The Evolving Role of Novel Biomarkers in Glomerular Disease

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Narrative Review

The Evolving Role of Novel Biomarkers in Glomerular Disease: A Review

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Outline

- Primary membranous nephropathy(MN)
  a. phospholipase A2 receptor 1 (PLA2R)
  b. thrombospondin type 1 domain containing 7A (THSD7A)
  c. Neural epidermal growth factor-like 1 protein (NELL-1)
  d. Exostosin 1/exostosin 2 (EXT1/EXT2)
- C3 glomerulopathy (C3G)
- Fibrillary glomerulonephritis (FGN)

Membranous Nephropathy

- Antibodies targeting autoantigens at the **podocyte cell membrane–basement membrane interface** resulting in immune complex formation.

- LM: thickened glomerular basement membrane(GBM) with “spikes” and “holes” on silver stain.

Membranous Nephropathy

- IF: granular capillary wall staining of **polyclonal immunoglobulin G (IgG)** with variable **C3** staining

- EM: podocyte effacement with **subepithelial** deposits.

Table 1. Biomarkers of Membranous Nephropathy in Adults

Biomarker	Disease	Method of Detection	Malignancy Screening and Rate	Incidence	Comments
Phospholipase A_2 receptor 1 (PLA_2R)	Primary MN	Serum: ELISA,[a] IIF,[a] WB Tissue: IHC, IF	Age-appropriate screening; rate of malignancy: ~9%[32]	~70%-80% of idiopathic MN	• Most common antigen in primary MN • Biopsy not necessary if eGFR > 60 without evidence of secondary/superimposed cause • IgG4 dominant
Neural epidermal growth factor-like 1 protein (NELL-1)	Primary MN	Serum: WB Tissue: IF, IHC	Search for malignancy; rate of malignancy: 11.7-33%[5,92]	~3.8%-16% of PLA_2R, THD7A-negative idiopathic MN	• 2nd most common antigen in MN • IgG1 dominant
Thrombospondin type 1 domain containing 7A (THSd7A)	Primary MN	Serum: ELISA, IIF,[a] WB Tissue: IHC, IF	Aggressive screening including urogenital and gastrointestinal/colorectal: rate of malignancy: 6%-20%[56,59,60]	1%-5% of idiopathic MN (~10% of PLA_2R negative)	• 3rd most common antigen in MN • ELISA not commercially available • IgG4 dominant
Exostosin 1/exostosin 2 (EXT1/EXT2)	Secondary MN	Tissue: IHC, IF	Limited data to recommend screening; rate of malignancy: 7.6%[6]	11.6% of PLA_2R-negative MN	• Tissue marker of class V lupus ~1/3 of cases & autoimmune disease, typically young, female • IgG1 dominant

Abbreviations: eGFR, estimated glomerular filtration rate (in mL/min/1.73 m^2); ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; IgG4, immunoglobulin G4; IHC, immunohistochemical; IIF, indirect immunofluorescence; MN, membranous nephropathy; PLA_2R, phospholipase A_2 receptor; WB, Western blot.
[a]Commercially available.

PLA2R MN

- "**Epitope spreading**" in PLA2R MN is thought to represent resistant disease.
- **Dominant primary epitope: cysteine-rich domain**
- Independent epitopes: CTLD1, CTLD7, CTLD8.

PLA2R MN

- Epitope spreading beyond the original cysteine-rich domain allows for diversification of the immunologic response to the antigen.

- **High titer (>369 RU/mL)**: resistant disease, more proteinuria, and lower eGFR

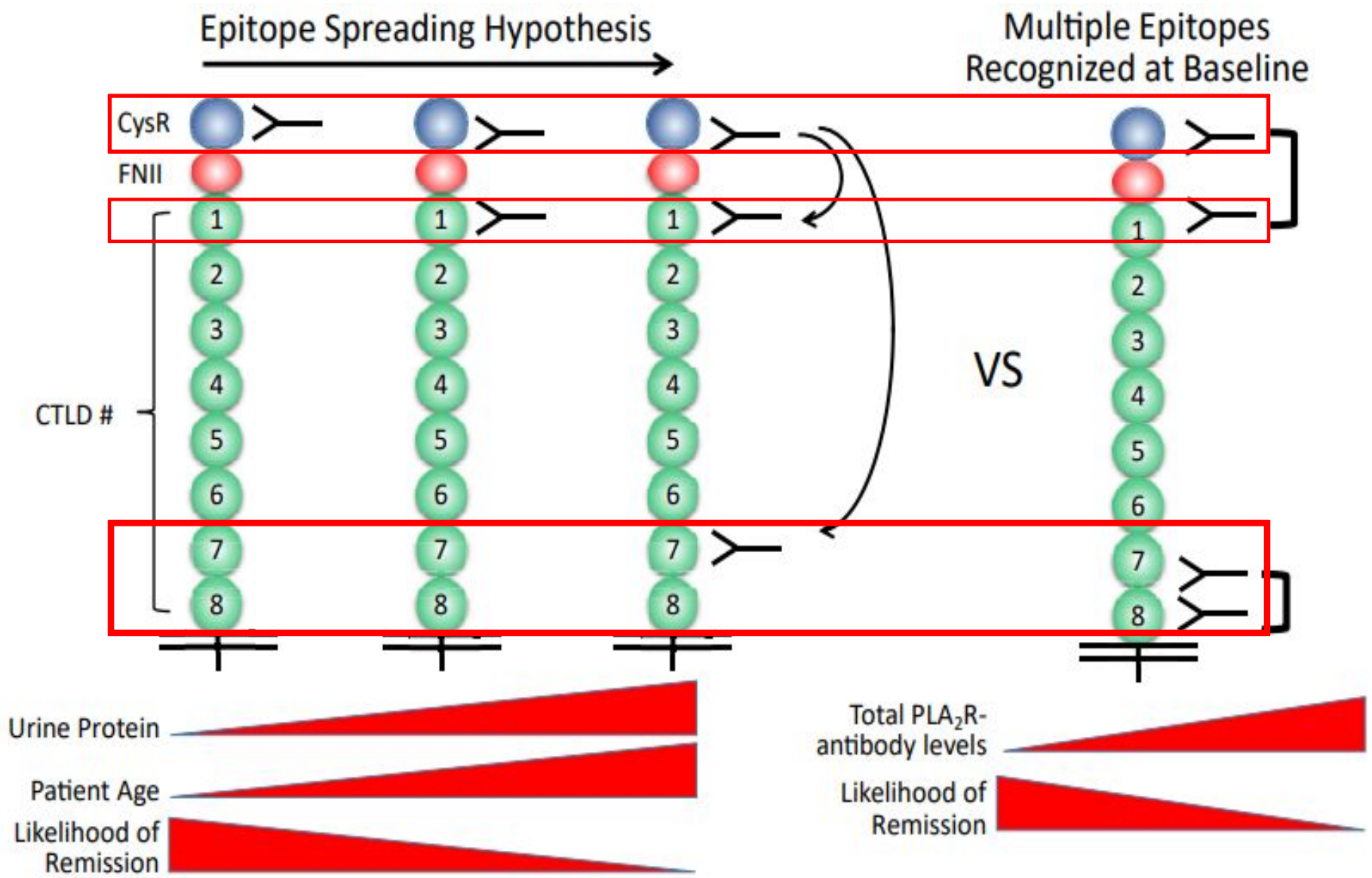


Figure 1. Intramolecular epitope spreading of the anti–phospholipase A2 receptor antibody (anti-PLA2R) versus baseline multidomain recognition. The reactivity of anti-PLA2R antibodies to a ubiquitous epitope in the cysteine-rich (CysR) domain (left) "spreads" to include subdominant epitopes of the first (center, C-type lectin domain [CTLD1]) and seventh and eighth CTLDs (right, CTLD7 and CTLD8) distinct from the CysR epitope. Disease progression is positively correlated with greater urinary protein excretion and patient age and inversely correlated with the likelihood of remission. An alternative hypothesis is that antibodies to multiple domains are present at the time of diagnosis, and progression of disease is correlated with total anti-PLA2R antibody levels. Abbreviation: FNII, fibronectin type II domain.

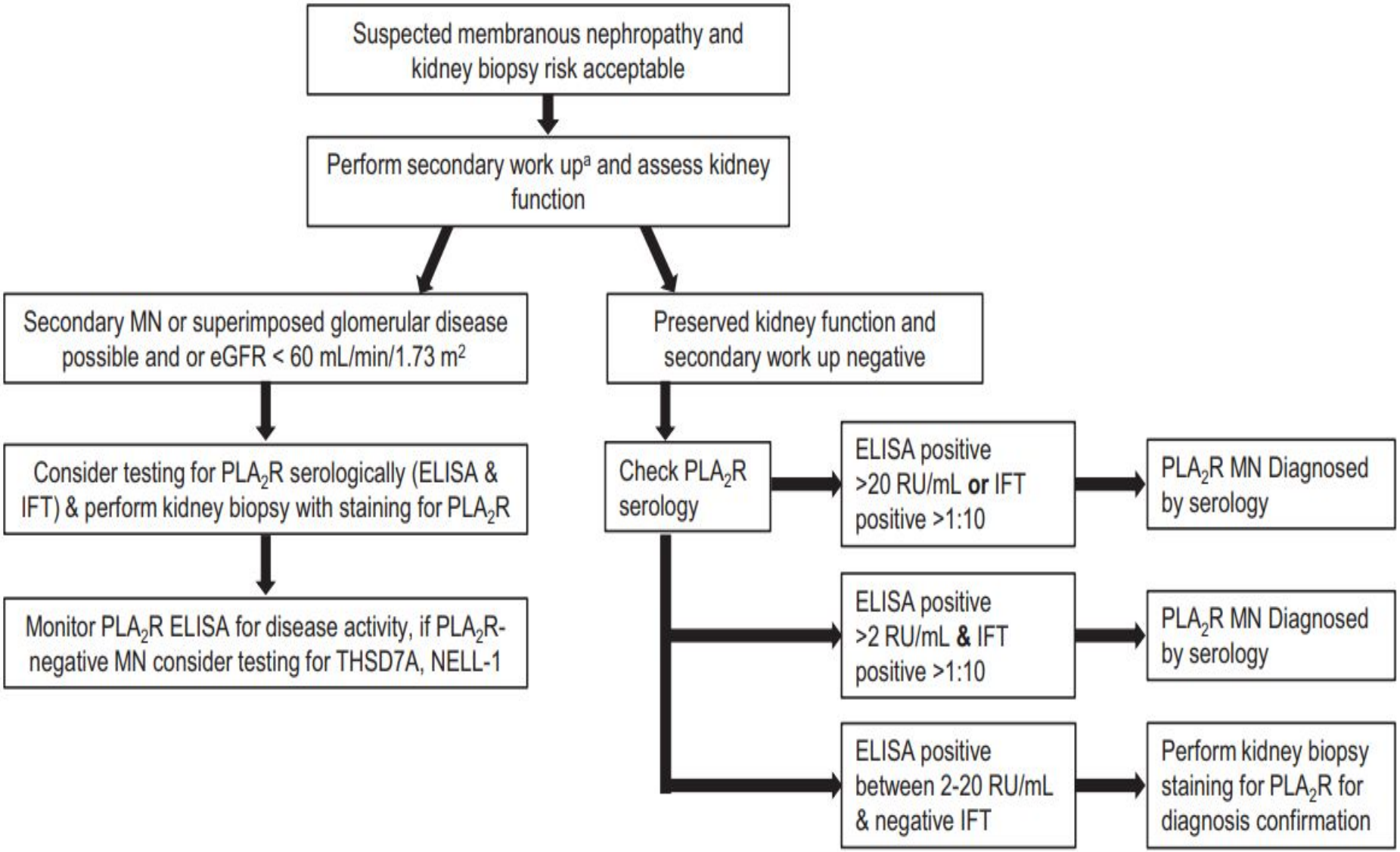
PLA2R MN, GFR > 60 mL/min/1.73 m^2

- ELISA can be obtained while simultaneously screening for secondary causes:

  $\longrightarrow$ viral hepatitis, antinuclear antibodies, IgG4, sarcoidosis, age-appropriate malignancy screening, medication and NSAIDs use.

PLA2R MN, GFR > 60 mL/min/1.73 m^2

- For patients with suspected MN and eGFR > 60 mL/min/1.73 m^2 with **no evidence of secondary causes**, a **biopsy** to prove serologic-positive PLA2R MN is **not necessary**.


Suspected membranous nephropathy and kidney biopsy risk acceptable
Perform secondary work up[a] and assess kidney function
Secondary MN or superimposed glomerular disease possible and or eGFR < 60 mL/min/1.73 m2
Consider testing for PLA2R serologically (ELISA & IFT) & perform kidney biopsy with staining for PLA2R
Monitor PLA2R ELISA for disease activity, if PLA2R-negative MN consider testing for THSD7A, NELL-1
Preserved kidney function and secondary work up negative
Check PLA2R serology
ELISA positive >20 RU/mL or IFT positive >1:10
PLA2R MN Diagnosed by serology
ELISA positive >2 RU/mL & IFT positive >1:10
PLA2R MN Diagnosed by serology
ELISA positive between 2-20 RU/mL & negative IFT
Perform kidney biopsy staining for PLA2R for diagnosis confirmation

PLA2R MN, GFR < 60 mL/min/1.73 m^2

- The severity of **tubulointerstitial scarring** is a prognostic indicator in glomerular disease. However, it is not clear if this is superior to clinical data in MN.

- PLA2R Ag presence does **not exclude** the possibility of superimposed disease.

PLA2R MN, GFR < 60 mL/min/1.73 m^2

- **Hypertensive damage** in cases of higher anti-PLA2R antibody titer is associated with progressive decline in kidney function in MN and **poor response to immunosuppression in diabetic patients**.

- Time to biopsy even high PLA2R (+): **crescentic disease in MN** is a rare (<1%)

- ANCA, anti–GBM or very rarely monoclonal gammopathy of unknown significance (MGUS).

PLA2R MN, GFR < 60 mL/min/1.73 m^2

- Mind the crescentic GN: heavy proteinuria, hematuria, and acute kidney injury(AKI)

- PLA2R MN has also been reported post–hematopoietic stem cell transplantation with crescent formation and as a manifestation of graft-versus-host disease that responded to corticosteroid therapy.

THSD7A MN

- A glycosylated 250-kDa type 1 transmembrane protein highly expressed on podocytes

- **Cancer screening**
  a. gallbladder
  b. colorectal
  c. endometrial
  d. breast

THSD7A MN

- **THSD7A antibodies** are thought to be **pathogenic** -> localizes to the slit diaphragm of podocyte foot processes -> **graft kidney failure**

- Serum indirect IF(IIF) has 92% diagnostic sensitivity and 100% specificity.

C3 Glomerulopathy (C3G)

- Dysregulation of alternative complement in the fluid phase, remarkably **heterogeneous**

- **Trigger events** such as infection, autoimmunity, or monoclonal gammopathy.

C3 Glomerulopathy (C3G)

- **Acquired drivers**: the autoantibodies C3 nephritic factor [C3Nef], C4Nef, C5Nef, monoclonal gammopathy, and anti-factor H

- **Genetic drivers**: mutations of C3 or the complement factor genes CFB, CFH, CFI, and CFHR1-CHR5

C3 Glomerulopathy (C3G)

- **Genetic testing** should be undertaken when familial causes are suspected (ie, CFHR5 nephropathy).

- Kidney failure in **36.5%** of patients at **10 years**

C3 Glomerulopathy (C3G)

- Decision to immunosuppression:
  - severity of proteinuria
  - kidney function
  - degree of tubular atrophy/interstitial fibrosis

- Treatment: **mycophenolate mofetil(MMF)** and corticosteroids

Tissue Biomarkers in C3G

- Glomerular deposition of complement **C3(most)**, C5, C6, C7, C8, and C9.

- Most detected -> **C3dg**, which is cleaved from surface bound C3b. -> opsonins and participate in adaptive immune stimulation.

Tissue Biomarkers in C3G

- Routine evaluation for C3 by IF detects **C3c**
- Most prevalent protein, disease activity: **factor H-related protein 5 (FHR5)**
- Negatively correlated with eGFR: **FHR5, C5b-9**

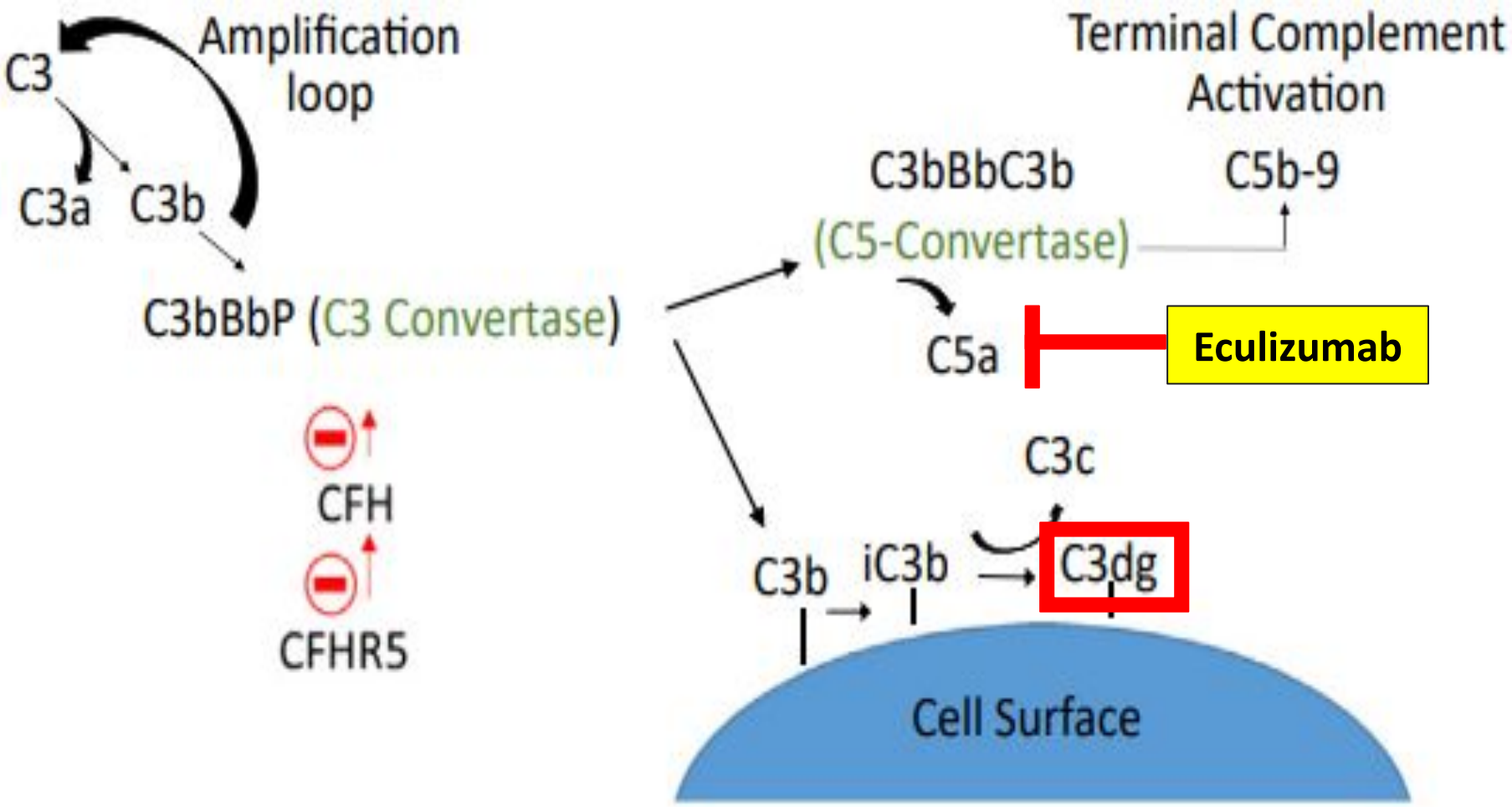


Figure 3. Alternative complement cascade and hypothesized role of complement factor H (CFH)-related protein 5 (CFHR5). Formation of C3 convertases leads to cleavage of C3 and formation of C5 convertase, creating potent anaphylatoxins (C3a and C5a) that mediate the inflammatory response. C3b is degraded into iC3b and C3dg, which mediate phagocytosis and an adaptive immune response. CFH is a strong inhibitor of C3 convertase, whereas CFHR5 preserves C3 convertase activity by inhibiting CFH. C5b causes terminal complement activation membrane attack complexes.

Fibrillary glomerulonephritis (FGN)

- Proliferative glomerulopathy defined ultrastructurally by haphazardly arranged fibrils that are **10 to 30 nm in diameter** and a **lack of Congo red staining** for amyloid.

  $\longrightarrow$ amyloid fibrils (8-12 nm) vs immunotactoid glomerulopathy fibrils (>35 nm)

Fibrillary glomerulonephritis (FGN)

- **DNAJ homolog subfamily B member 9 (DNAJB9), sen. 67-98%, spe. 98-99% for FGN,**

  → amyloidosis, myeloma, non-FGN glomerular disease and healthy individuals

DNAJB9

- A molecular chaperone to bind to immunoglobulins that assist in folding and degrading misfolded proteins to **protect cells from stress apoptosis**.
-
- Present in **podocytes, mesangial and endothelial cells** in the glomerulus.

DNAJB9

- Serum level is negatively associated with eGFR.
- Biomarker for diagnosis and potentially **longitudinal monitoring**.

Conclusions

- MN
  - **PLA2R**
  - THSD7A
  - NELL-1
  - EXT1/EXT2
- C3G: **FHR5**
- FGN: **DNAJB9**

Take home messages

- Novel biomarkers in GNs: MN, C3G, FGN.

Thank you!