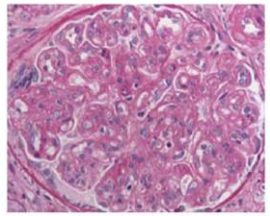
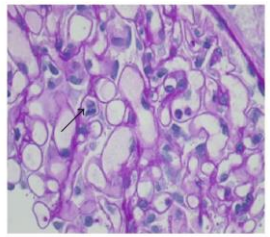
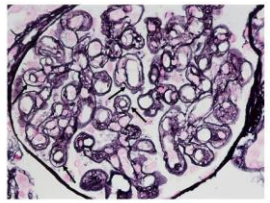
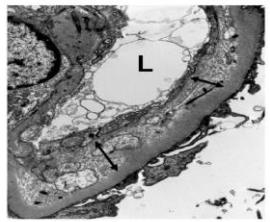
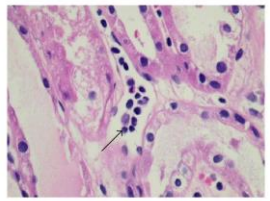
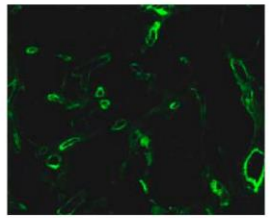












Chronic Antibody Mediated Rejection (cAMR)

SHOKOUFEH SAVAJ MD

PROFESSOR OF MEDICINE, HASHEMI NEJAD KIDNEY HOSPITAL

IRAN UNIVERSITY OF MEDICAL SCIENCES

Introduction

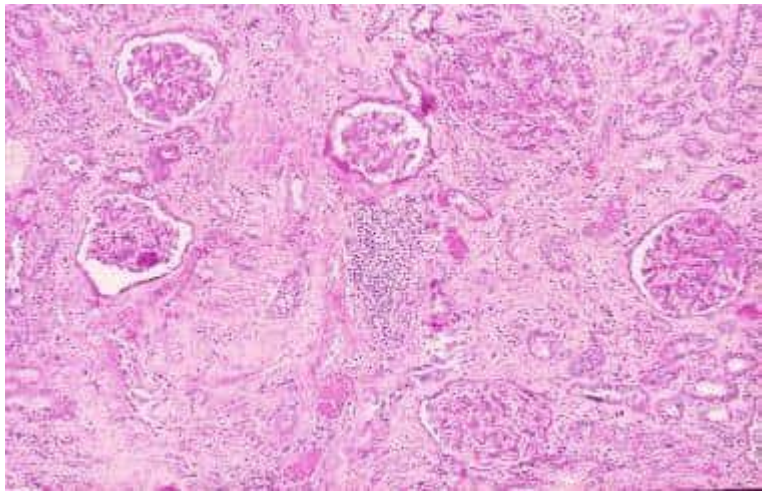
cAMR is the most important cause of late kidney allograft failure and a major unmet therapeutic challenge.

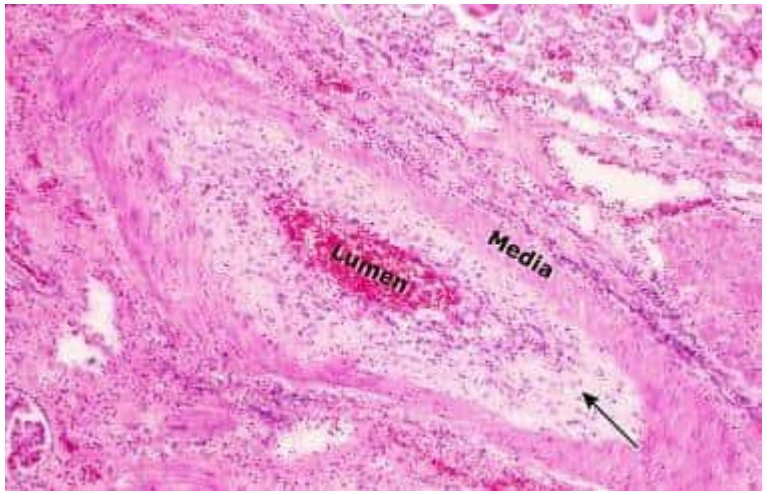
Detected in approximately 20–30% of kidney transplant recipients over time and is one of the leading causes of late graft dysfunction.

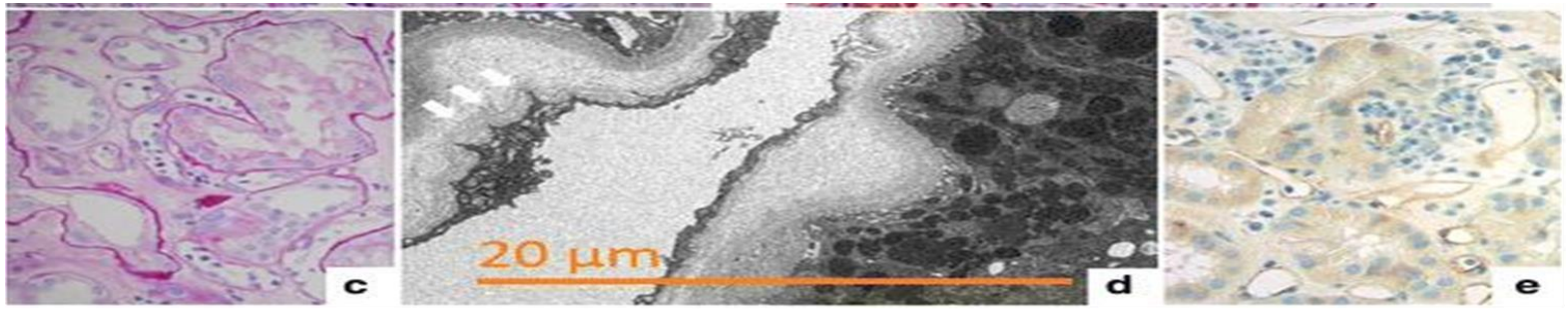
Up to 40–60% of patients with cAMR progress to graft loss within 3–5 years, especially high- risk group with persistent high-titer DSA and late diagnosis

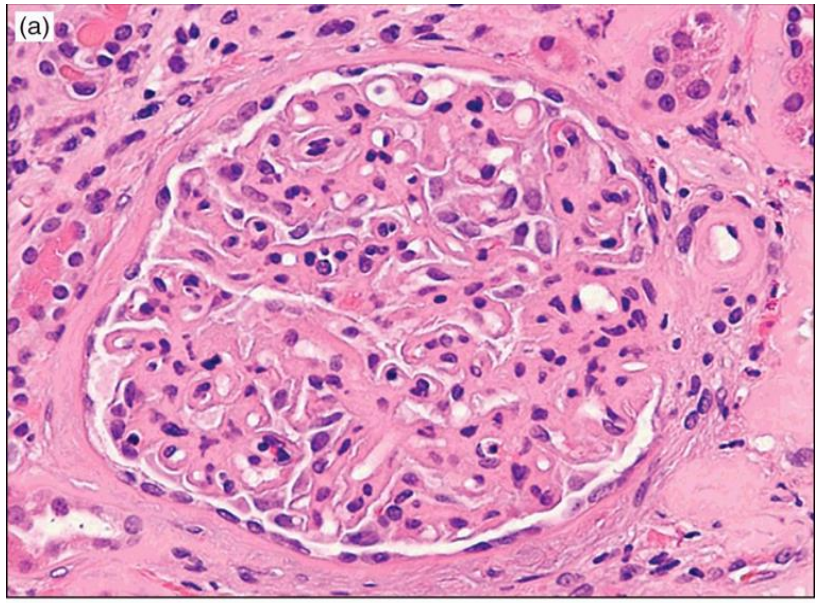
Definition cAMR : Evidence of chronic tissue injury

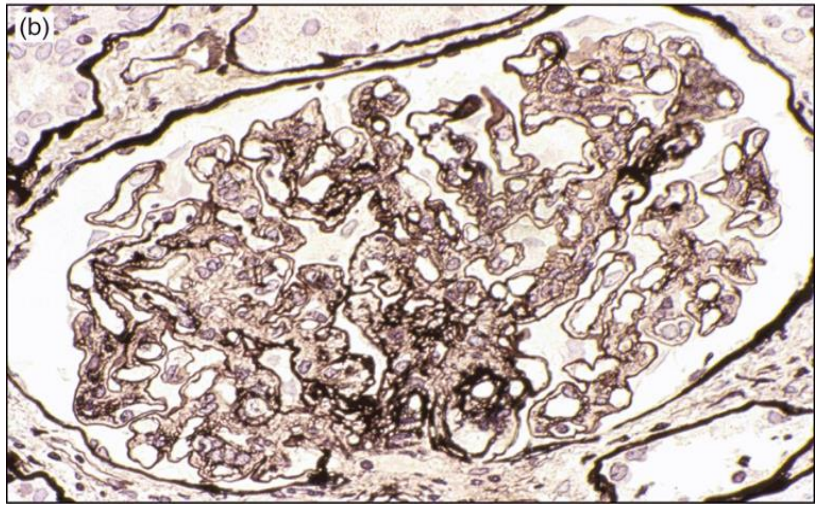
Transplant glomerulopathy (cg > 0)
Severe peritubular capillary basement membrane Multilayering
Interstitial fibrosis / Tubular atrophy (IFTA)±
Arterial intimal fibrosis (chronic vasculopathy)











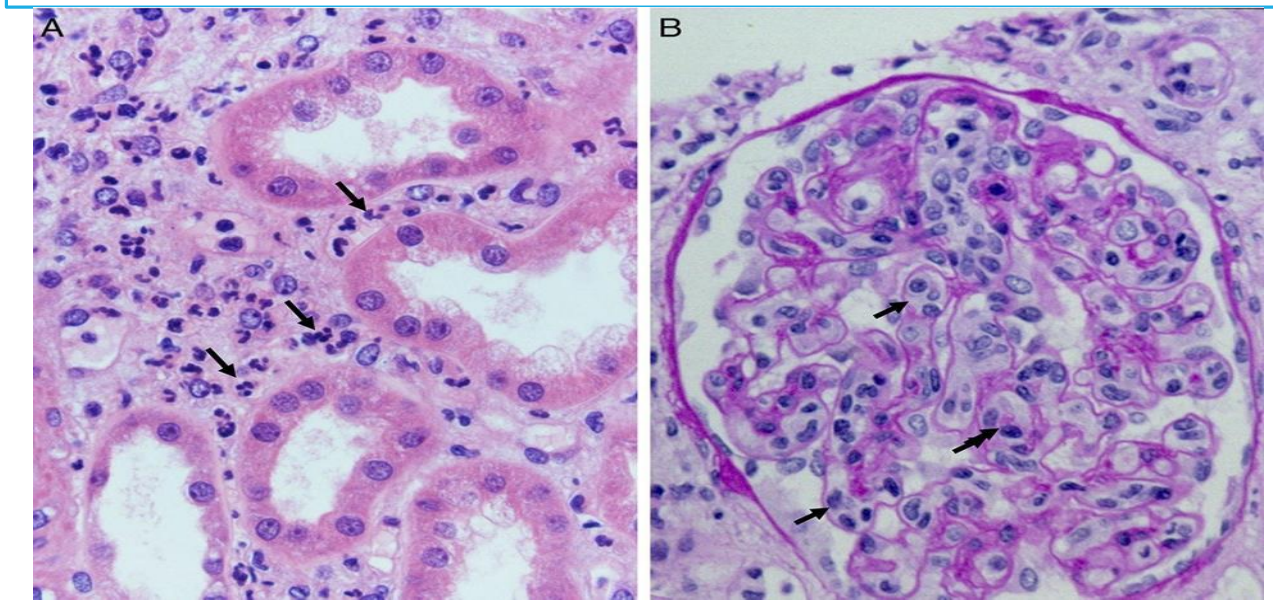
Definition cAMR : Evidence of Antibody–Endothelium Interaction

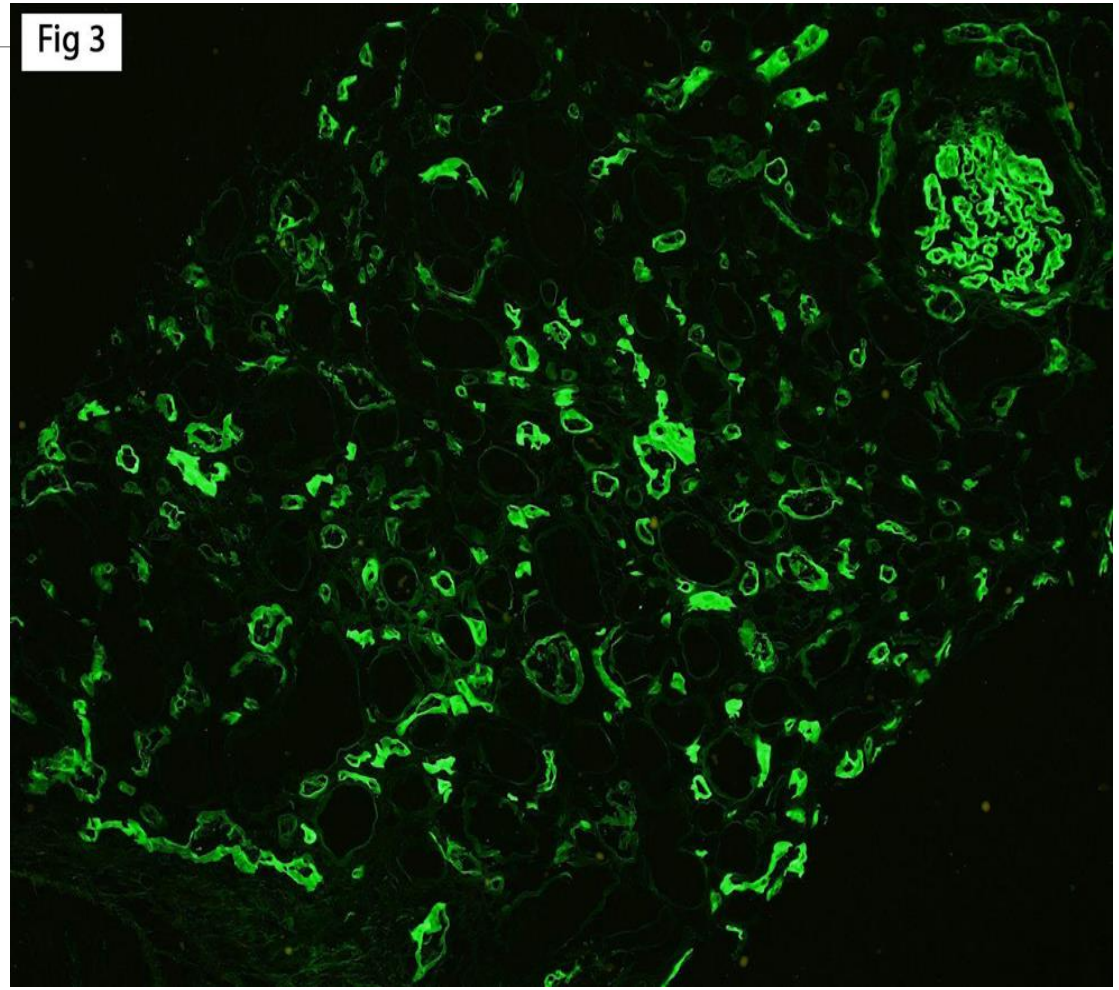
At least ONE of the following:

C4d deposition in peritubular capillaries

Microvascular inflammation (MVI):Glomerulitis (g)Peritubular capillaritis (ptc)

Molecular markers of endothelial activation(ENDATs / transcriptomics)





Molecular markers of endothelial activation (ENDATs / transcriptomics)

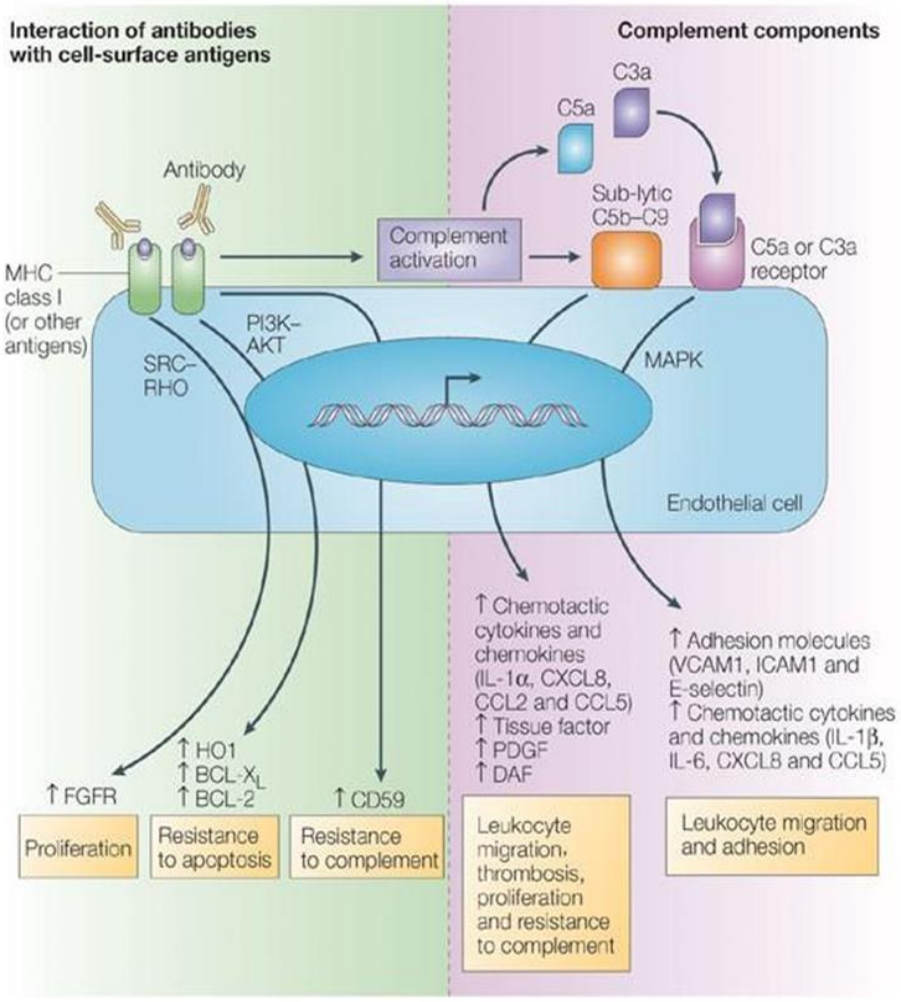
Endothelial activation & adhesion:VCAM1 (Vascular Cell Adhesion Molecule-1), ICAM1 (Intercellular Adhesion Molecule-1),E-selectin

Interferon signaling pathway:STAT1, IRF1

Interferon-γ–inducible chemokines : CXCL9,CXCL10 (IP-10), CXCL11

NK cell / cytotoxicity-associated transcripts : GNLY (Granulysin),GZMB (Granzyme B), PRF1 (Perforin)

Endothelial injury / activation markers : VWF (von Willebrand factor),PECAM1 (CD31), CDH5 (VE-cadherin)



ENDAT Workflow: From Biopsy to Clinical Diagnosis

- Kidney Allograft Biopsy
- Tissue Allocation (Histology + Molecular core)
- RNA Extraction
- Gene Expression Profiling (CXCL9, CXCL10, VCAM1, STAT1)
- Bioinformatics Analysis
- ENDAT Score + AMR Probability
- Integrated Interpretation (Banff + DSA)
- Final Diagnosis (e.g., Chronic Active AMR)

Halloran PF. Nat Rev Nephrol. 2016.

MOLECULAR DIAGNOSTICS LABORATORY

MOLECULAR TRANSPLANT REPORT

Endothelial Activation & Rejection Profiling (MMDx®)

REPORT DATE
May 06, 2024
LAB ID
MDL24-050612345

PATIENT INFORMATION

Patient ID:	TXP-123456
Name:	John Doe
DOB / Sex:	08/12/1972 (M)
Transplant Date:	11/15/2020
Time Post-Transplant:	3.5 years
Indication:	Rising creatinine, suspected rejection

SPECIMEN INFORMATION

Specimen Type:	Kidney Allograft Biopsy
Collection Date:	05/01/2024
Received Date:	05/02/2024
Specimen Number:	S24-98765
Requesting Physician:	Dr. A. Smith
Center:	University Transplant Center

TEST PERFORMED

Molecular Microscope for Endothelial Activation & Rejection (MMDx®)

Method: RNA expression profiling (Microarray)

RESULT SUMMARY

PROBABILITY OF ANTIBODY-MEDIATED REJECTION

82%

HIGH

Interpretation: High probability of antibody-mediated rejection.

ENDOTHELIAL ACTIVATION (ENDAT) SCORE

HIGH

Interpretation: Increased endothelial activation consistent with antibody–endothelium interaction.

OTHER MOLECULAR SCORES

	Score	Interpretation
TCMR Score	22%	LOW
Injury Score	67%	MODERATE-HIGH
Fibrosis / Atrophy Score	46%	MODERATE
Microvascular Inflammation Score	78%	HIGH

KEY ENDOTHELIAL ACTIVATION TRANSCRIPTS

Gene Group / Pathway	Representative Genes	Result
IFN-γ Inducible Chemokines	CXCL9, CXCL10, CXCL11	↑↑↑
Adhesion Molecules	VCAM1, ICAM1, SELE	↑↑↑
Cytotoxic / NK Cell Pathway	GZMB, PRF1, GNLY	↑↑
Endothelial Activation Markers	VWF, PECAM1, CDH5	↑↑
IFN-γ Signaling Pathway	STAT1, IRF1	↑↑

RESULT KEY

↑↑↑	Markedly Increased	↔	No Significant Change
↑↑	Moderately Increased	↓	Decreased
↑	Mildly Increased		

MOLECULAR DIAGNOSTIC INTERPRETATION

The molecular profile demonstrates a high probability of antibody-mediated rejection with significant endothelial activation (ENDAT high). There is upregulation of IFN-γ–inducible chemokines and adhesion molecules, along with cytotoxic/NK cell-associated transcripts, consistent with active antibody–endothelium interaction and microvascular injury.

These findings support the diagnosis of antibody-mediated rejection in the appropriate histologic and clinical context.

RECOMMENDED CORRELATION

- Correlate with histology (Banff scores: g, ptc, cg, ptcml, ci, ct)
- Correlate with donor-specific antibody (DSA) testing (HLA ± non-HLA)
- Correlate with clinical findings and graft function
- Consider C4d staining results

OVERALL MOLECULAR IMPRESSION

Consistent with Chronic Active Antibody-Mediated Rejection (cAMR)

Confidence: HIGH

Test performed and interpreted by:
Molecular Diagnostics Laboratory
CLIA ID: 12D3456789
CAP Accredited

Authorized by:
Dr. Michael Lee, MD, PhD
Medical Director

MOLECULAR DIAGNOSTICS
Advancing Transplant Care Through Molecular Insight

Practical Use of ENDAT in the Clinic

POSITVE POINTS

1. **Clarifying "uncertain" biopsies**

When histology is borderline (e.g., mild **g/ptc**, unclear chronicity), When **C4d is negative**

2. Diagnosing C4d-negative AMR

A common real-world problem, ENDAT can act as a **surrogate for antibody activity**

3. DSA-negative but suspicious cases

4. Risk stratification / prognosis

5. Complementing Banff (not replacing it)

LIMITATIONS

1. Lack of specificity

ENDAT reflects **inflammation**, not only AMR , Can be elevated in

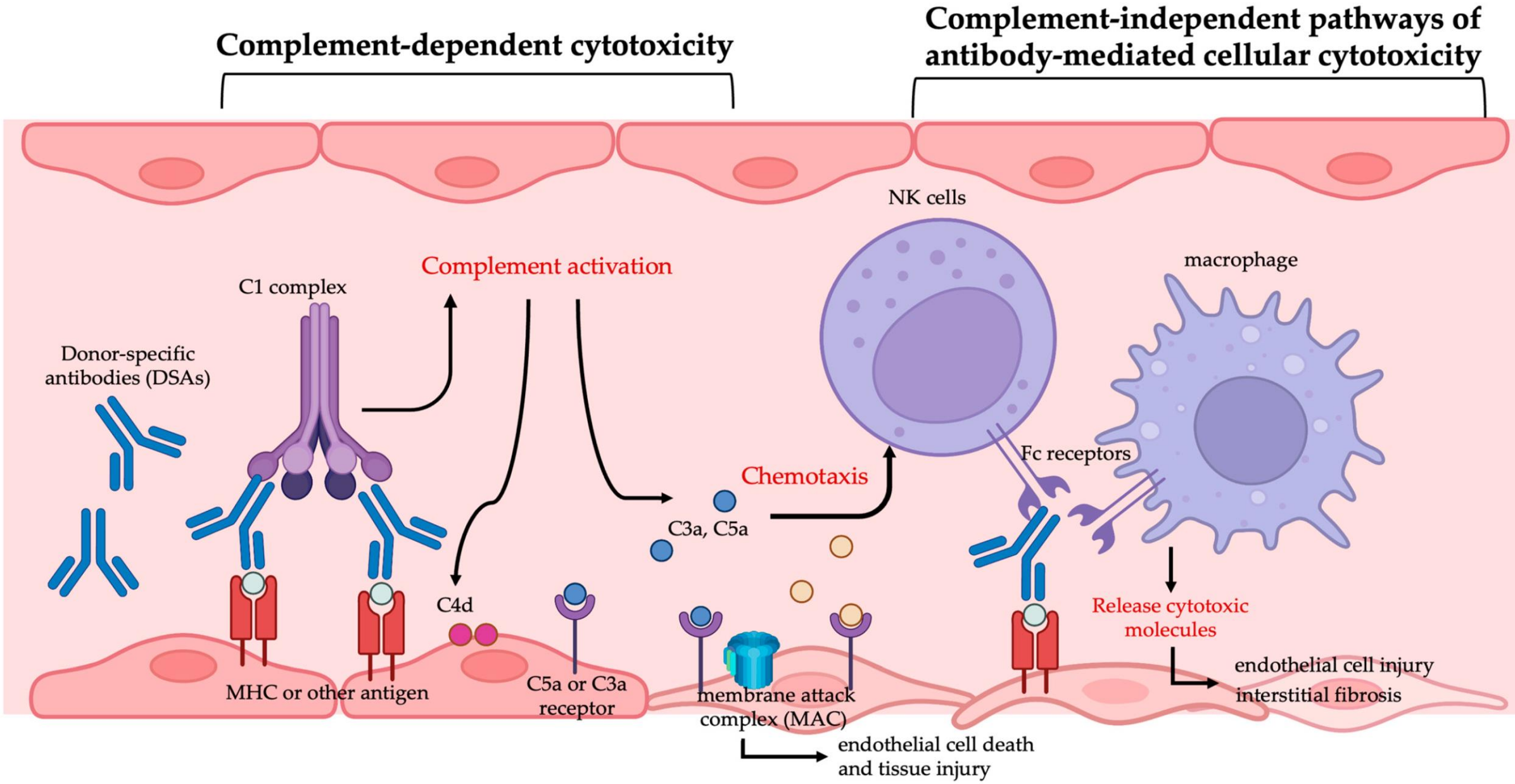
- TCMR
- Viral infections (e.g., BK)
- Ischemic injury

2. No universal cut-off

3. Limited availability & cost

4. Not yet fully guideline-integrated

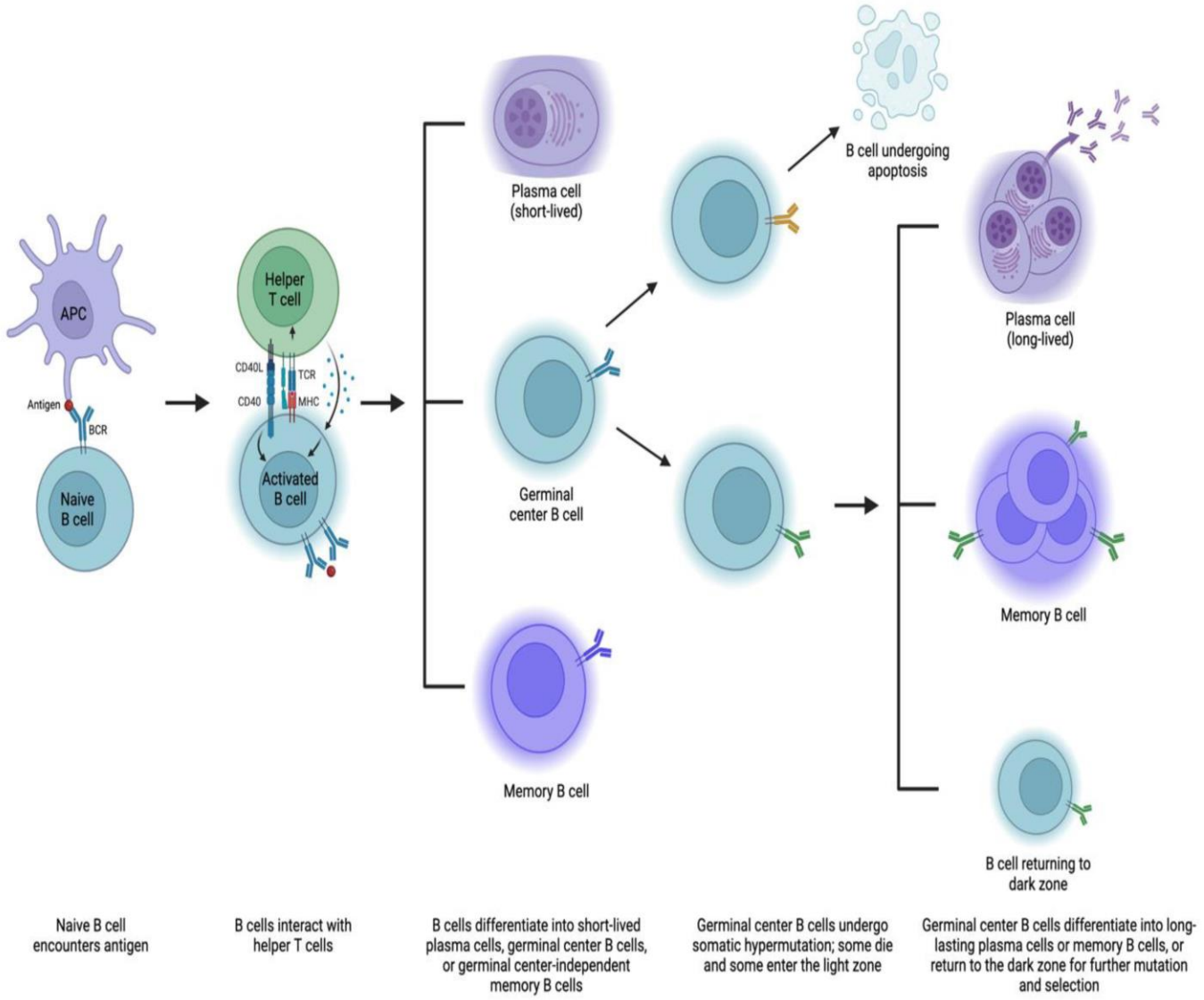
5. Unclear role in therapy monitoring


Complement-dependent cytotoxicity
Complement-independent pathways of antibody-mediated cellular cytotoxicity
NK cells
macrophage
Complement activation
C1 complex
Donor-specific antibodies (DSAs)
Chemotaxis
C3a, C5a
Fc receptors
C4d
Release cytotoxic molecules
endothelial cell injury
interstitial fibrosis
MHC or other antigen
C5a or C3a receptor
membrane attack complex (MAC)
endothelial cell death and tissue injury

Hidden Players In Chronic Antibody-mediated Rejection (cAMR): Explaining Therapeutic Resistance”

- **Long-lived plasma cells (LLPCs)**→ Continuous DSA production, resistant to conventional therapy
- **Memory B cells**→ Rapid reactivation and regeneration of antibody response
- **T follicular helper (Tfh) cells**→ Sustain B-cell maturation and antibody production
- **Natural killer (NK) cells**→ Mediate antibody-dependent cellular cytotoxicity (ADCC)
- **Non-HLA antibodies**→ e.g., anti-AT1R, anti-ETAR → complement-independent injury
- **Endothelial-to-mesenchymal transition (EndMT)**→ Link between immune injury and chronic fibrosis
- **Innate immune activation (macrophages, monocytes)**→ Amplification of microvascular inflammation
- **Extracellular vesicles & microRNAs**→ Intercellular signaling, propagation of injury

In (cAMR), the “memory” of the immune system is a central driver of persistent and treatment-resistant injury. Two main cellular players are involved: memory B cells and long-lived plasma cells, both shaped by prior antigen exposure (donor HLA or non-HLA).



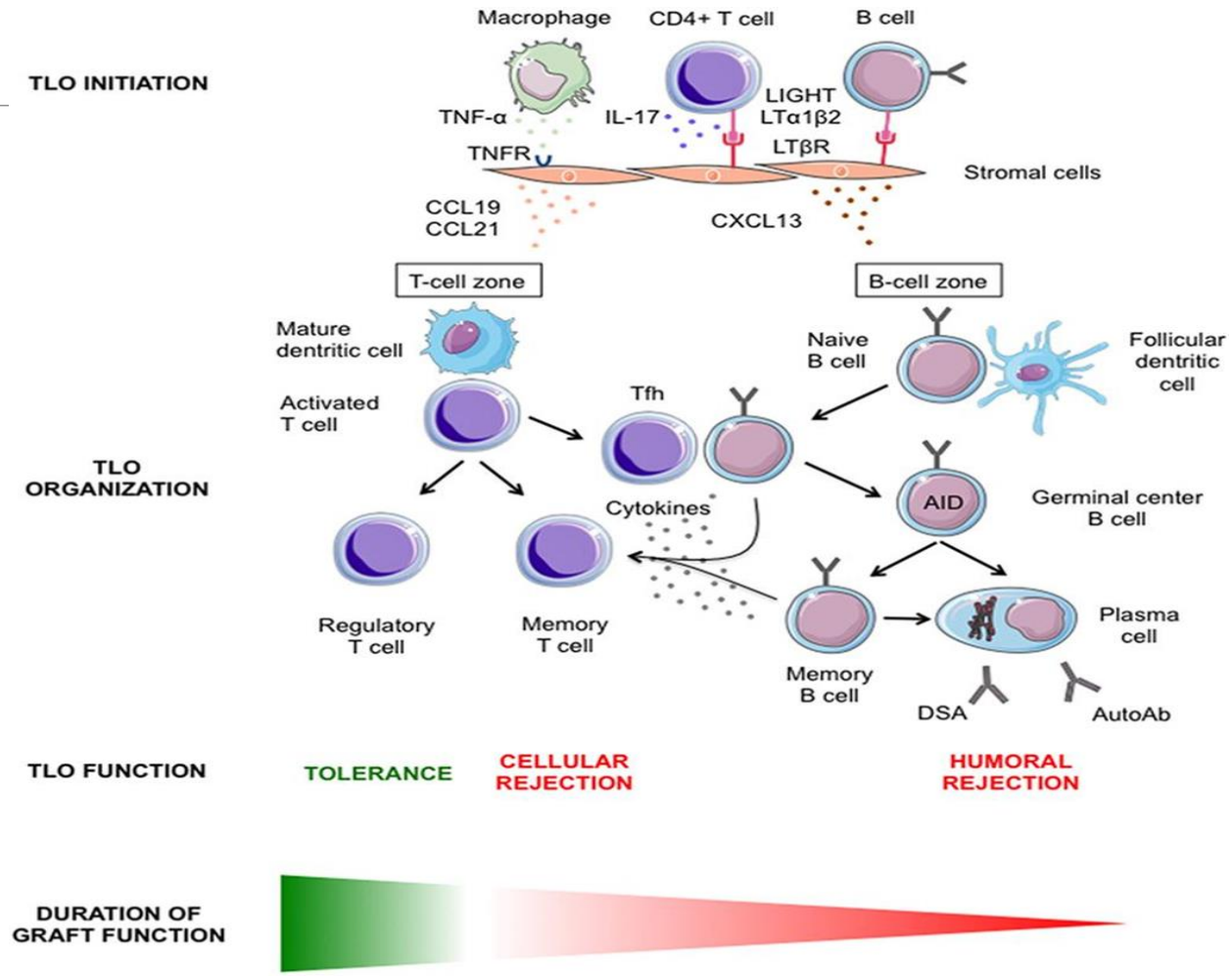
Memory B cells - the hidden reservoir

After initial sensitization (transplant, transfusion, pregnancy),Memory B cells specific for donor antigens persist long-term.

They are usually quiescent, not detected in routine assays, not producing antibodies continuously.

But **when reactivated**, rapidly differentiate into plasma cells and cause **a rapid rise in (DSA)**, drive progression of cAMR even years after transplant.

Key point: They explain why DSA can reappear suddenly after being negative



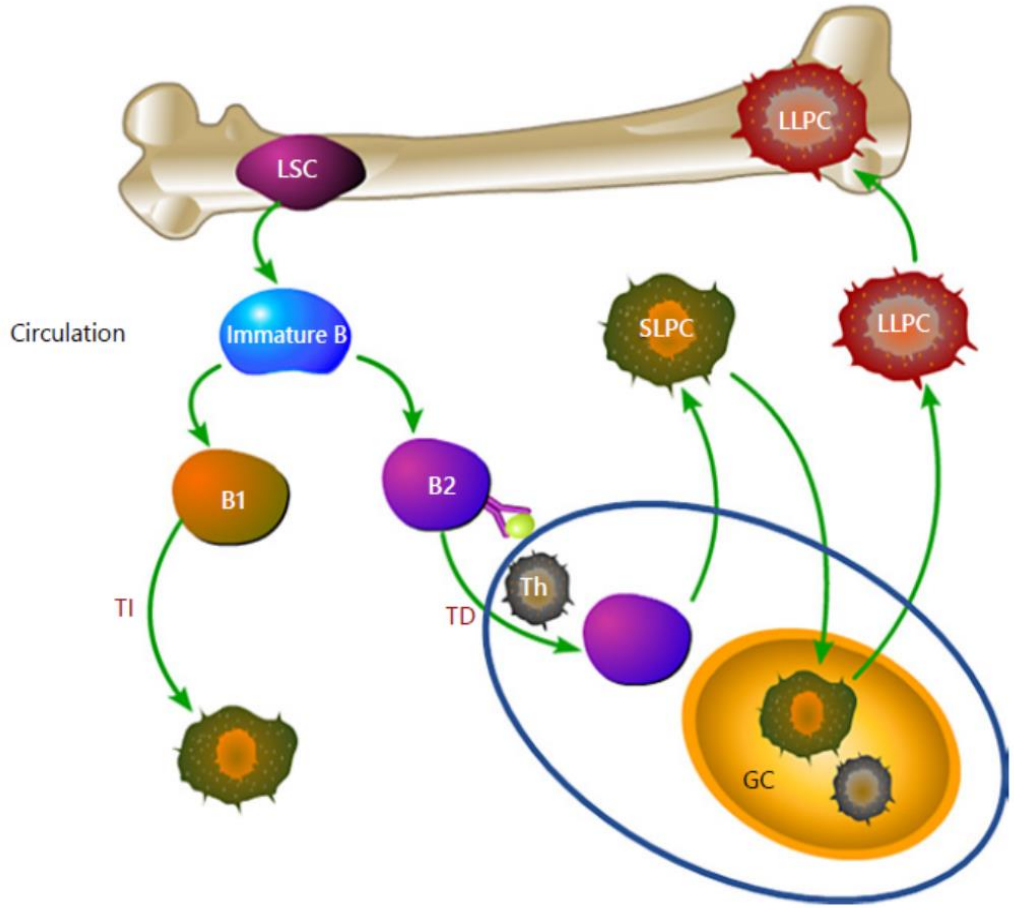
Long-lived plasma cells (LLPCs)

LLPC represent a minority of plasma cell pool (10-20%m), These cells are the major effector in cAMR:Reside in bone marrow and inflamed graft tissue , Continuously produce DSA (anti-HLA and non-HLA antibodies).

Do NOT require antigen stimulation once established.

Clinical importance: Resistant to standard therapies like: Rituximab (targets CD20, but plasma cells are CD20-negative)

Maintain chronic endothelial injury



Hua Su , kidney diseases 2019

a

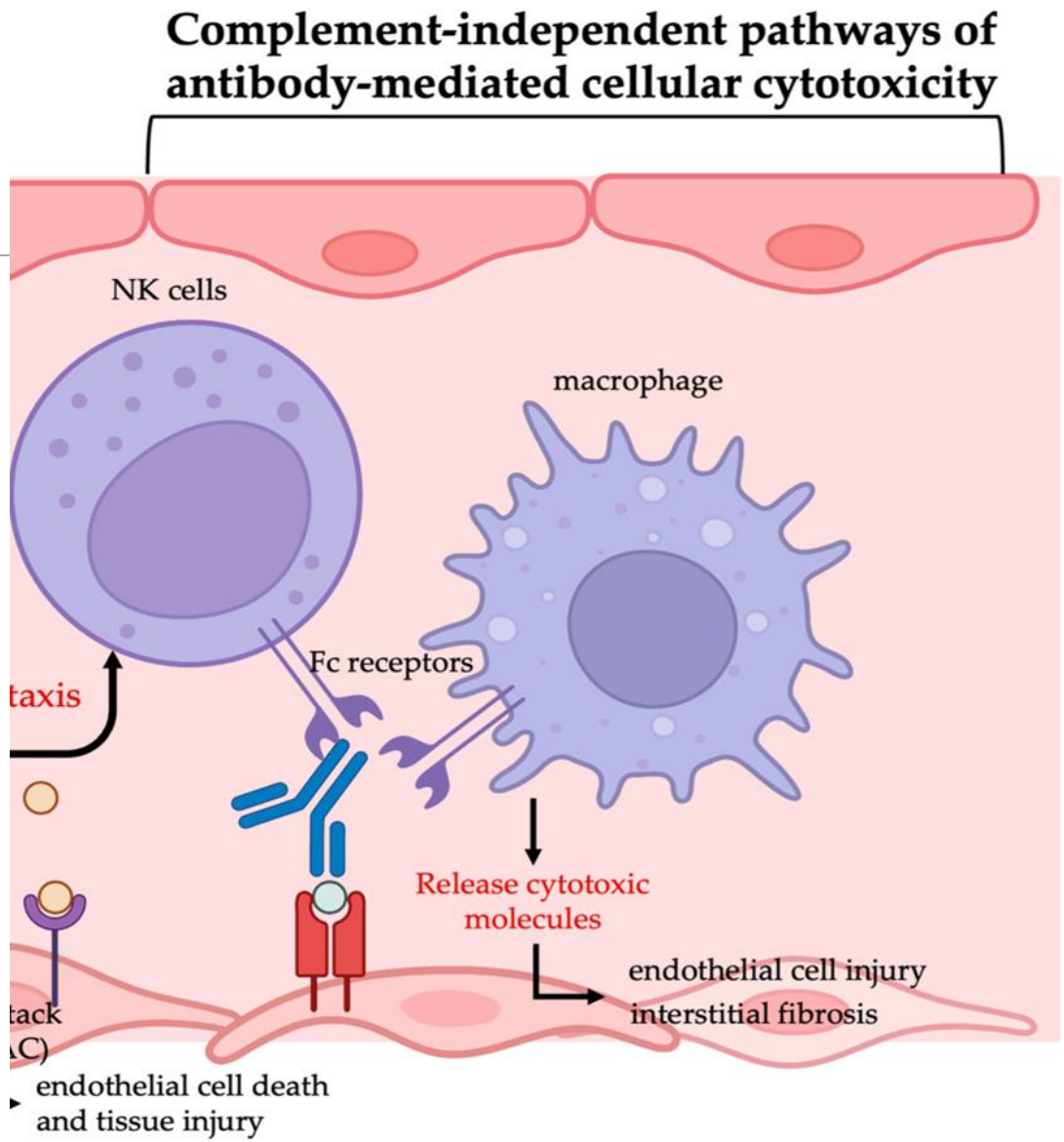
Natural killer (NK) cells

Natural killer (NK) cells :a key effector arm in cAMR—sometimes even more important than complement.

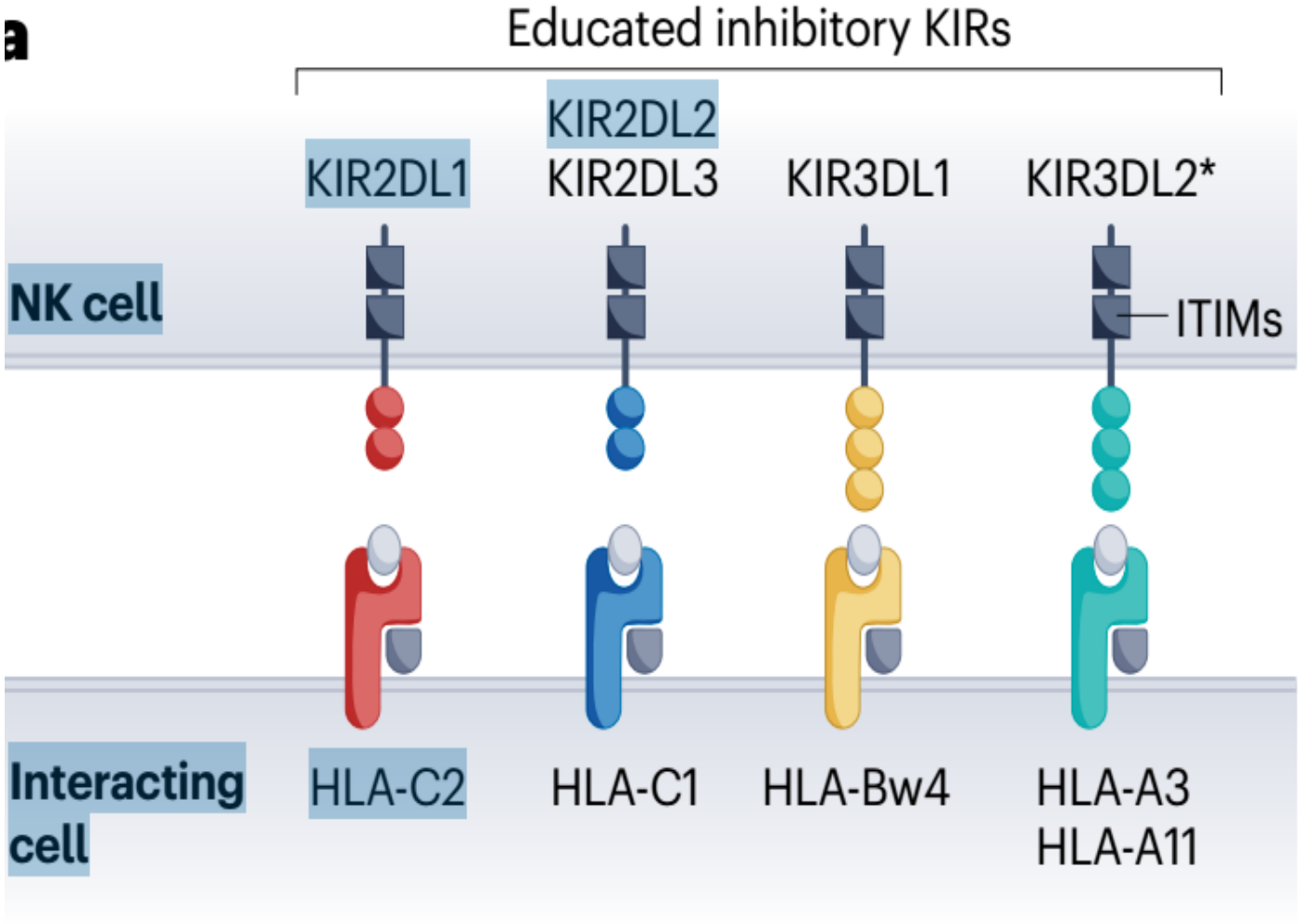
NK cells express CD16 (FcγRIII), which binds antibodies already attached to graft endothelium.

Sequence: Donor-specific antibodies (DSA) bind endothelial HLA (or non-HLA targets)NK cells attach via CD16, become activated → release: Perforin + granzyme → endothelial cell injury, IFN-γ → inflammation

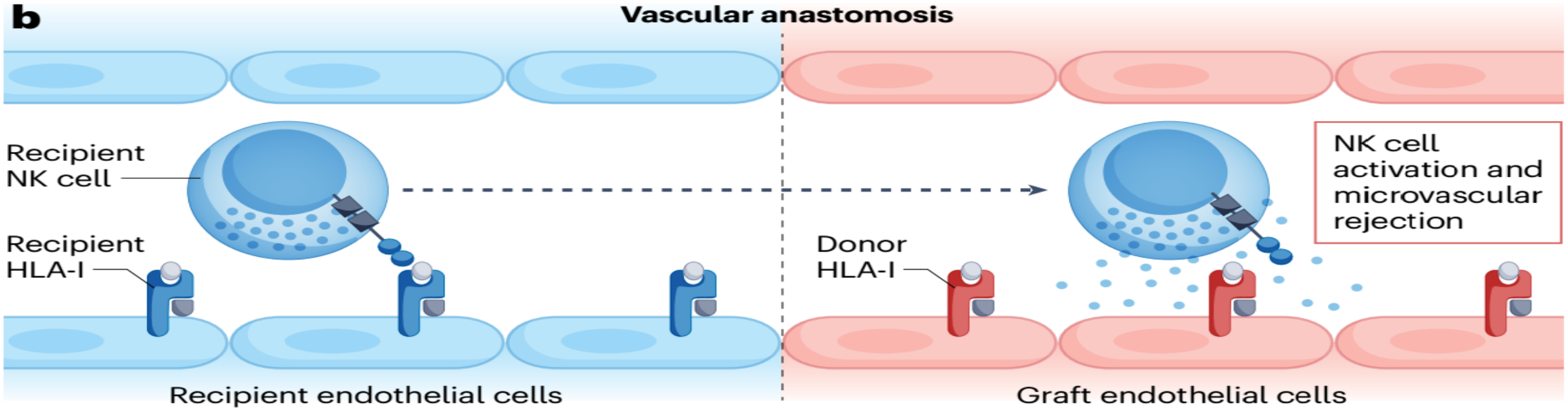
It is complement-independent



Missing-self-induced NK-mediated innate microvascular rejection



Schematic of the molecular interactions between **the five types of inhibitory killer immunoglobulin-like receptors (KIRs)** expressed on the surface of natural killer (NK) cells and the type I human leukocyte antigen (HLA) molecules expressed on the surface of interacting cells



HLA mismatches between donors and recipients create a situation in which graft endothelial cells are unable to provide HLA-I-mediated inhibitory signals to recipient circulating NK cells. This situation of missing self triggers activation of the recipient's NK cells, which initiates a pathophysiological cascade similar to antibody-mediated rejection (albeit in the absence of donor-specific antibodies).

Non HLA Antibody (C4d Negative , DSA negative)

Terasaki deduced that non-HLA immunological factors contribute more to graft failure than HLA antibodies do (40% and 20%, respectively).

It is still debated whether these antibodies can act alone or whether they result in worse allograft outcome together with DSAs.

- Major histocompatibility complex class I-related chain A (MICA) and MICB
- Antibodies against G-coupled receptors present on the endothelium: AT1R and endothelin A receptor (ETAR).
- Antibodies to Collagen I, Collagen V, and k-alpha Tubulin
- Anti- perlecan/ LG3 fragment

Tineke Kardol-Hoefnagel, BSc1 and Henny G. Otten, PhD, transplantation, 2021

1. Trigger antibody binds endothelial targets

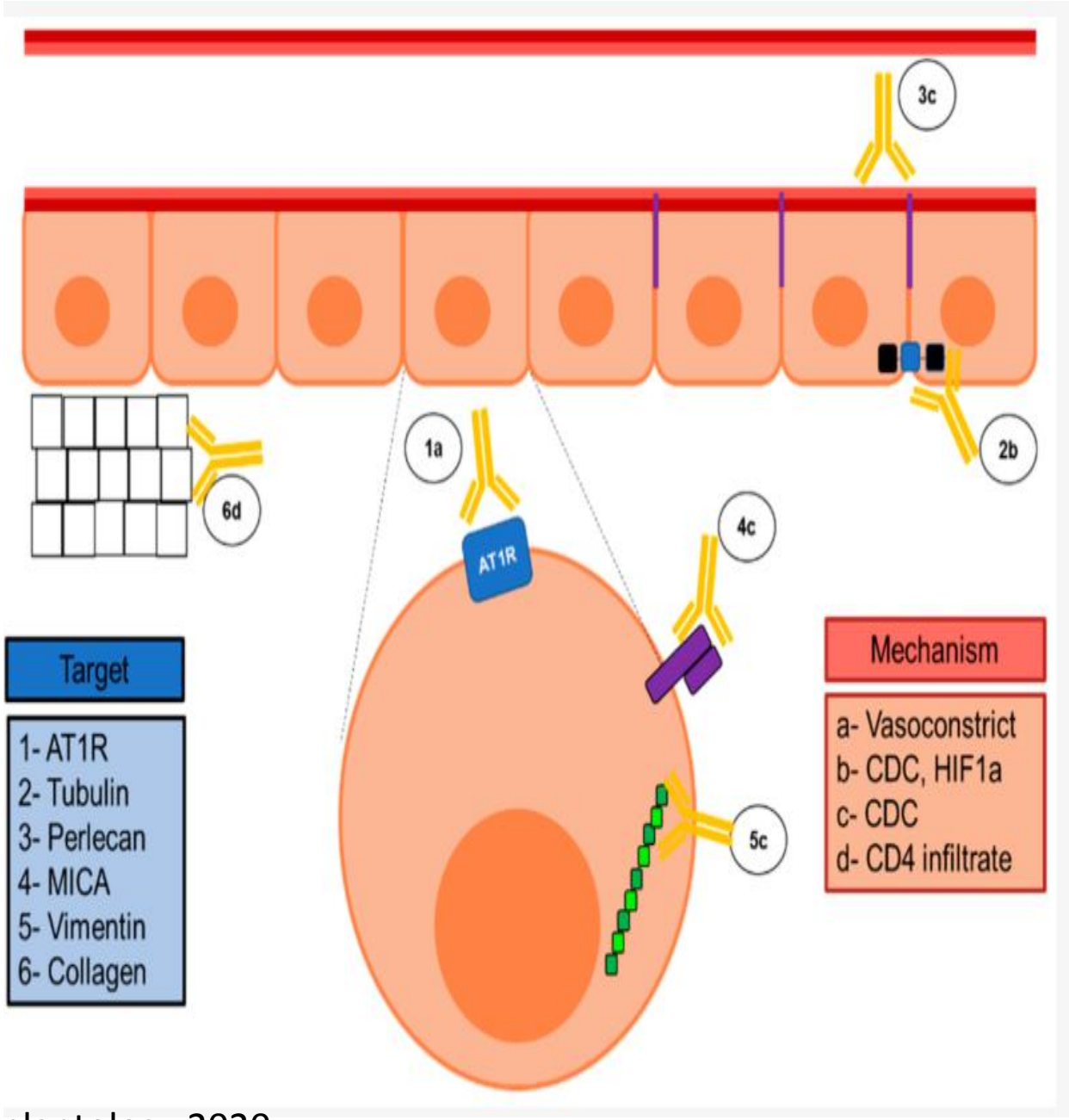
2. Direct endothelial activation :Turn on intracellular pathways →Vasoconstriction, Inflammation (IL-6, MCP-1), Pro-fibrotic signaling

3. Immune amplification via NK cells

4. Complement (secondary, not dominant): Some non-HLA antibodies activate complement

5. Injury → autoimmunity loop

Final outcome in the graft :Persistent endothelial activation, Capillaritis +glomerulitis, Transplant glomerulopathy and Progressive fibrosis



María Gutiérrez-Larrañaga, Transplantology 2020

Practical clinical insight

Think of non-HLA antibodies when you see:

C4d-negative AMR

DSA-negative rejection

Disproportionate vascular injury

Progressive fibrosis despite treatment

Zhang Q, Reed EF. Nat Rev Nephrol 2016

IL-6 in Chronic Antibody-Mediated Rejection (CAMR)

Central Role of IL-6

Produced by endothelial cells, macrophages, and lymphocytes , Links innate immunity to humoral alloimmunity, Promotes Tfh expansion and plasma cell survival.

Sustains donor-specific antibody (DSA) production and amplifies endothelial inflammation and fibrosis

IL-6–Driven CAMR Cascade

DSA → Endothelial Injury

Macrophage Activation

↑ IL-6 Release

↑ Tfh + Plasma Cells

Persistent DSA Production

Microvascular Inflammation

Fibrosis & Transplant Glomerulopathy

Key Concept: IL-6 acts as a bridge cytokine linking endothelial injury, innate immunity, humoral alloimmunity, and chronic graft fibrosis.

References: Choi et al. Am J Transplant 2017; Doberer et al. JASN 2021; Jordan et al. Kidney Int 2022.

Innate immune activation (monocytes & macrophages) in cAMR

1. Recruitment

2. Activation pathways

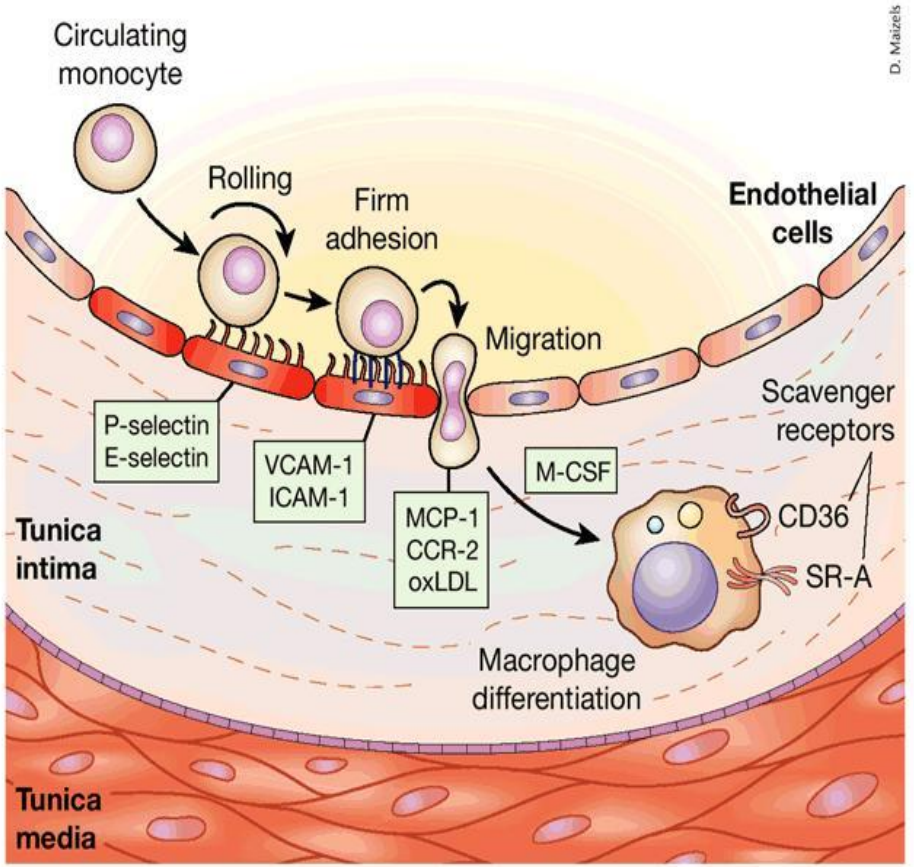
3- Complement fragments

4- Release: TNF-αIL-1,Reactive oxygen species, Sustains vascular damage Bridge to fibrosis (critical role)

Macrophages produce: TGF-β → drives, EndoMT & fibrosis, PDGF →

fibroblast activation, Matrix components, Central to:Transplant

glomerulopathy, Interstitial fibrosis



Macrophage polarization

M1 (pro-inflammatory)

Early phase

Cytotoxic, cytokine-rich

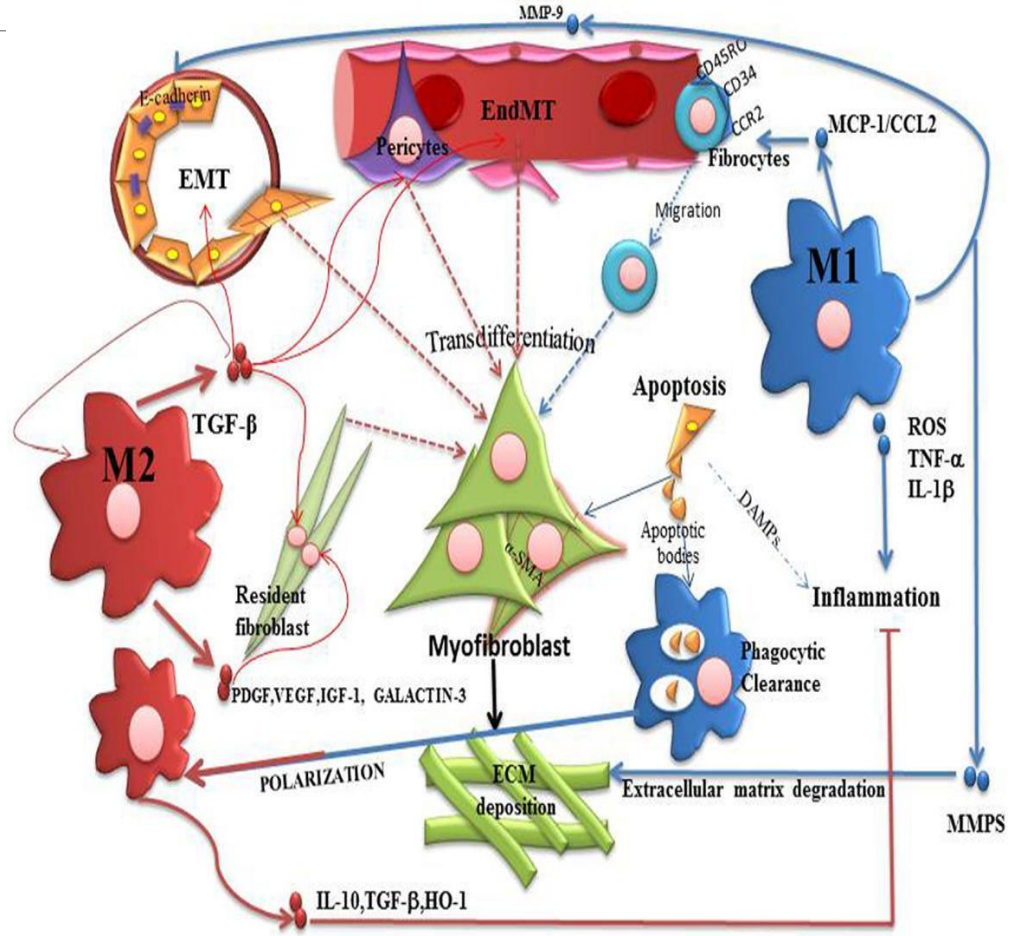
M2 (pro-fibrotic)

Chronic phase

Promote:

- Tissue remodeling
- Fibrosis

In cAMR:
Shift from M1 → M2 → chronic fibrotic phenotype



Tarcio Teodoro Braga et al, Molecular Innate Immunity 2015

Endothelial-to-mesenchymal transition (EndoMT) in cAMR

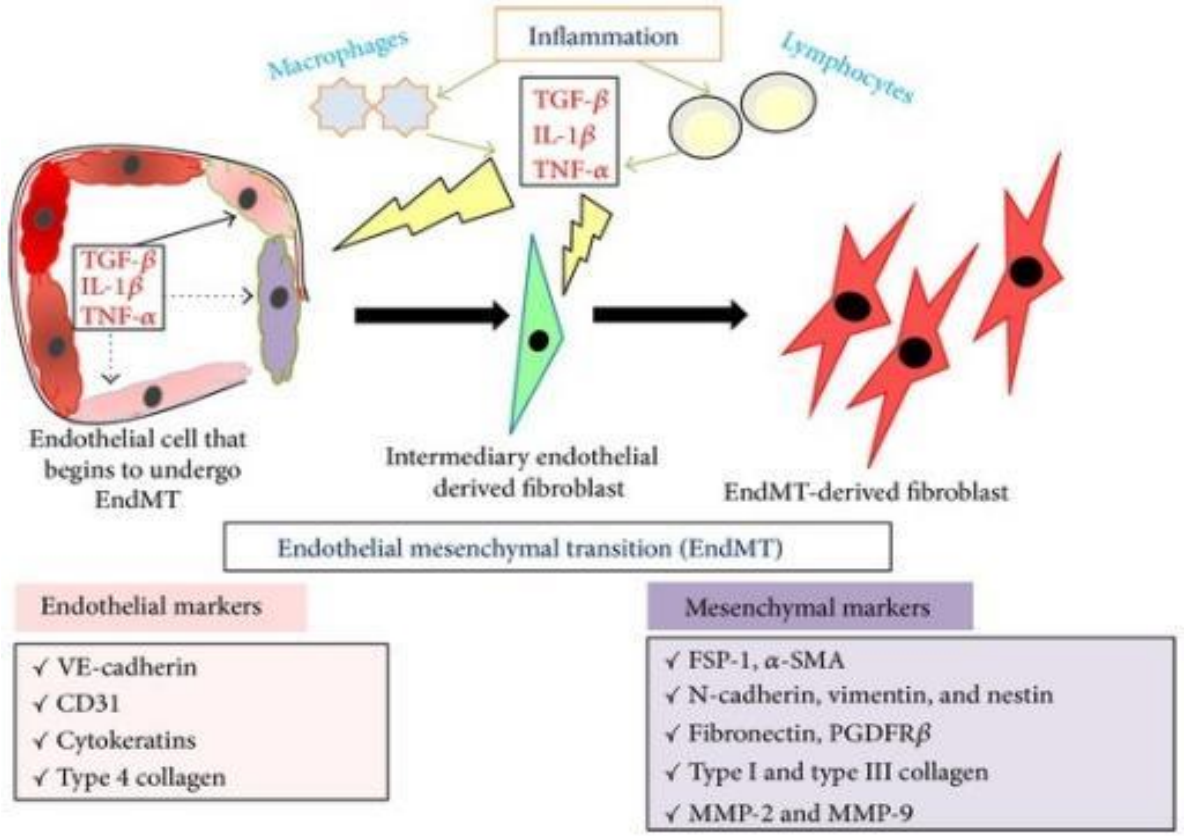
In chronic antibody-mediated rejection:

Persistent endothelial injury (HLA + non-HLA antibodies)

NK-cell–mediated damage

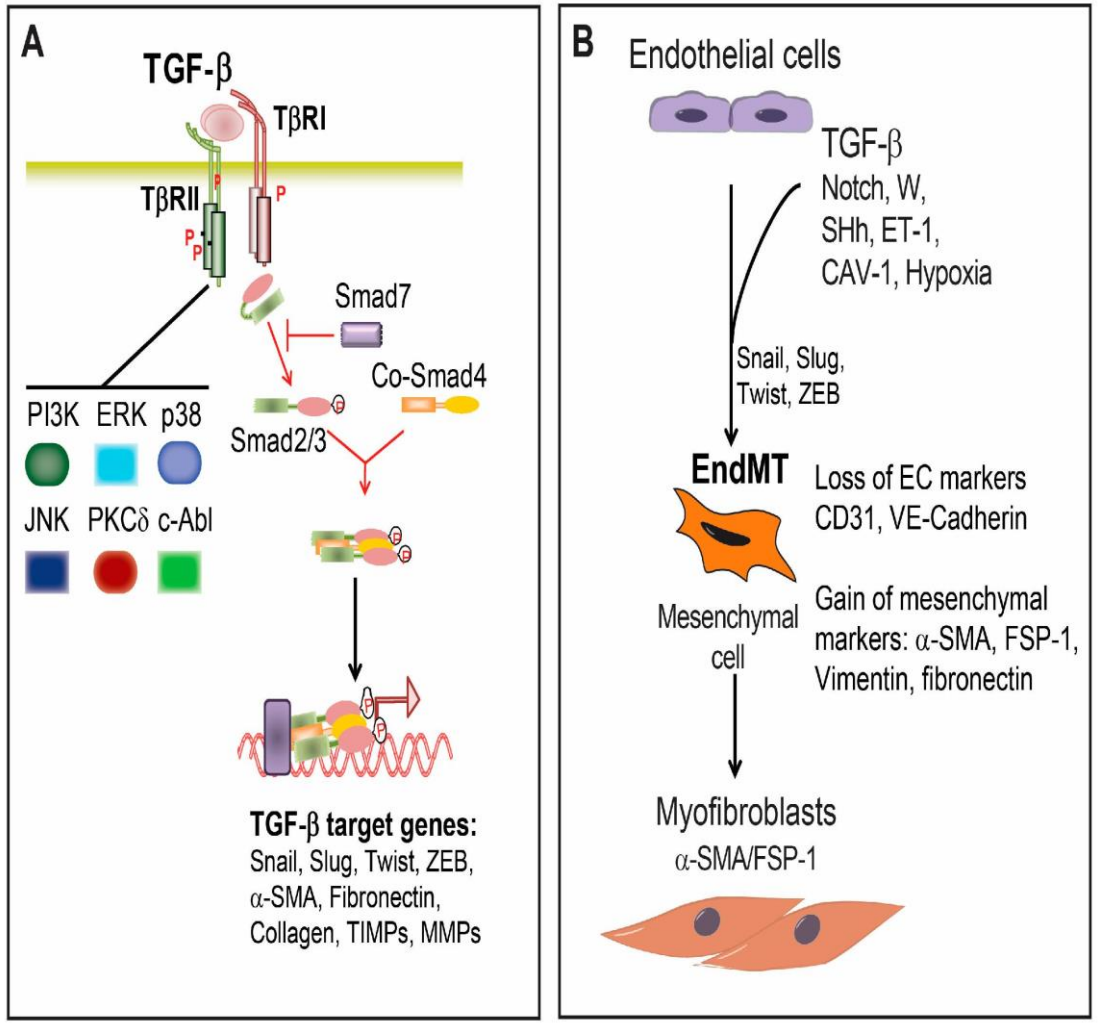
Cytokine-rich environment

Drives **progressive EndoMT → fibrosis**



Mechanistic cascade

- Antibodies bind endothelium
- NK cells + inflammation injure vessels
- Endothelium becomes activated
- TGF-β signaling turns on
- Endothelial cells transition → mesenchymal phenotype
- Fibroblast-like cells produce matrix
- Leads to:Transplant glomerulopathy, Interstitial fibrosis.



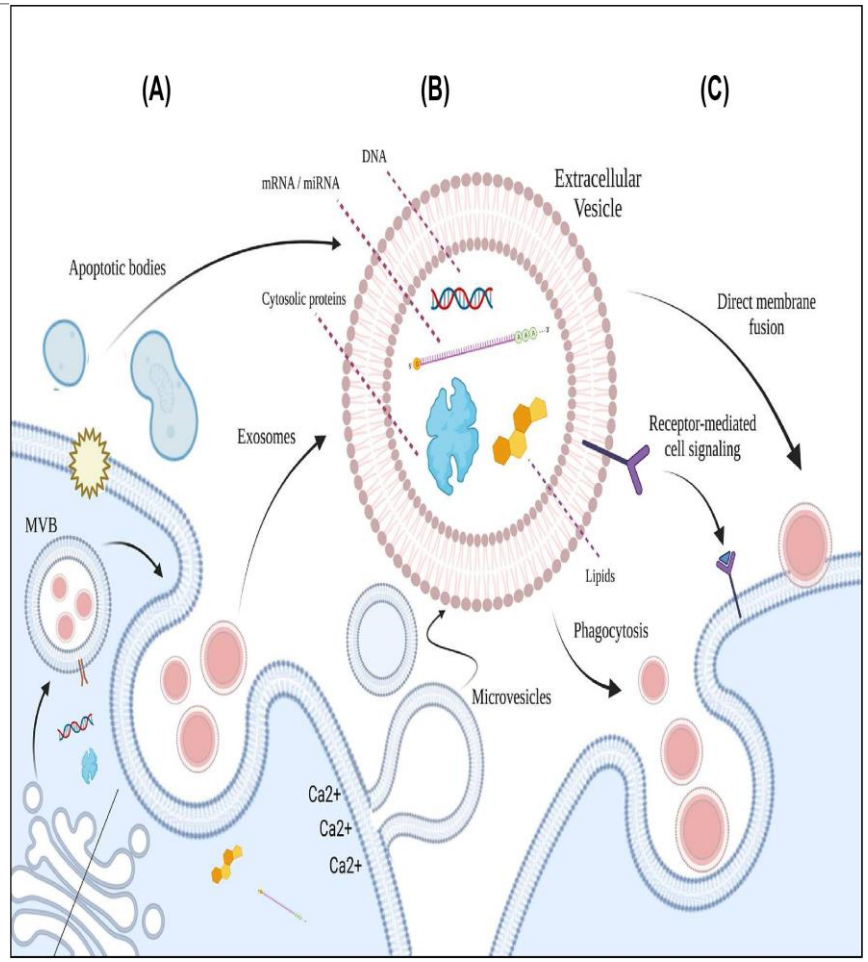
Evangelia Pardali, Int. J. Mol. Sci. 2017,

Extracellular vesicles (EVs) & microRNAs in cAMR

EVs (especially exosomes) are “message carriers” that shuttle microRNAs and proteins between endothelial cells and immune cells → amplifying inflammation and fibrosis in cAMR.

EVs sit in the middle of the network: Antibodies → endothelial injury, Endothelium → releases Evs, EVs → activate: NK cells, Macrophages, Fibrosis pathways, Creates a long-range communication system sustaining cAMR.

Clinical implications: Non-invasive biomarkers Blood or urine EV-miRNA profiles may detect cAMR early, monitor treatment response

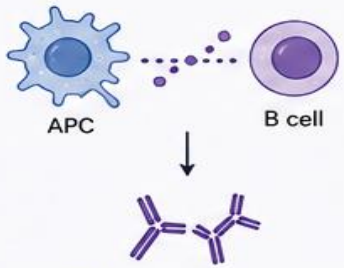


Nassar et al , Front. Transplant., 2023

WRAP-UP: PATHOGENESIS OF cAMR

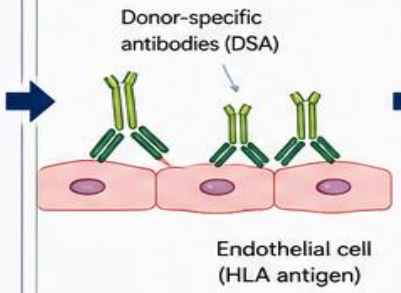
From sensitization to chronic allograft injury and loss

1 SENSITIZATION
Formation of DSA



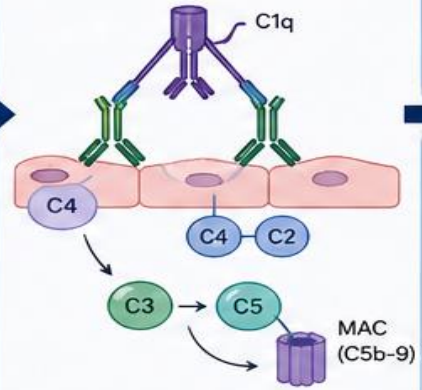
- Alloantigen exposure (transplant, transfusion, pregnancy)
- Activation of B cells → differentiation to plasma cells → production of DSA

2 DSA BINDING
To donor endothelium



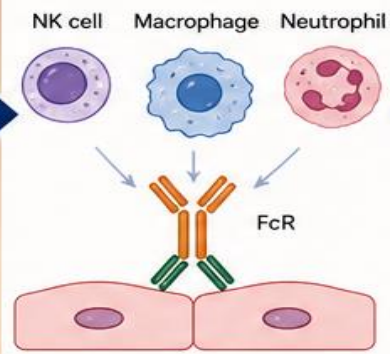
- DSA binds to HLA antigens on endothelial cells
- Activates complement and Fc-mediated pathways

3 COMPLEMENT ACTIVATION
Classical pathway



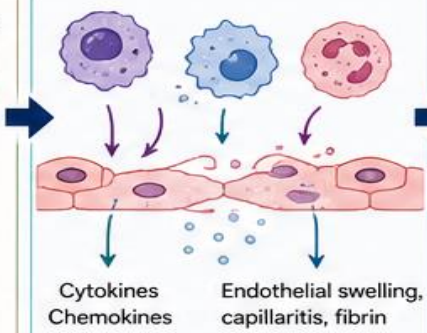
- C1q binding → C4d deposition
- Generation of C3a, C5a (inflammation)
- Terminal complement → MAC formation (endothelial injury)

4 EFFECTOR CELL ACTIVATION
Fc receptor–mediated



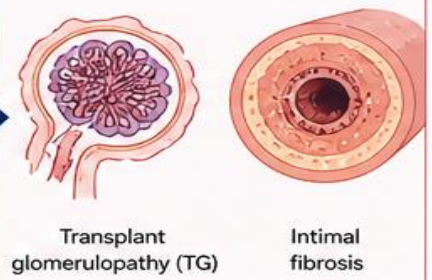
- DSA–Fc binding activates NK cells, macrophages, neutrophils
- Release of cytokines, proteases, ROS → endothelial injury

5 MICROVASCULAR INJURY
& Inflammation



- Microvascular inflammation (g, ptc) and endothelial cell injury
- Capillary leak, thrombosis, ischemia
- C4d deposition (if complement-mediated)

6 CHRONIC ALLOGRAFT INJURY
& Remodelling

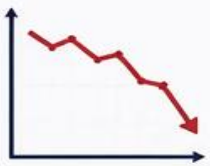


- Persistent injury → fibrosis
- Transplant glomerulopathy (TG)
- Intimal fibrosis of arteries
- Progressive decline in graft function → graft loss

KEY DRIVERS

- **Persistent DSA** (especially de novo and complement-binding DSA)
- **Complement activation** (C1q, C3d, C4d)
- **Fc receptor–mediated cellular injury**
- **Pro-inflammatory cytokines & chemokines** (e.g., IL-6, IL-8, TNF-α)
- **Endothelial activation & microvascular inflammation**
- Genetic, non-adherence, infections, alloantigen load (HLA-DQ mismatch)

CONSEQUENCES



Progressive allograft dysfunction and chronic damage

- Decline in GFR
- Proteinuria
- Fibrosis & scarring
- Graft failure

MODULATING FACTORS

Genetic susceptibility (donor & recipient)

Immunosuppression intensity & adherence

Infections (e.g., CMV, BK) & inflammation

Donor factors (age, HLA mismatch, ischemia-reperfusion)

Alloantigen load & epitope spreading

KEY TAKEAWAY

cAMR results from a complex interplay of **DSA, complement activation, Fc-mediated effector mechanisms,** and **endothelial injury,** leading to progressive microvascular damage, fibrosis, and ultimately graft loss.

REFERENCES:

Loupy A, Haas M, Solez K, et al. The Banff 2019 Kidney Meeting Report: Antibody-mediated rejection. *Kidney International* (2020) 98, 76–107.

Reeve J, Einecke G, Loupy A. Antibody-mediated rejection — an update. *Nat Rev Nephrol*. 2018;14(2):105–118.

Sethi S, Haas M, Loupy A, et al. Pathologic features of chronic antibody-mediated rejection. *Curr Opin Nephrol Hypertens*. 2012;21(3):305–310.

DSA in Chronic AMR – Decision Tree

When to Request & How to Monitor

WHEN TO REQUEST DSA

1. ROUTINE SURVEILLANCE
(even if stable)

- Baseline (pre-transplant / immediate post-Tx)
- 3 months
- 6 months
- 12 months
- Annually thereafter

Goal: detect de *novo* DSA early (before damage)

2. TRIGGER-BASED TESTING
(Request immediately if)

GRAFT DYSFUNCTION
- Rising creatinine
- New or worsening proteinuria

IMMUNOLOGIC RISK EVENTS
- Non-adherence
- Reduction of immunosuppression
- Infection requiring ↓ immunosuppression

SENSITIZING EVENTS
- Blood transfusion
- Pregnancy
- Re-transplantation

BIOPSY FINDINGS
- Microvascular inflammation
- Early transplant glomerulopathy (TG)
- C4d ±

HOW TO INTERPRET DSA

STRONG RISK FEATURES
- De *novo* DSA
- Class II (especially DQ)
- High MFI
- Complement-binding (C1q+, C3d+)
- Persistent over time

LIMITATIONS
- DSA alone ≠ diagnosis
- Some DSA-positive patients have no injury *(accommodation)*
- Some cAMR cases are DSA-negative (non-HLA Ab)
- MFI is semi-quantitative, not absolute

PEARLS
- De *novo* DSA = strongest predictor of cAMR
- Class II (DQ) = most dangerous long-term
- Trend > absolute MFI
- DSA precedes histologic damage

DSA TEST RESULT

DSA NEGATIVE

Continue routine surveillance

Repeat as per schedule or clinical triggers

DE NOVO DSA POSITIVE (New DSA detected)

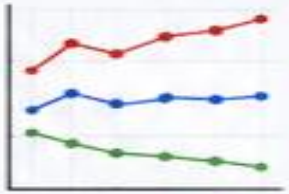
1. **Confirm result**
   - Repeat test
2. **Characterize**
   - MFI level
   - Class (I vs II; especially DQ)
   - Complement-binding (if high risk)

KIDNEY BIOPSY
(Even if creatinine is normal)

Assess biopsy
- cAMR present → **TREAT**
- No significant injury → **Close monitoring**

DSA POSITIVE (Established)

Monitor trends, not single values



- **Increasing MFI** → Ongoing immune activation
- **Stable** → Indolent or controlled
- **Decreasing MFI** → Possible response *(not always histologic improvement)*

What to do?
- If graft dysfunction or rising DSA → Biopsy
- If stable and no injury → Continue monitoring

MONITORING FREQUENCY (After DSA is Positive)

HIGH-RISK DSA	STABLE DSA	DSA NEGATIVE
(De novo, high MFI, DQ, complement-binding, rapidly rising)	(Low/Moderate risk, stable levels)	(Stable patient)
Every 1–3 months	Every 3–6 months	As per routine schedule

IMPORTANT PITFALLS

- **Do NOT treat numbers alone** (High MFI without injury ≠ automatic treatment)
- **MFI is not absolute** (Lab variability, prozone effect, interference)
- **DSA-negative ≠ safe** (Consider non-HLA antibodies, technical false negatives)

PRACTICAL ALGORITHM – SUMMARY

1. **Request DSA** (Routine or triggered)
2. **Interpret** (MFI, Class, Persistence, Complement-binding)
3. **Biopsy if**
   - De novo DSA
   - Rising DSA
   - Graft dysfunction
4. **Integrate** (DSA + Biopsy + Clinical)
5. **Manage** (Treat if cAMR; Optimize IS; Monitor trends)

Key Takeaway: Request DSA routinely and with any clinical trigger; monitor trends over time, and always combine with biopsy for diagnosis and management decisions.

DONOR-DERIVED CELL-FREE DNA (dd-cfDNA) IN CLINICAL PRACTICE

A non-invasive biomarker of allograft injury to guide transplant care

1. WHAT IS dd-cfDNA?

- Fragments of DNA released from injured donor allograft cells into recipient plasma
- Measured as % of total cfDNA or copies/mL plasma
- Reflects active graft injury from any cause

Examples of available assays:

AlloSure® CareDx | Prospera® Natera

2. CLINICAL USES

Early detection of rejection
Often precedes change in creatinine

Differentiate injury vs stable graft
Helps determine need for biopsy

Rule-out tool
High negative predictive value (NPV)

Risk stratification
Best when combined with DSA and clinical context

3. INTERPRETATION (KIDNEY TRANSPLANT)

dd-cfDNA (% of total cfDNA)	Interpretation
< 0.5%	Low probability of active rejection
0.5 – 1.0%	Indeterminate ("gray zone")
> 1.0%	Increased suspicion for rejection (esp. AMR)

Rising trend over time is more important than a single value

4. dd-cfDNA + DSA: HOW TO INTERPRET

dd-cfDNA	DSA	Interpretation	Suggested Action
Elevated (>1.0% or rising)	DSA Positive (de novo or persistent)	High risk of ANTIBODY-MEDIATED REJECTION (Active endothelial injury)	Biopsy recommended Treat if biopsy-proven
Elevated (>1.0% or rising)	DSA Negative	Graft injury from other causes (TCMR, BK, infection, ischemia, drug toxicity)	Biopsy recommended to identify cause
Normal / Low (<0.5%)	DSA Positive (de novo or persistent)	Possible SUBCLINICAL REJECTION (esp. AMR) Injury may be below detection threshold	Close monitoring Consider biopsy based on risk & clinical context
Normal / Low (<0.5%)	DSA Negative	Low probability of active rejection	Continue routine monitoring

5. WHEN TO USE dd-cfDNA

- Routine surveillance (e.g., 1, 3, 6, 12 months)
- Any graft dysfunction (creatinine ↑ or proteinuria)
- New de novo DSA
- Post-treatment monitoring of rejection

6. MONITORING

- Stable patient: every 3–6 months
- High-risk / abnormal: every 1–2 months
- Focus on trend, not single value

7. LIMITATIONS

- Not rejection-specific: Infection (e.g., BK virus), ischemia, biopsy injury, other graft injuries
- Early post-transplant levels may be elevated
- Cannot distinguish AMR vs TCMR alone
- Cost and accessibility limitations

8. KEY TAKEAWAY

dd-cfDNA is a **non-invasive biomarker** of allograft injury with **high negative predictive value.** It is best used **alongside DSA and clinical data** to guide biopsy decisions and optimize management of transplant recipients.

REFERENCES:

Bloom RD, Bromberg JS, Poggio ED, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. *Circulation*. 2017;136(23):230–241.

Position Statement on Use of Donor-Derived Cell-Free DNA in Transplantation. *Am J Transplant*. 2023.

(Kidney International (2020) 98, 76–107).

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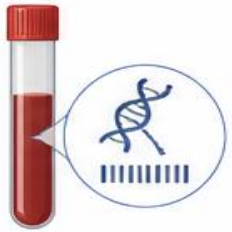
GENE EXPRESSION PROFILING (GEP) IN TRANSPLANTATION

A molecular "snapshot" of immune activity to guide transplant care

1. WHAT IS GEP?



- Measures **mRNA expression** patterns from peripheral blood or allograft biopsy tissue
- Reflects **immune activation** status (rejection vs quiescence)
- Uses **multi-gene** algorithms to generate a risk result

SOURCES

- Peripheral blood
- Allograft biopsy tissue

2. COMMON PLATFORMS / TESTS

AlloMap®
Heart transplant (blood test)
- Identifies low risk of acute cellular rejection

TruGraf®
Kidney transplant (blood test)
- Detects immune quiescence vs subclinical rejection

Tissue-based platforms
Molecular microscope / transcriptomics (e.g., MMDx)
(biopsy tissue; research/advanced use)

3. CLINICAL USES

- **Non-invasive surveillance** — Monitor immune status over time
- **Detect subclinical rejection** — Identify immune activation before clinical dysfunction
- **Rule-out tool** — High negative predictive value to reduce unnecessary biopsies
- **Works best in combination with** DSA, dd-cfDNA and clinical data

4. INTERPRETATION (CONCEPTUAL)

GEP REPORT (Typical Output)

IMMUNE QUIESCENCE SIGNATURE
Low probability of rejection
(Stable / not-at-risk)

REJECTION / INJURY SIGNATURE
Increased risk of rejection
(At-risk / further evaluation needed)

Trend over time is more important than a single result

5. INTEGRATING GEP WITH OTHER BIOMARKERS

GEP RESULT	dd-cfDNA	DSA	INTERPRETATION	SUGGESTED ACTION
Normal (Quiescence)	Normal (<0.5%)	Negative	Very low risk of active rejection	Continue routine monitoring
Abnormal (Rejection signature)	Elevated (>1.0%)	Positive or Negative	Active injury likely	Biopsy recommended Treat if biopsy-proven
Normal (Quiescence)	Elevated (>1.0%)	Positive (de novo or persistent)	Possible early / subclinical AMR	Consider biopsy Close monitoring
Abnormal (Rejection signature)	Normal (<0.5%)	Negative	Immune activation without injury (e.g., early TCMR, viral, other)	Clinical correlation Repeat testing / Consider biopsy

GEP complements, but does not replace, clinical judgment and biopsy when indicated.

6. WHEN TO USE GEP

- **Routine surveillance** (e.g., months 1, 3, 6, 12...)
- **Stable patient with clinical suspicion**
- **Discordant DSA / dd-cfDNA results**
- **To avoid biopsy in low-risk patients**
- **Post-treatment monitoring**

7. LIMITATIONS

- Not rejection-specific (infection, inflammation viral, offect)
- Less validated than biopsy in kidney transplant
- Variable performance in TCMR vs AMR
- Platform-dependent algorithms
- Cost and availability limitations

8. KEY TAKEAWAY

Gene expression profiling provides a non-invasive assessment of immune activity. It is best used to rule out rejection and reduce unnecessary biopsies, especially when combined with DSA and dd-cfDNA.

9. PRACTICAL ALGORITHM (How to Use GEP in Clinical Practice)

1. **Identify Patient** — Stable surveillance or clinical trigger
2. **Order Tests** — GEP + dd-cfDNA ± DSA (consider clinical context)
3. **Interpret Together** — Integrate GEP result with dd-cfDNA, DSA and clinical data
4. **Decide Next Step** — Biopsy if high risk / discordant
Monitor if low risk
5. **Act & Reassess** — Treat if biopsy-proven
Monitor trends over time

REFERENCES

- Bloom RD, Bromberg JS, Poggio ED, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. *Circulation*. 2017;136(23):230–241.
- Halloran PF. Molecular diagnostics in transplantation: gene expression profiling. *Am J Transplant*. 2014,14(4):730–738.
- Deng MC, Eisen HJ, Mehra MR, et al. Noninvasive detection of rejection in cardiac allograft recipients using gene expression profiling. *J Heart Lung Transplant*. 2006;25(1):22–31.
- American Society of Transplantation (AST) Reviews on Molecular Diagnostics in Transplantation.

cAMR DIAGNOSIS WITHOUT BIOPSY

A Practical Diagnostic Algorithm

KIDNEY TRANSPLANT RECIPIENT

Clinical trigger: ↑ Serum creatinine / proteinuria / ↓ eGFR / unexplained graft dysfunction

↓

1 INITIAL RISK ASSESSMENT

Review history and baseline parameters

- Time from transplant
- Previous rejection episodes
- Non-adherence / under-immunosuppression
- Recent infections / comorbidities
- Immunologic risk (sensitization, high PRA)
- Trend of kidney function (stable vs progressive)

↓

2 BASIC NON-INVASIVE SCREENING (ALL PATIENTS)

DSA TESTING

- Anti-HLA DSA
- Consider non-HLA (AT1R, ETAR, MICA)

DONOR-DERIVED CELL-FREE DNA (dd-cfDNA)

- e.g., AlloSure®, Prospera®

GENE EXPRESSION PROFILING (GEP)

- e.g., TruGraf®, kSORT®
- Detects immune activation signatures

ENDOTHELIAL BIOMARKERS

- CXCL9, CXCL10
- VCAM-1, E-selectin
- (± C4d signature)

3 INTEGRATE RESULTS

HIGH PROBABILITY OF cAMR

Any of the following:

- Strong DSA (high MFI) **PLUS**
- dd-cfDNA significantly ↑ (>1% or above assay cut-off)
- GEP with ABMR probability above threshold
- Endothelial markers markedly ↑ (CXCL9/10, VCAM-1)

→ **LIKELY ACTIVE cAMR** (High non-invasive probability)

INTERMEDIATE PROBABILITY

Some of the following:

- Low/Moderate DSA or non-HLA antibodies
- dd-cfDNA mildly ↑ or borderline
- GEP borderline / inconclusive
- Mild elevation of endothelial markers

→ **POSSIBLE cAMR** (Indeterminate risk)

LOW PROBABILITY

All of the following:

- No DSA / non-HLA Ab
- dd-cfDNA low (<0.5%)
- GEP not suggestive of rejection
- Endothelial markers normal or minimally ↑

→ **LOW LIKELIHOOD OF cAMR** (Consider other causes)

CONFLICTING RESULTS

Discordant findings (e.g., DSA negative but dd-cfDNA high or GEP positive)

→ **UNCERTAIN** (Needs further evaluation)

4 NEXT STEPS

Likely active cAMR

- Consider this as probable active cAMR
- Optimize immunosuppression
- Treat if clinically indicated (per center protocol)
- **STRONGLY CONSIDER** BIOPSY to confirm & grade severity (Banff)

Possible cAMR

- Close clinical monitoring
- Repeat tests in 1–2 weeks
- Search for other causes (drug toxicity, infection, BK, obstruction)
- **CONSIDER BIOPSY** if persistent dysfunction or rising biomarkers

Low likelihood of cAMR

- Investigate non-immune causes (dehydration, drug toxicity, UTI, obstruction, BK, etc.)
- Routine monitoring
- Repeat panel if kidney function worsens

Uncertain

- Reassess clinically
- Repeat panel
- If uncertainty persists or kidney function declining → **PROCEED TO BIOPSY**

5 WHEN TO PROCEED DIRECTLY TO BIOPSY (Regardless of non-invasive results)

- Rapid rise in creatinine (>20–30% within 1–2 weeks)
- Nephrotic-range proteinuria or rapidly increasing proteinuria
- Declining graft function with hemodynamic instability
- New structural abnormality on imaging (if available)
- High clinical suspicion of rejection despite negative tests

KEY POINTS

- No single non-invasive test can replace biopsy.
- Use a combination of tests to increase diagnostic confidence.
- Serial testing and trends are more informative than single values.
- Biopsy remains gold standard for definitive diagnosis, staging and prognosis.

ABBREVIATIONS

cAMR: chronic antibody-mediated rejection
DSA: donor-specific antibody **GEP:** gene expression profiling
dd-cfDNA: donor-derived cell-free DNA
MFI: mean fluorescence intensity **Ab:** antibody

NON-INVASIVE TESTS GUIDE – BIOPSY DECIDES – INTEGRATED CARE IMPROVES OUTCOMES

WRAP-UP: INTEGRATED NON-INVASIVE DIAGNOSIS OF cAMR

No single test is sufficient → Combine biomarkers + biopsy when needed

1. STEP 1: SCREEN (NON-INVASIVE BIOMARKERS)

DSA
Immunologic trigger

- Detect de novo DSA
- Monitor trend
- Class II (DQ) highest risk

dd-cfDNA
Graft injury

- Reflects allograft injury
- Rising levels increase risk

GEP
Immune activation

- Gene expression signature
- Detects immune activation

2. STEP 2: INTERPRET PATTERN (TRIAD APPROACH)

DSA	dd-cfDNA	GEP	INTERPRETATION	SUGGESTED ACTION
Positive (de novo or persistent)	Elevated (>1.0% or rising)	Abnormal (Rejection signature)	**HIGH PROBABILITY AMR** Active antibody-mediated injury likely	Biopsy recommended
Positive (de novo or persistent)	Normal (<0.5%)	Normal (Quiescence signature)	**POSSIBLE SUBCLINICAL AMR** No current injury but immune risk present	Monitor closely Consider biopsy (based on risk)
Negative	Elevated (>1.0% or rising)	Abnormal (Rejection signature)	**NON-DSA INJURY** Consider TCMR, infection, drug toxicity, ischemia, etc.	Biopsy recommended
Negative	Normal (<0.5%)	Normal (Quiescence signature)	**LOW RISK** Very low probability of active rejection	No biopsy Continue routine monitoring

3. STEP 3: CONFIRM

KIDNEY BIOPSY = GOLD STANDARD

- Microvascular inflammation
- Transplant glomerulopathy (TG)
- C4d staining (±)
- Plasma cell infiltration
- Chronic changes

Histology + clinical context = Definitive diagnosis

4. STEP 4: RISK STRATIFICATION

High risk of cAMR if:

- De novo DSA (especially DQ)
- Rising dd-cfDNA (trend)
- Persistent abnormal GEP
- Complement-binding DSA (C1q+, C3d+)
- Prior rejection or non-adherence

5. DIAGNOSTIC PITFALLS

- Treating biomarkers without biopsy
- Ignoring discordant results
- Assuming DSA-negative = no AMR
- Relying on a single value (trend is more important)

6. FINAL TAKEAWAY

Early detection of cAMR requires an integrated approach:
DSA (trigger) + dd-cfDNA (injury) + GEP (immune activity)
→ Biopsy confirmation

7. PRACTICAL ALGORITHM FOR DIAGNOSIS OF cAMR

1. **Clinical Trigger or Routine Surveillance** — Stable or changes in creatinine / proteinuria
2. **Order Biomarkers** — DSA + dd-cfDNA + GEP
3. **Interpret Triad Pattern** — Use triad table above to interpret
4. **Decide on Biopsy** — Biopsy if high risk or discordant results
5. **Integrate All Data** — Biopsy + Biomarkers + Clinical context
6. **Management & Monitoring** — Treat if indicated, monitor trends over time

REFERENCES:

- Loupy A, Haas M, Solez K, et al. The Banff 2019 Kidney Meeting Report: Antibody-mediated rejection. *Kidney International* (2020) 98, 76–107.
- Bloom RD, Bromberg JS, Poggio ED, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. *Circulation*. 2017;136(23):230–241.
- American Society of Transplantation (AST) Reviews on Biomarkers in Transplantation.