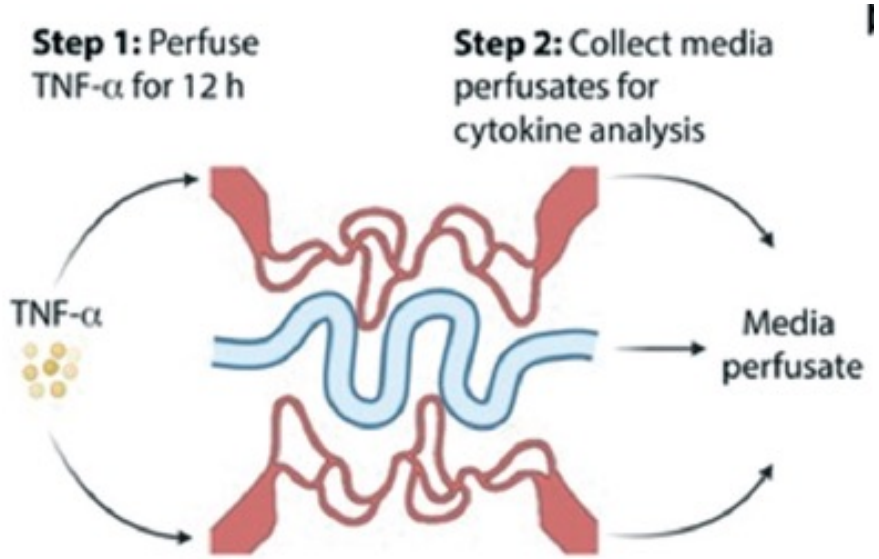


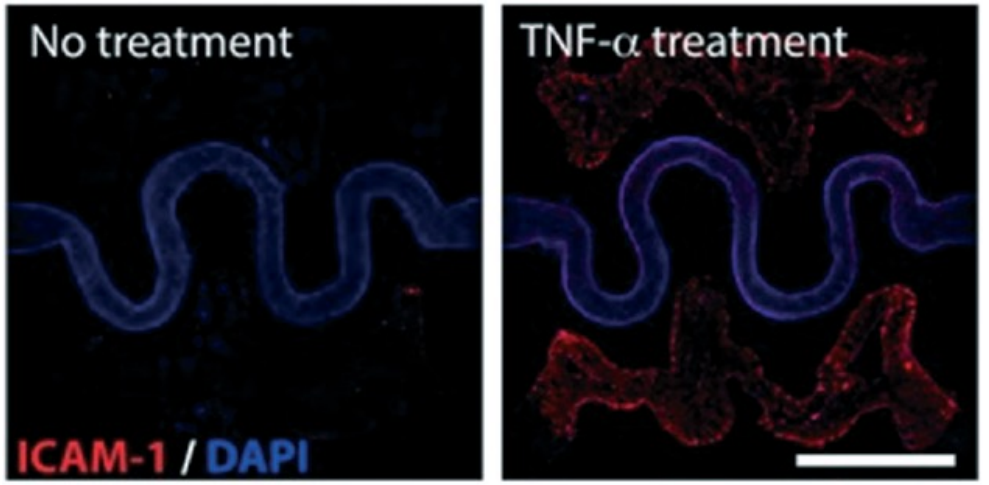






PERITUBULAR CAPIILLARY WITH PROXIMAL TUBULE





Rajasekar, *et al.*, *Lab on a Chip*, 2022

CONCLUSIONS

- We have identified common and compartment-specific changes in the basement membrane proteins in the absence of transplant glomerulopathy
- LGALS1 may be a potential therapeutic target as it communicates with the basement membrane proteins and immune cells and can modify inflammation
- Novel models to study AMR in peritubular or glomerular capillary bed will enable us to integrate immune cells with endothelial cells and to perform drug screening

Gaps in knowledge

- The role of kidney tissue in antibody mediated rejection is poorly understood
- The most important non-HLA antibodies to be included in routine clinical monitoring are unknown
- Determination of pathogenic DSA is elusive
- Effective therapies are lacking

Non-HLA Antibodies

AT-1 receptor agonistic antibodies

Dragun et al. *NEJM* 2005

Anti-Angiotensinogen Abs

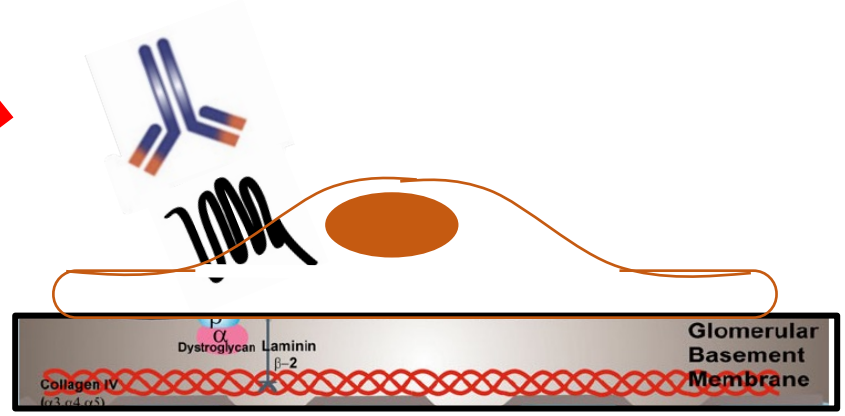
Li et al. J Proteome Res 2010

LG3 perlecan fragment antibodies

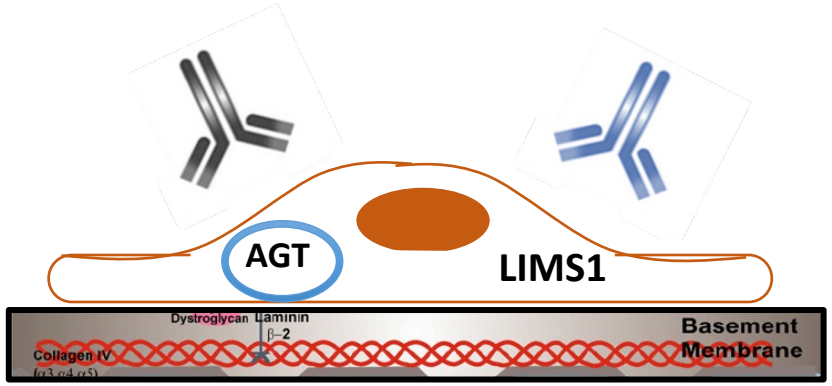
Cardinal et al. *AJT* 2013, Hebert *Circ Res* 2012

Anti-LIMS1 Abs in kidney allograft rejection

Steers et al. N Engl J Med 2019



Donor **endothelial** cell



Donor **epithelial** cell

UHN Ajmera Transplant Centre

Story # 3

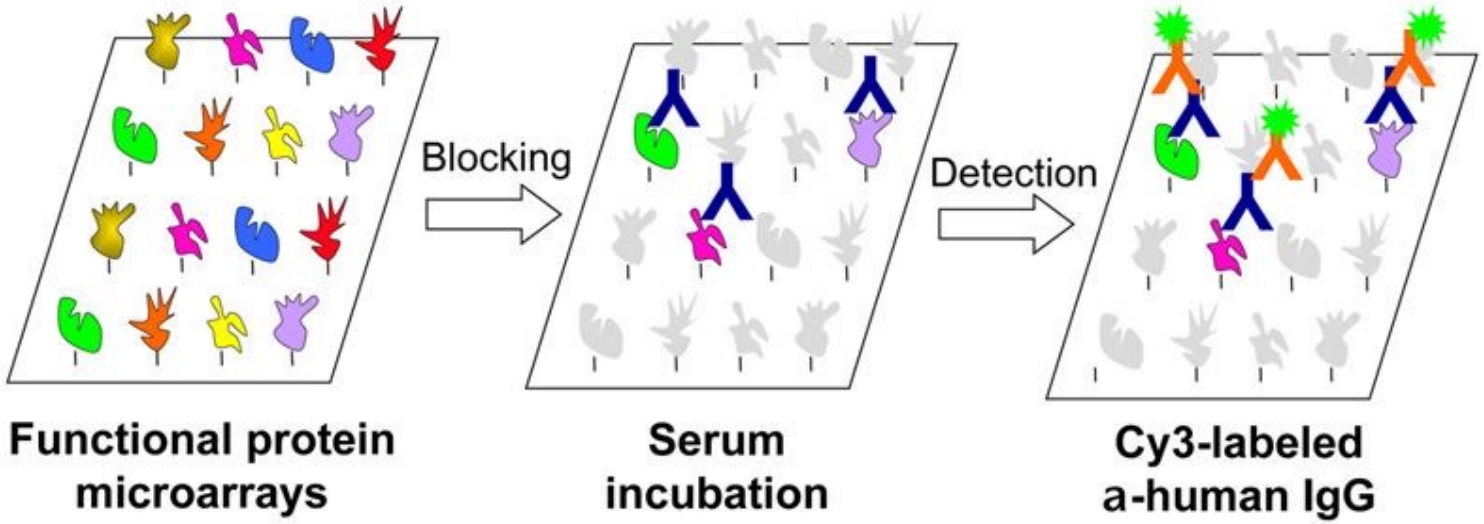
Assess circulating non-HLA antibodies in patients with AMR compared to other forms of graft injury

UHN Ajmera Transplant Centre

Dr. Sonia Rodriguez Ramirez
Post-doctoral fellow

Clotet-Freixas, *et al.*, *Transplant Direct*, 2021

Measuring non-HLA antibodies



Dr. Andrzej Chruscinski

Zhu, H.; *et al*. Applications of functional protein microarrays in basic and clinical research. *Adv Genet*. 2012, 79: 123-155

Designed protein microarrays to measure antibodies against 134 antigens implicated in autoimmune disease or solid organ transplant rejection

Dr. Sergi Clotet-Freixas
Post-doctoral fellow

RESULTS

Antibody name	Antigen specificity	Antibody levels: Before transplant: AMR/mixed, median (IQR)	ACR, median (IQR)	P
IgG M2	M2 (PDC-E2 + OGDC-E2 + BCOADC-E2)	267 (0–513)	0 (0–0)	0.0051
IgG CENP-B	Major centromere autoantigen B	312.5 (0–877.2)	0 (0–235.2)	0.0097
IgG Ro/SS-A (52 kDa)	Ro/SS-A (52 kDa)	315 (0–915.7)	0 (0–257.5)	0.0112
IgG gliadin	Gliadin	3209 (1485.8–5474)	1618.5 (793–3110.6)	0.0157
IgG PDH	Pyruvate dehydrogenase	238 (0–507)	0 (0–226)	0.0232
IgG smooth muscle	Smooth muscle actin	382.5 (304.5–532.8)	310.8 (230.5–374.1)	0.0457
		AMR/mixed, median (IQR)	**ATN, median (IQR)**	**P**
IgM PL-12	Alanyl-tRNA synthetase	1049.5 (544.2–1672.8)	352 (236.5–601)	0.0074
IgM HGMEC lysate	Glomerular endothelial cells	1393.5 (881–2234.5)	752 (522–1131.5)	0.0224
IgM PM/Scl-100	PM/Scl-100	317.5 (0–451.5)	0 (0–0)	0.0234
IgM OGDC-E2	M2 (OGDC)	426 (104.8–766)	0 (0–307.5)	0.0298
IgG HCEC cytoplasm	Cardiac endothelial cells	380.5 (229.2–532.7)	222 (0–294.5)	0.0309
IgM LG3	Basement membrane-specific heparan sulfate proteoglycan core protein	402.5 (238.3–607.8)	0 (0–401)	0.0365
IgM LKM 1 hp	LKM 1 hp antigen of cytochrome P450 2D6	210.5 (0–314)	0 (0–0)	0.0393
IgG Ro/SS-A (60 kDa, R)	Ro/SS-A (60 kDa)	0 (0–233.5)	296 (0–559.5)	0.0403
IgM Sm (NR, B)	Small nuclear ribonucleoprotein Sm	311 (0–495.7)	0 (0–299.5)	0.0470
IgG PDH	Pyruvate dehydrogenase	238 (0–507)	0 (0–0)	0.0494

Dr. Max Kotlyar

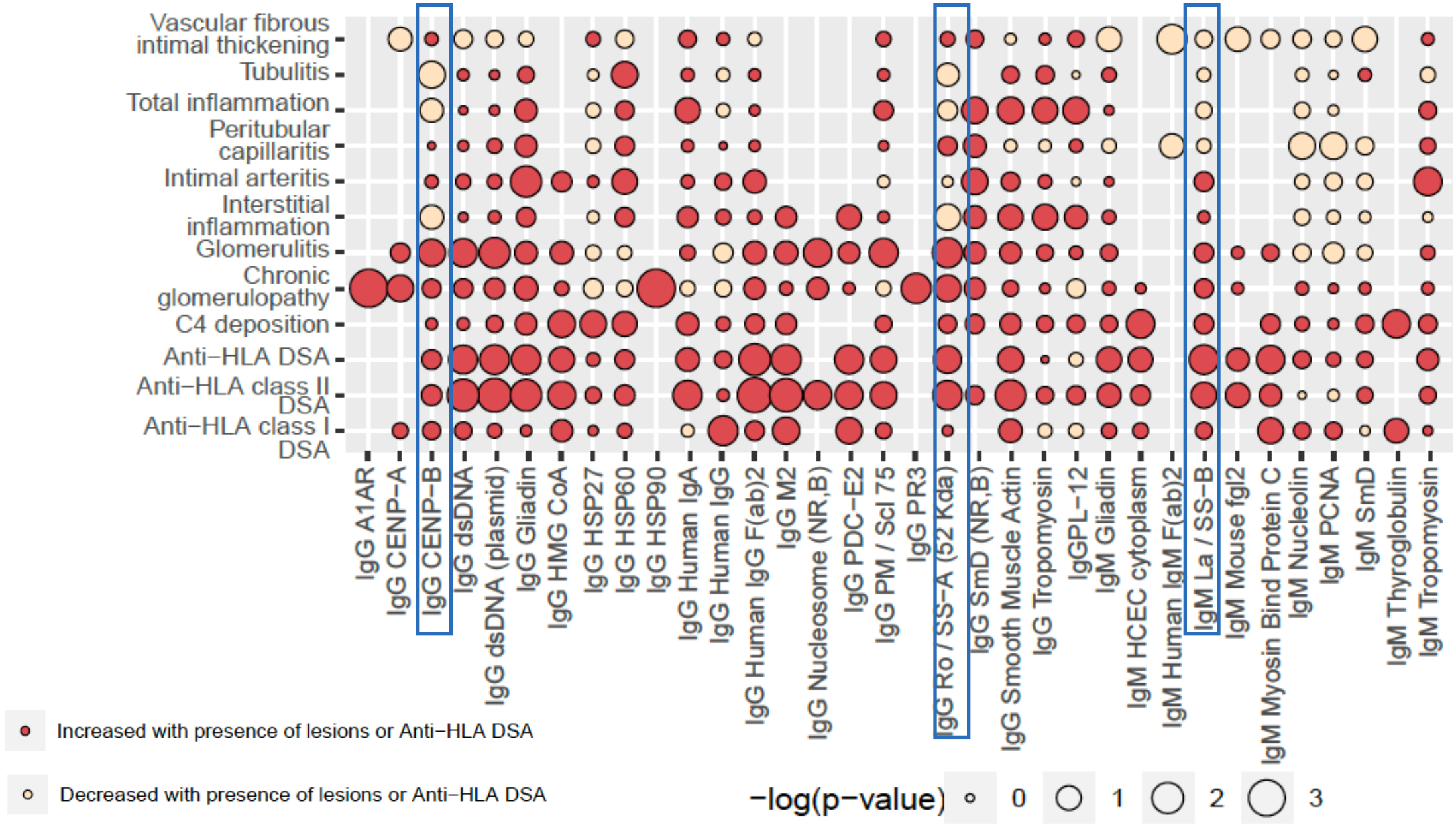
Clotet-Freixas, *et al.*, *Transplant Direct*, 2021

RESULTS – Sera at the time of diagnosis

		Antibody levels		
		At diagnosis		
Antibody name	**Antigen specificity**	**AMR/mixed, median (IQR)**	**ACR, median (IQR)**	***P***
IgG CENP-B	Major centromere autoantigen B	510 (0–877.8)	0 (0–0)	0.0126
IgG Ro/SS-A (52 kDa)	Ro/SS-A (52 kDa)	353.5 (0–1494)	0 (0–0)	0.0325
IgM La/SS-B	La/SS-B	735.5 (390.8–1843)	296 (139.5–411)	0.0261
IgM CENP-B	Major centromere autoantigen B	666.5 (313.5–1889.5)	262 (100.2–429)	0.0447
IgM PDH	PDH	251 (0–684.5)	0 (0–0)	0.0472
		AMR/mixed, median (IQR)	**ATN, median (IQR)**	***P***
IgG M2	M2 (PDC-E2 + OGDC-E2 + BCOADC-E2)	219 (0–596.5)	0 (0–0)	0.0313
IgG human IgA	Human IgA	750.5 (229.5–1322)	0 (0–337.5)	0.0495

Clotet-Freixas, *et al.*, *Transplant Direct*, 2021

Do non-HLA antibodies correlate with microvascular lesions?



Validation of top non-HLA antibodies in an external cohort

60 kidney transplant recipients from Montreal previously described with serum samples analyzed using the same platform

Cardinal et al. Am J Transplant 2013

1. Antibody mediated or Mixed rejection

2. Acute cellular rejection

3. Controls without rejection

Dr. HELOÏSE CARDINAL

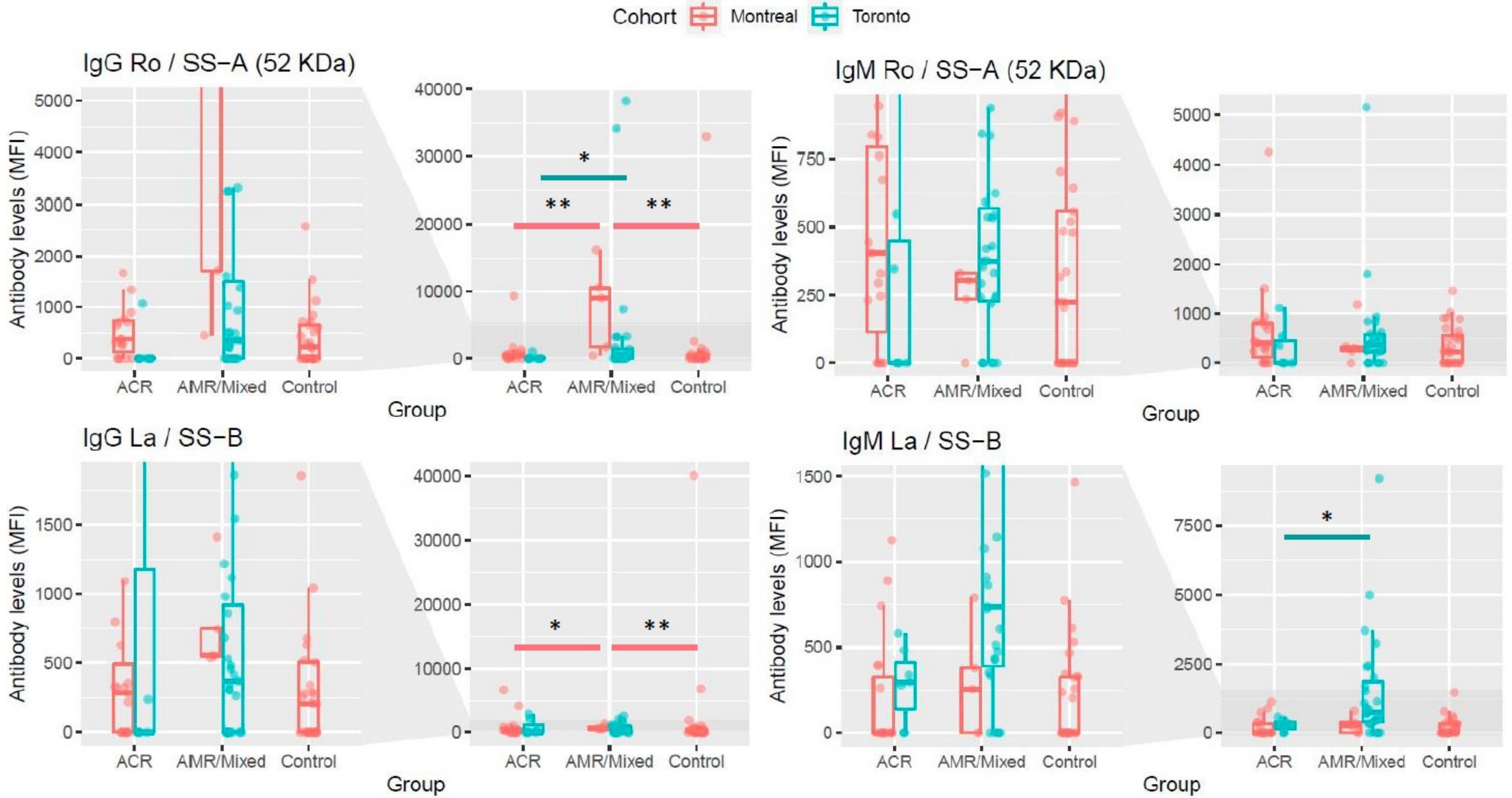
Dr. Marie-Josée Hébert

Dr. MÉLANIE DIEUDÉ

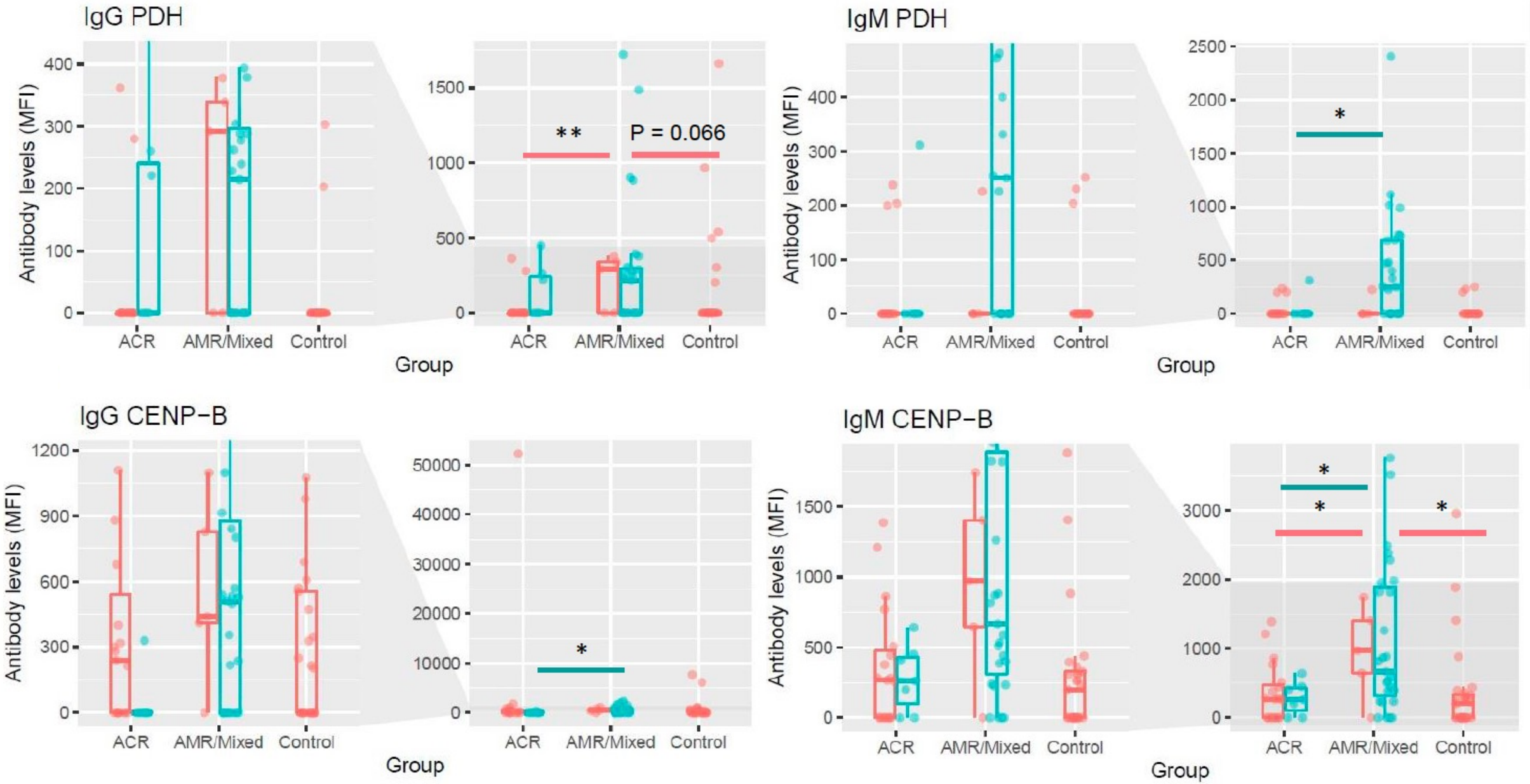
Applied the same method of non-HLA Ab measurement

Examine only antibodies most significant in our cohort:
Anti-Ro, Anti-La, Anti-CENPB, Anti-PDH

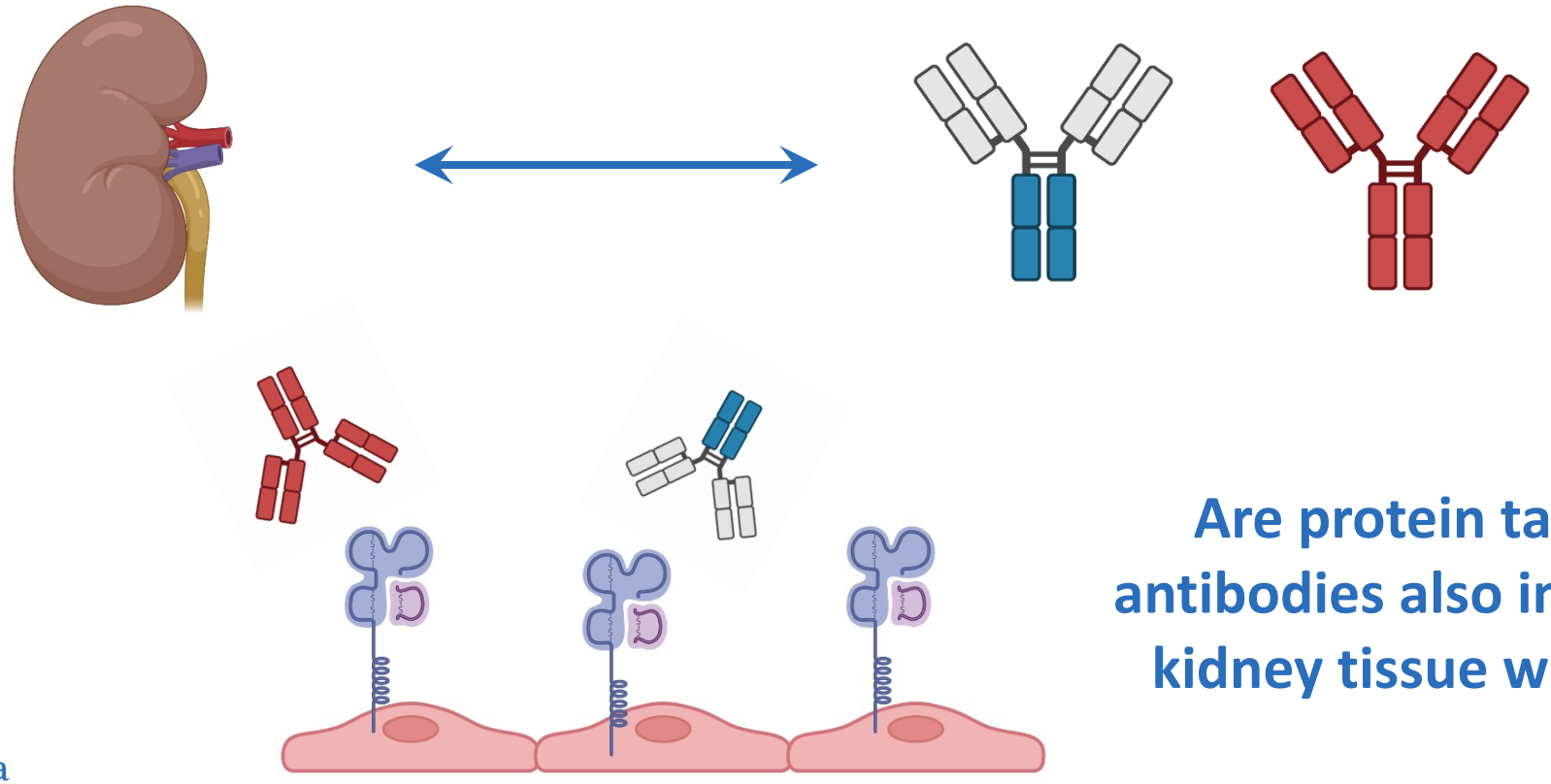
Validation of Results



Validation of Results



Integration of circulating antibody specificity and kidney tissue protein expression



Are protein targets of antibodies also increased in kidney tissue with AMR?

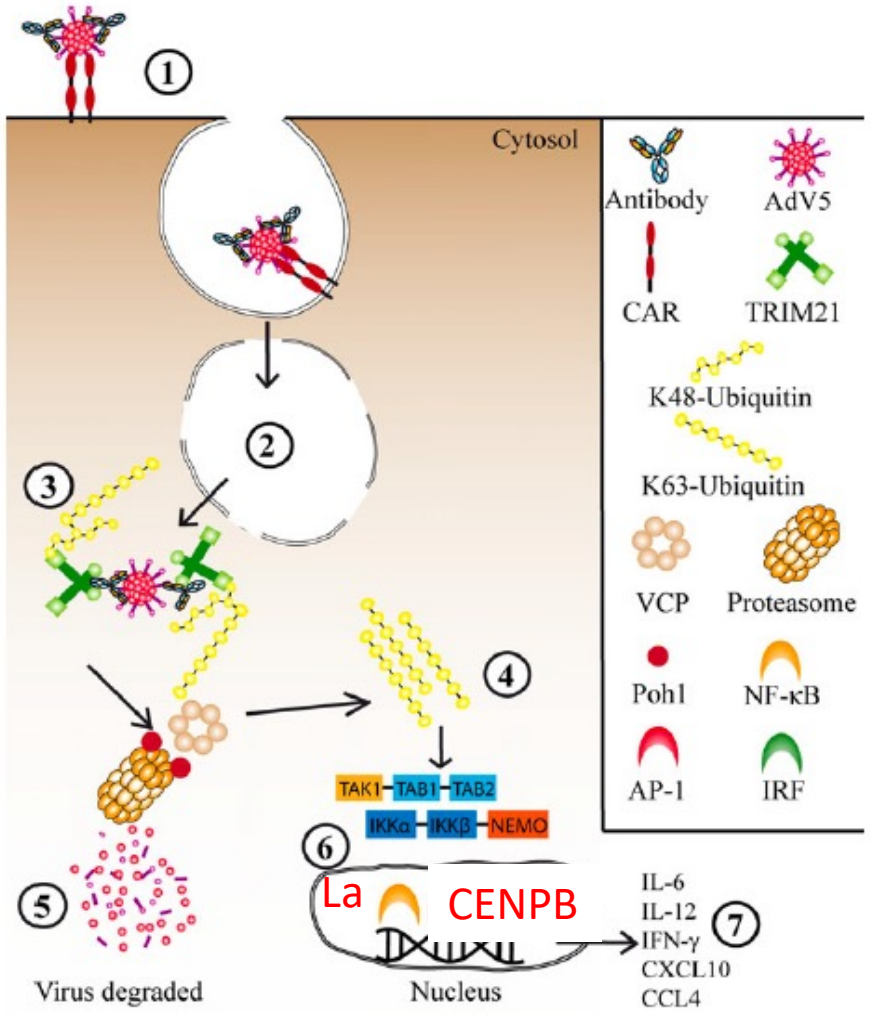
UHN Ajmera Transplant Centre

Dr. Chiara Pastrello

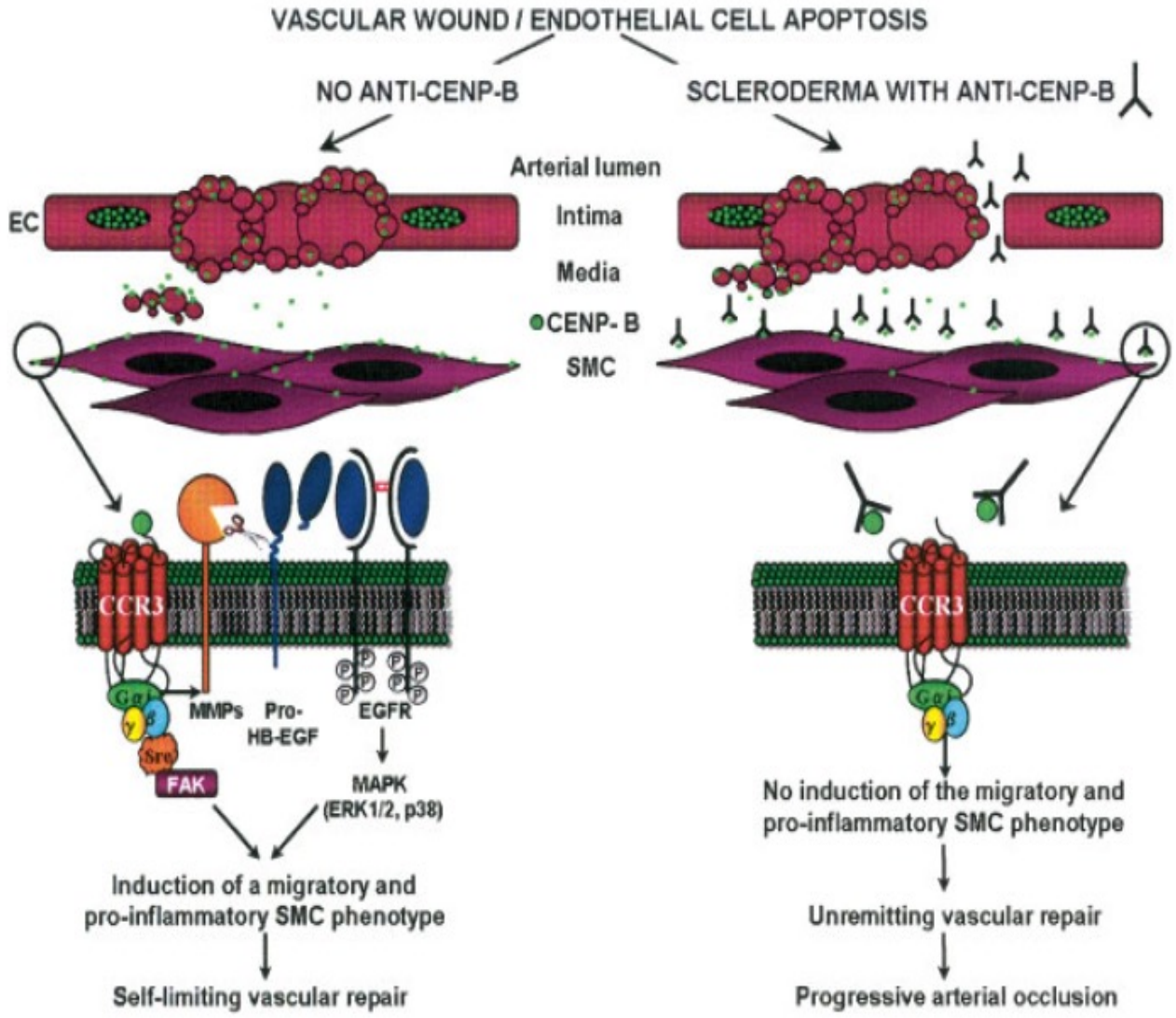
Clotet-Freixas, *et al.*, *Transplant Direct*, 2021

ARTHRITIS & RHEUMATISM
Vol. 60, No. 9, September 2009, pp 2805–2816
DOI 10.1002/art.24765

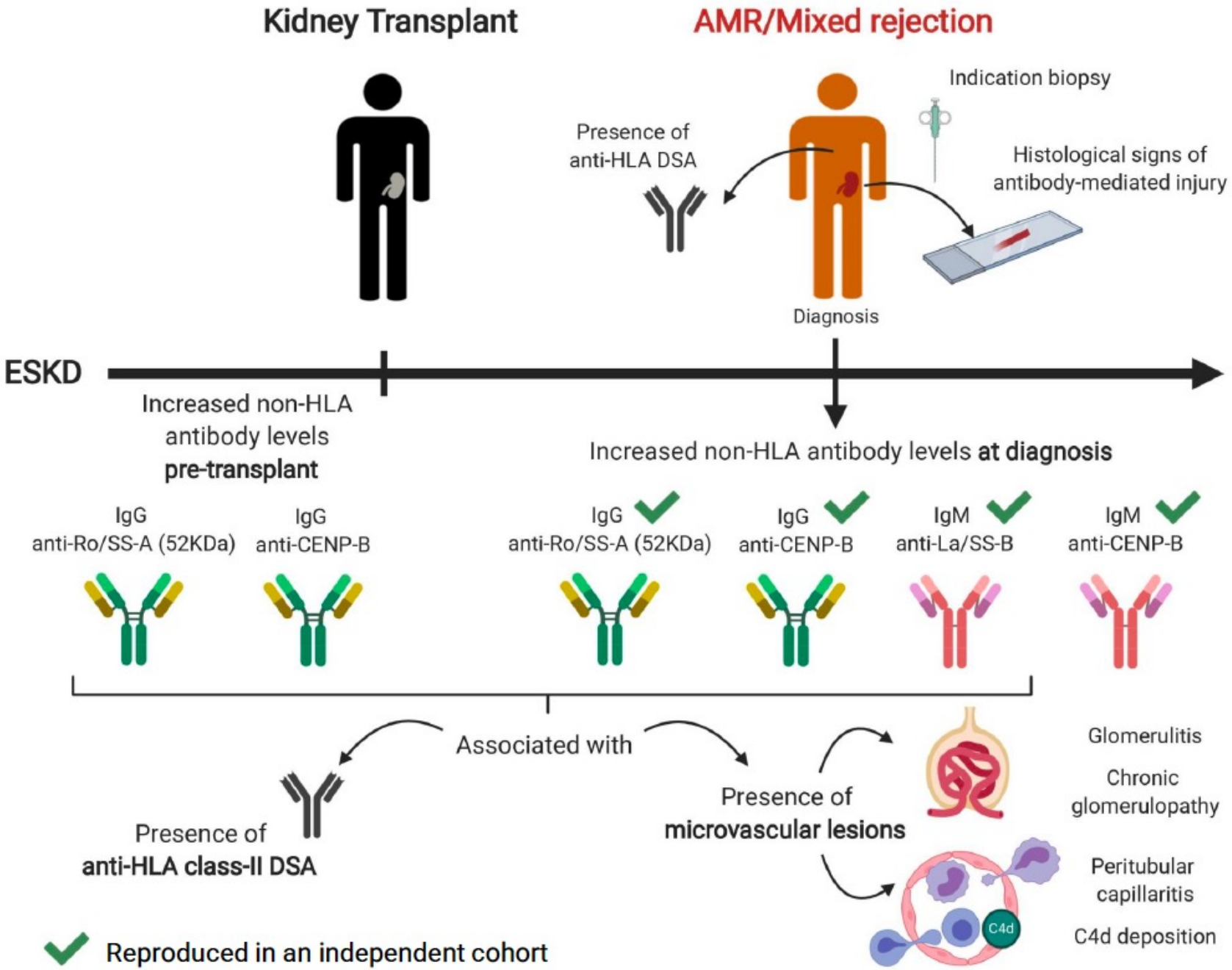
What do these antibodies target?



Immunological Reviews 2015
Vol. 268: 328–339



UHN Ajmera Transplant Centre



Clotet-Freixas, *et al.*, *Transplant Direct*, 2021

Gaps in knowledge

- The role of kidney tissue in antibody mediated rejection is poorly understood
- The most important non-HLA antibodies to be included in routine clinical monitoring are unknown
- Determination of pathogenic DSA is elusive
- Effective therapies are lacking

30-60% of patients with DSA do not develop rejection

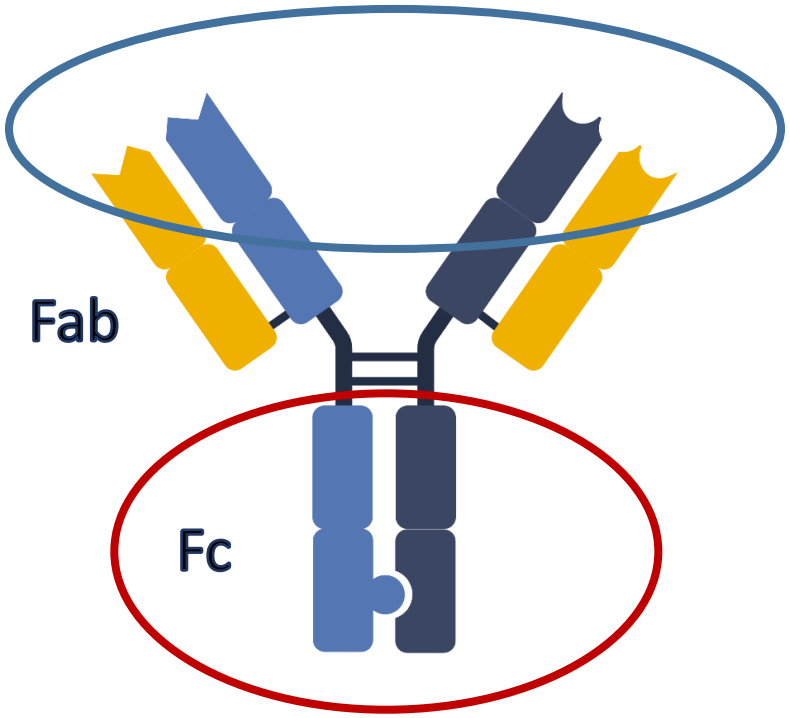
FUTURE DIRECTIONS

Decipher donor specific antibody biochemical and functional properties linked to its pathogenicity

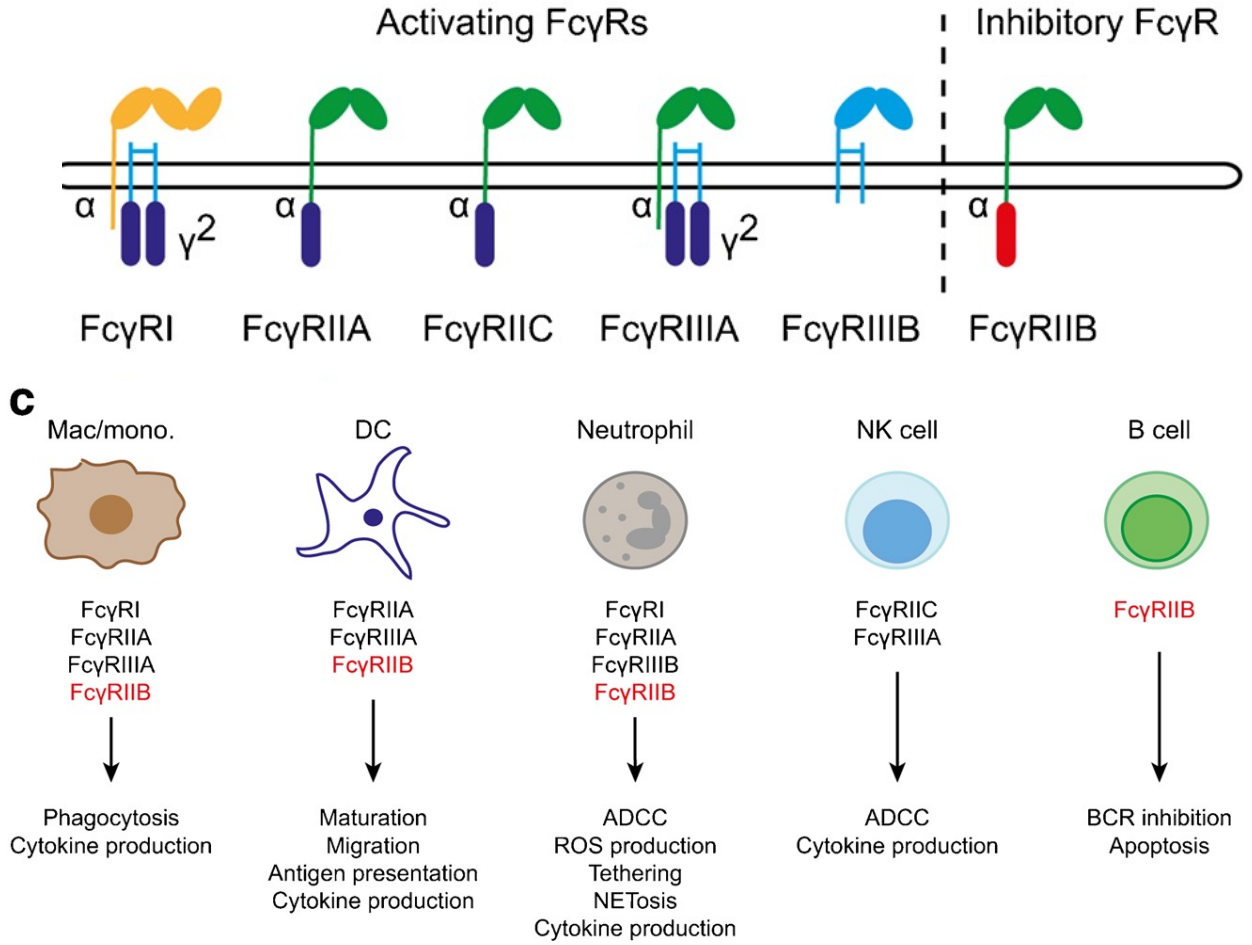
UHN Ajmera Transplant Centre

Donor Specific Antibodies (DSA) and Fc Receptors

Binding specificity



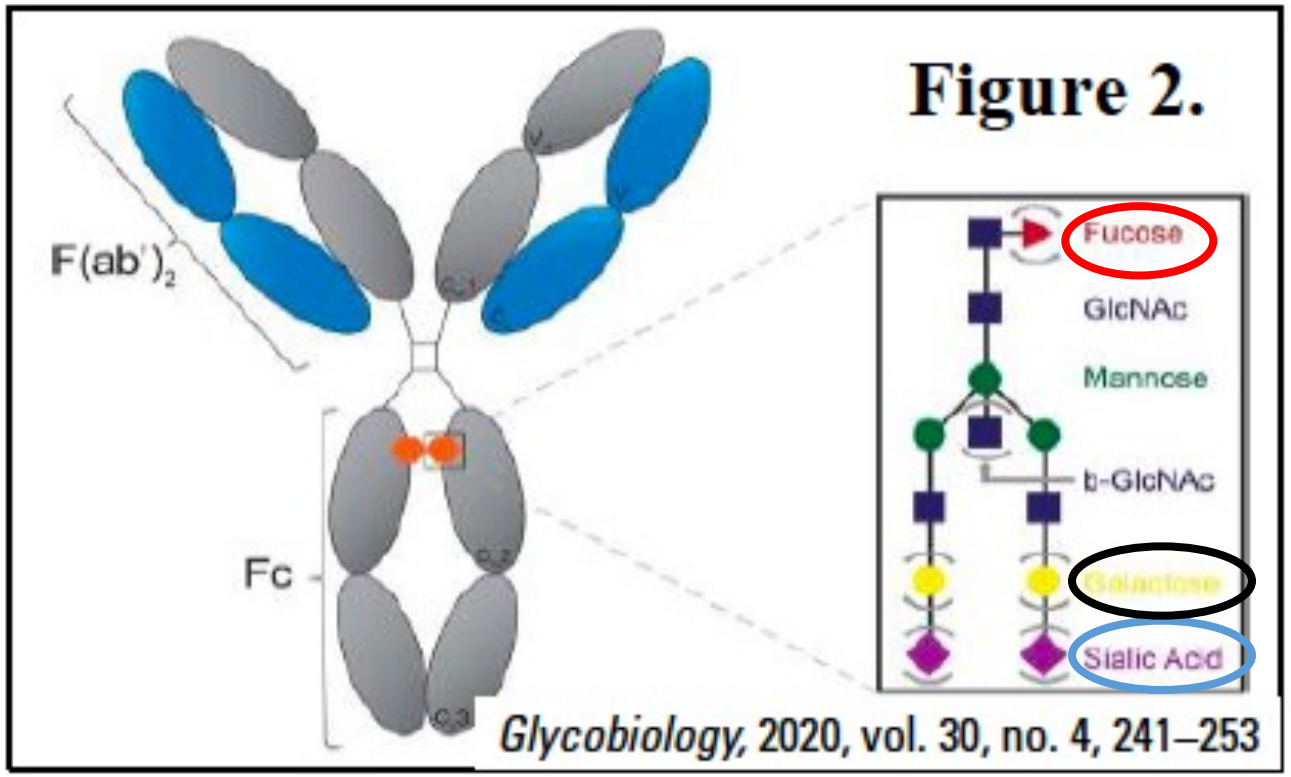
Functional interactions with Fc receptors



Curr Transpl Rep (2016) 3:284–293
DOI 10.1007/s40472-016-0116-7

UHN Ajmera Transplant Centre

Fc Glycosylation



Lack of fucose activates FcGR3A on NK cells and monocytes/macrophages resulting in cytotoxicity

Lack of terminal galactose results in complement activation

Sialic acid activates inhibitory FcGR2B on monocytes, macrophages, B-cells

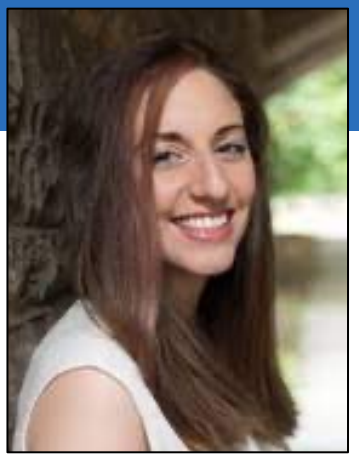
How can we decipher these glycans when DSA is different in every patient?

Dr. Sergi Clotet-Freixas
Research Associate

Dr. Caitriona McEvoy
Staff Nephrologist, Dublin

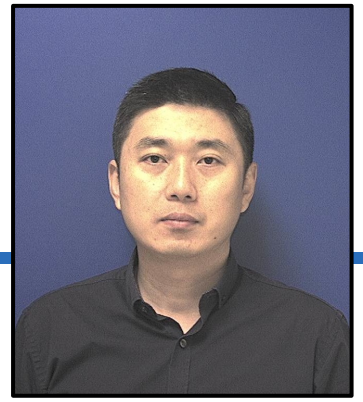
Dr. Sofia Farkona
Post-doctoral fellow

Alex Boshart
PhD student



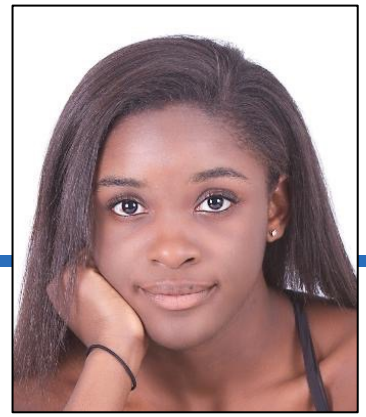
Dr. Kieran Manion
Post-doctoral fellow

KONVALINKA LAB

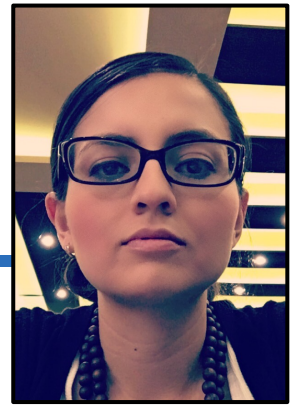


Shenghui Su
ATC Biobank Manager

Slaghaniya Neupane
Master's degree student



Maya Allen
Master's degree student



Dr. Sonia Rodriguez Ramirez
Post-doctoral fellow

UHN Ajmera Transplant Centre

UHN Multi-Organ Transplant

Konvalinka lab:

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Dr. Caitriona McEvoy

Dr. Sofia Farkona

Dr. Kieran Manion

Alex Boshart

Aninda Saha

Slaghaniya Neupane

Maya Allen

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Dr. Tomas Tokar

Dr. Max Kotlyar

Dr. Chiara Pastrello

Dr. Jishnu Das

Mass Spectrometry group:

Dr. Eleftherios Diamandis

Ihor Batruch

Dr. Andrea Bozovic

Dr. Vathany Kulasingam

Pathology / Transplant:

Dr. Rohan John

Dr. Andrzej Chruscinski

Dr. S. Joseph Kim

Segun Famure (Kim)

Dr. Yanhong Li (Kim)

Engineering:

Dr. Milica Radisic

Dr. Boyang Zhang

Dr. Aniruddh Sarkar

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- Evaluation survey will be sent to you after this webinar. Please complete the evaluation so to get your CPD credits.
- CSN members will receive 1 hr Section 1 Group Learning credits

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