Impact of Factor H Related Proteins (FHR) on the Pathogenesis of IgA Nephropathy

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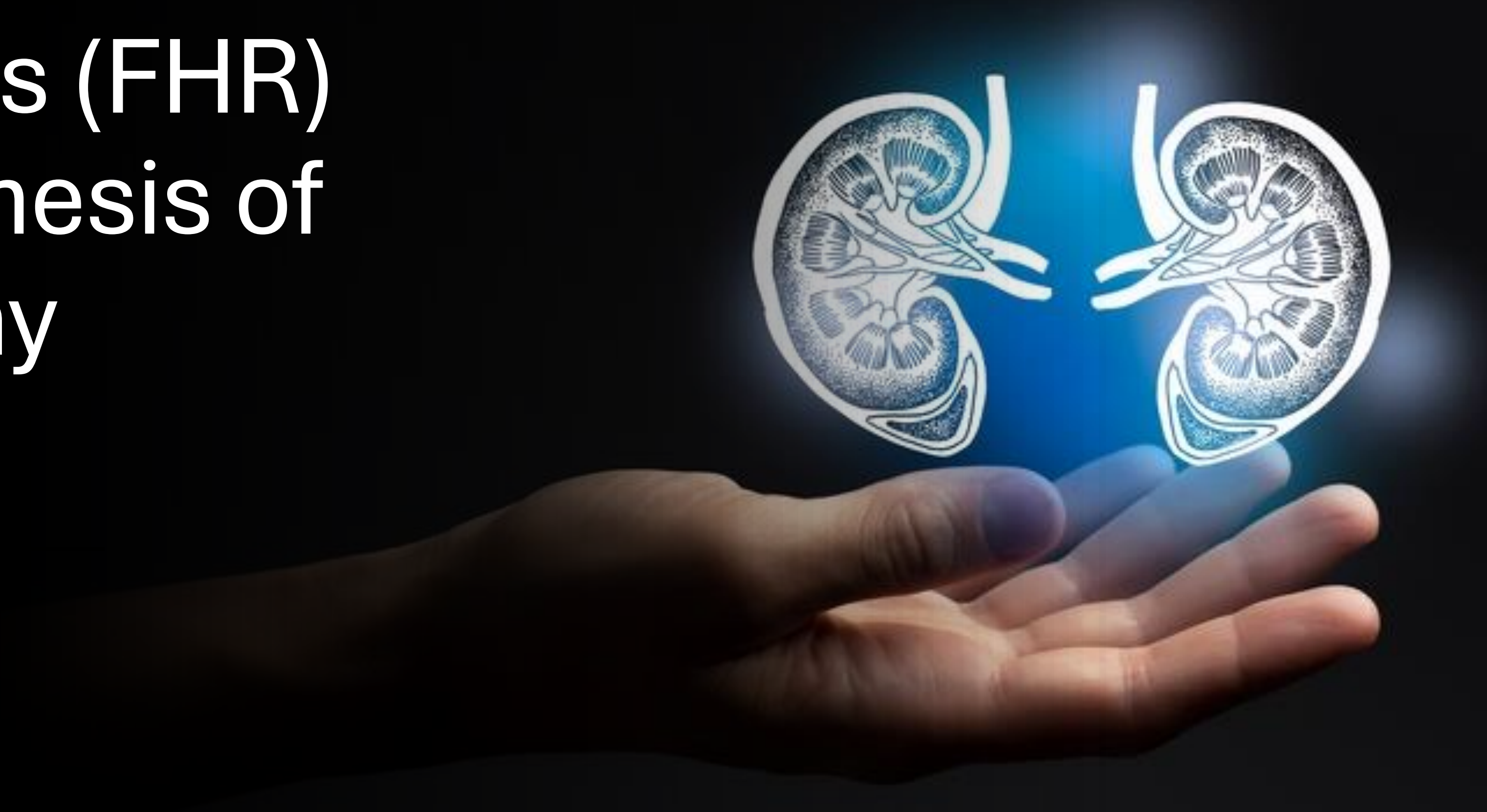

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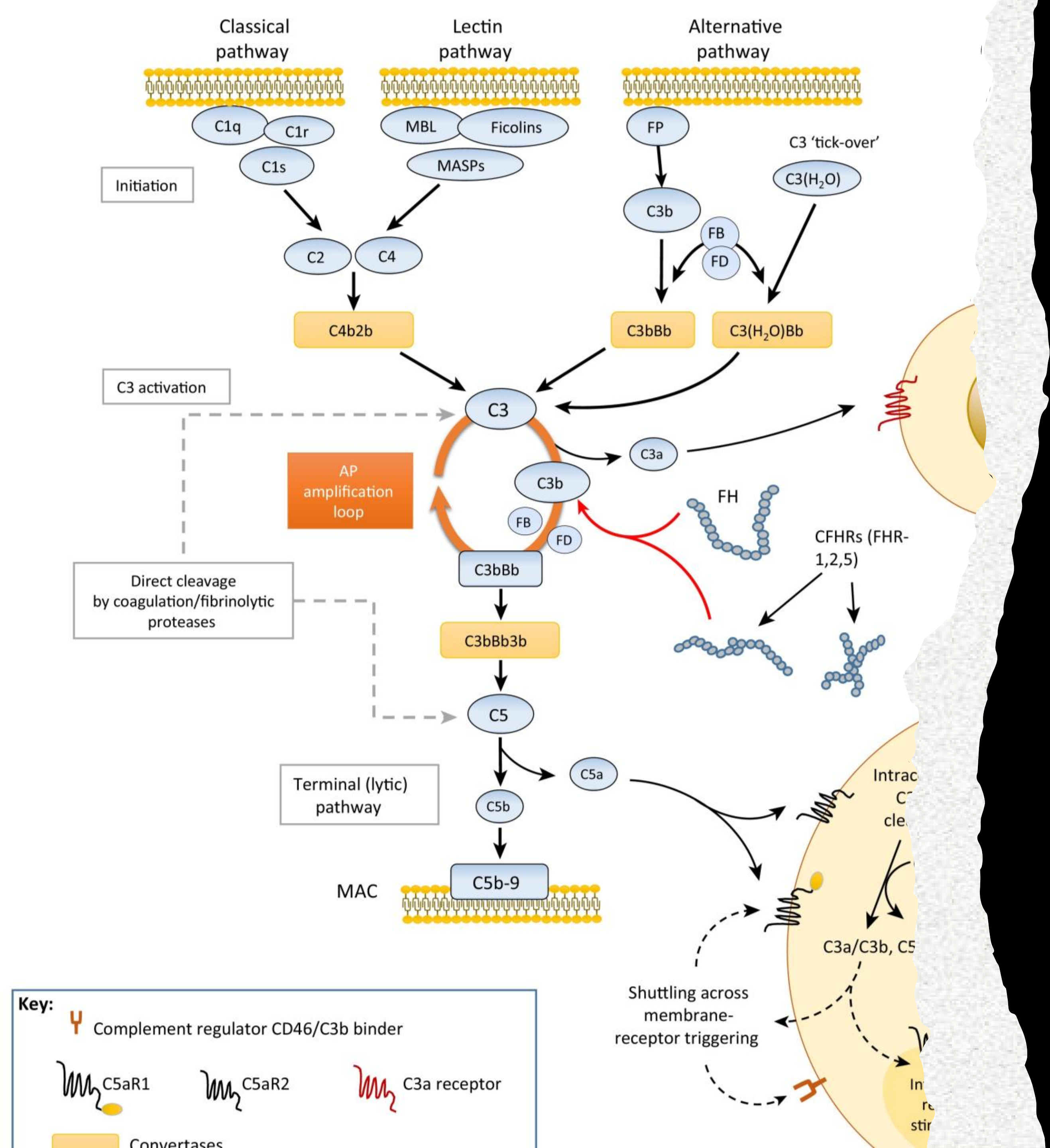


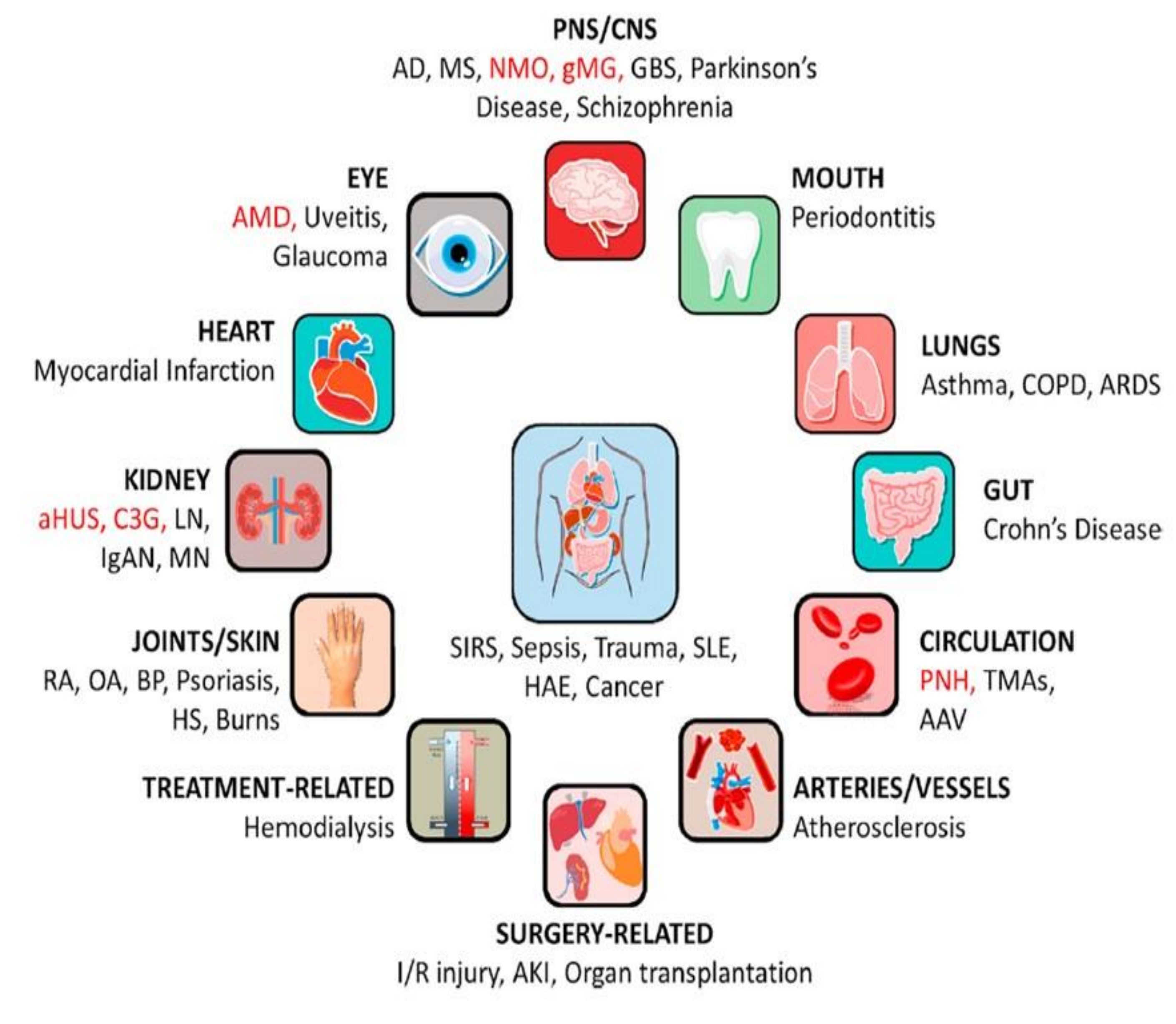
The Complement System

Complement Related Diseases and Indications

- **H-Guard® fusion protein** designed for AKI-CRRT and extracorporeal acute indications.

  - Phase2a Completed
  - Phase2b MHRA Approved
  - US Orphan Designation for Pediatric AKI *in progress* along with major US Children's Hospital

- **H-Guard-*Lite* (PspCN mimic)** designed for systemic and other delivery routes appropriate for multiple acute and non-acute applications.



H-Guard® controls complement activation to reduce inflammation

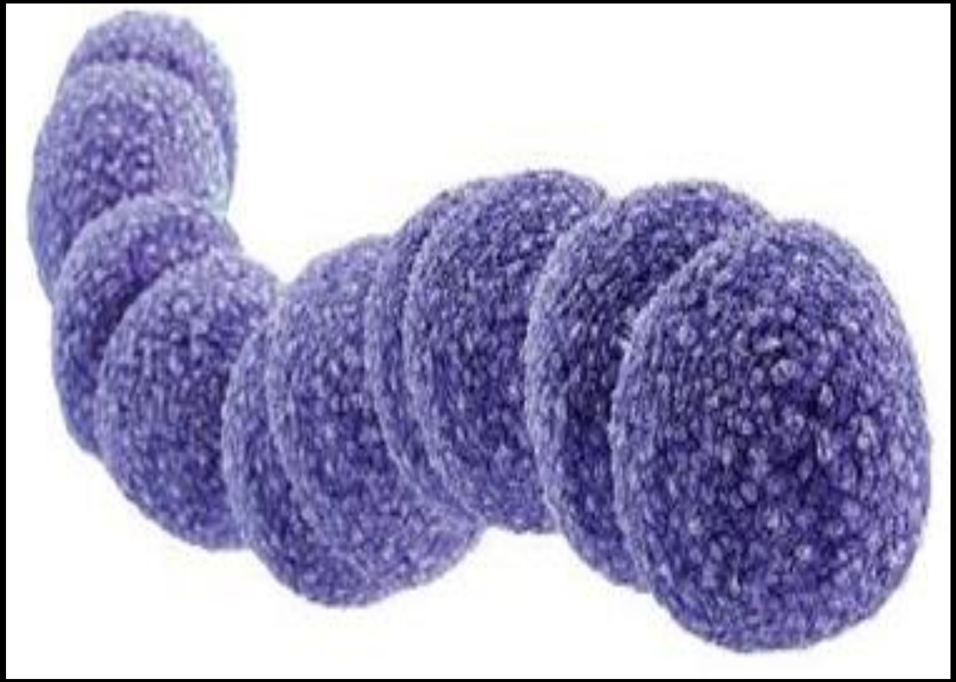
- Complement encompasses a powerful amplification loop that requires tight regulation to avoid over-activation

- All three Complement pathways converge at the central C3 molecule through the alternative pathway amplification loop.

- H-Guard® =PspCN Fusion Protein was designed to stick to HD membranes surfaces: **Invisibility Cloak**

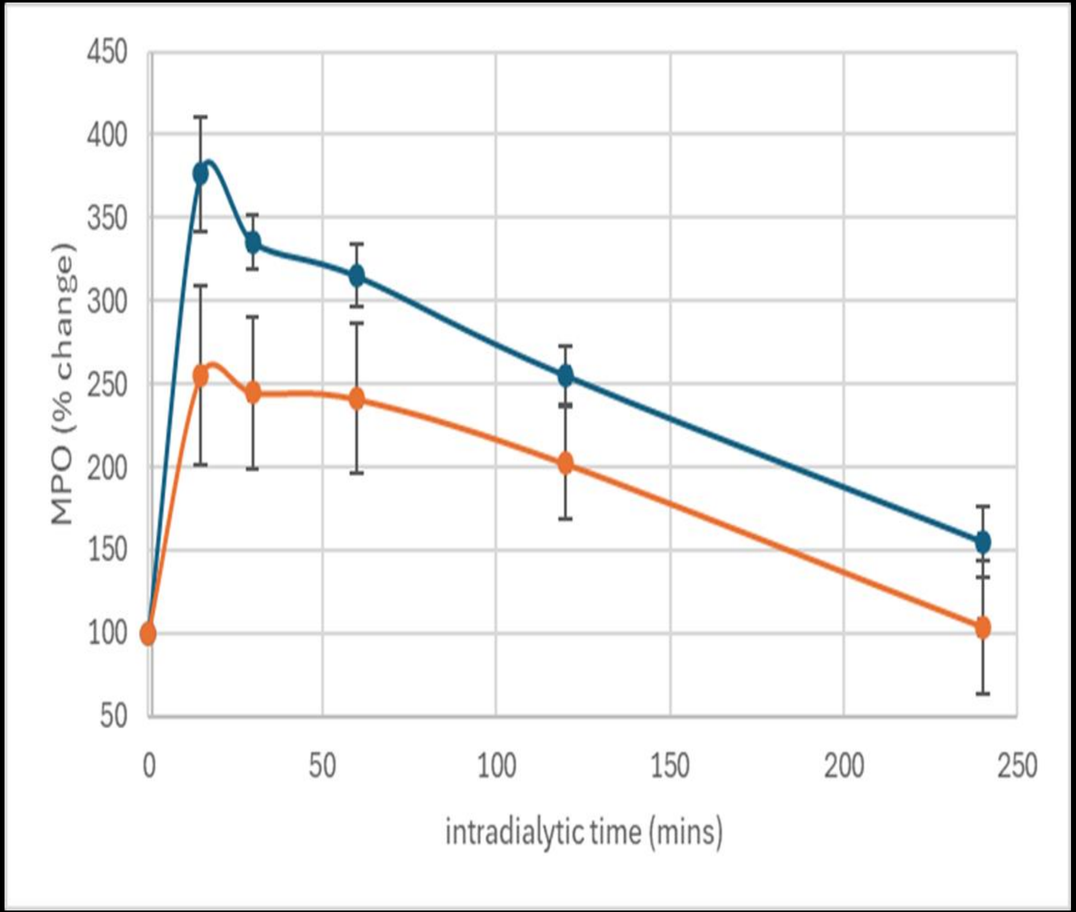
- **H-Guard *Lite* technology enhances the body's own Factor H activity by 500% and regulates the alternative pathway by targeting C3b & the C3 and C5 convertases**

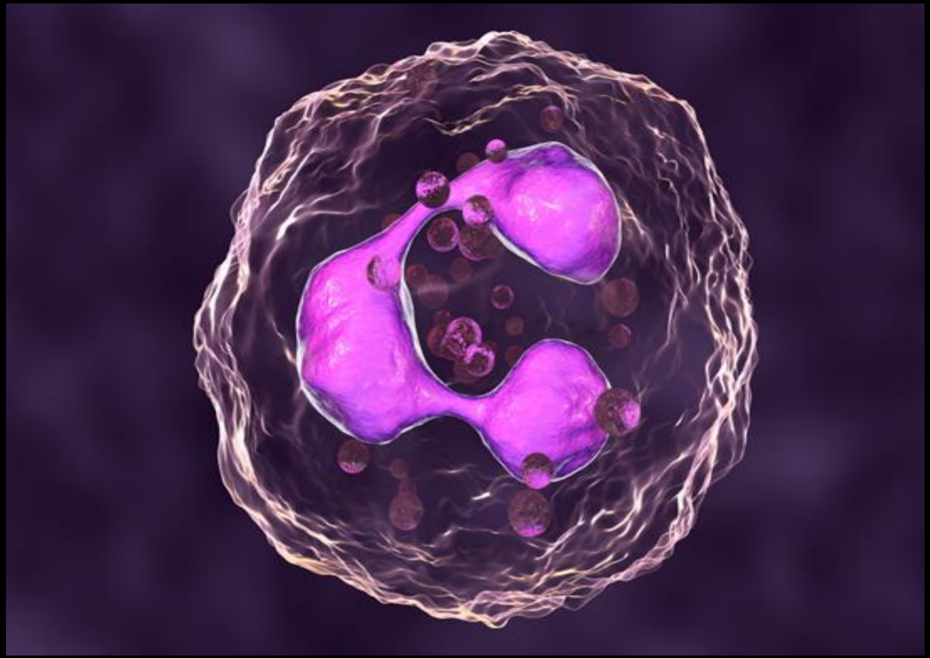
- **H-Guard is a negative modulator and not direct inhibitor of the Complement cascade**



PspCN (the active part of H-Guard®) mimics a bacterial virulence factor from *Streptococcus Pneumoniae*

H-Guard® Ph2a – Consistent Reduction in C3a,C5a,C5b9 & MPO





Without H-Guard: higher MPO (more inflammation)

With H-Guard: lower MPO (less inflammation)

- What should be the next therapeutic indication for our technology based on upregulating Factor H activity and downregulating Complement activity?

Complement & IgA Nephropathy

- IgAN is the commonest form of glomerulonephritis & important cause of kidney failure; > 50 % of patients will eventually progress to end-stage renal disease[1]
- IgAN is characterized by deposition of polymeric IgA1, which triggers complement activation through the Alternative (AP) and the Lectin (LP) pathways; key drivers of pathology in IgAN [2,3]
- Recent complement-related discoveries important for understanding its involvement in IgAN[1]:
- Cross-talk between the lectin and alternative pathways→ MASP-3
- **Factor H-related proteins (FHRs) antagonize the ability of Factor H to regulate complement activation[3]**

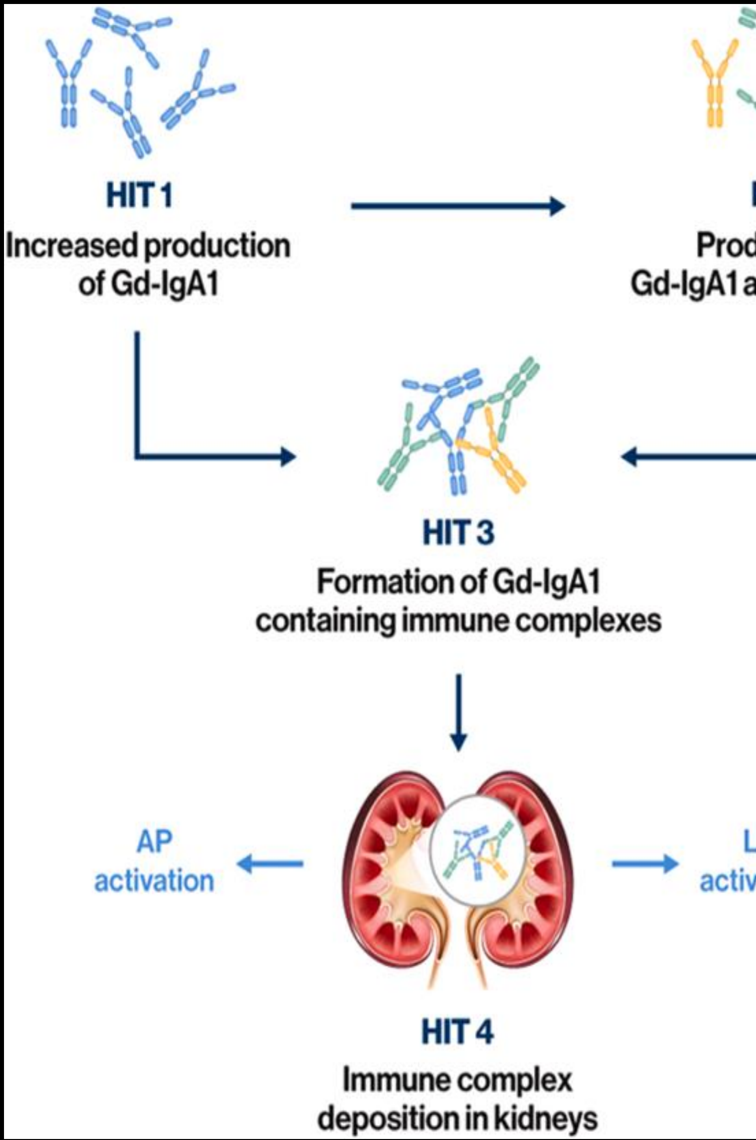


Figure from Tesař V. et al., 2023, DOI: 10.1016/j.ekir.2023.06.010

1Pitcher D. et al., 2023, DOI: 10.2215/CJN.0000000000000135 • 2Dobó J. et al., 2018, DOI: 10.3389/fimmu.2018.01851 • 3Poppelaars F. et al., 2021, DOI: 10.3390/jcm10204715 • 4Lee M. et al., 2023, DOI: 10.1093/ ckj/sfad199

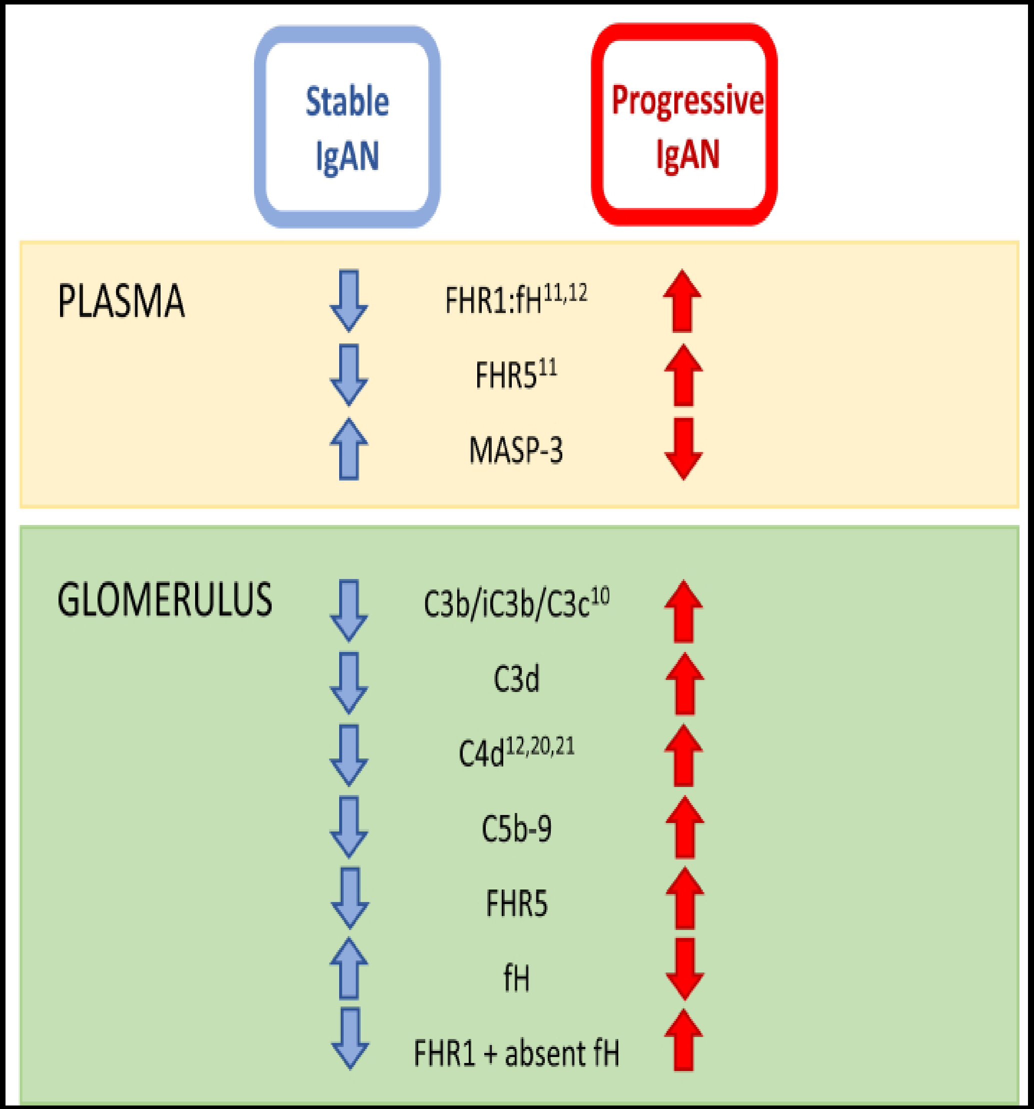
- **Complement Markers & IgA Nephropathy Severity**

- Plasma FHR5 levels & FHR5 glomerulus deposition in IgAN patients are significantly higher *vs.* healthy controls; FHR5 has a more prominent role for complement activation in IgAN vs. FHR1[5,6]

- FHR5 can function as competitive antagonist of FH for C3b binding (FH deregulation) & enhance C3 deposition[7]

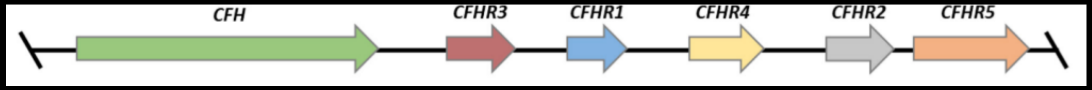
- IgAN cases characterized by low-C3 and low-C4 might imply a prevalent activation of the LP (C4-mediated, *"lectin-pathic"*).

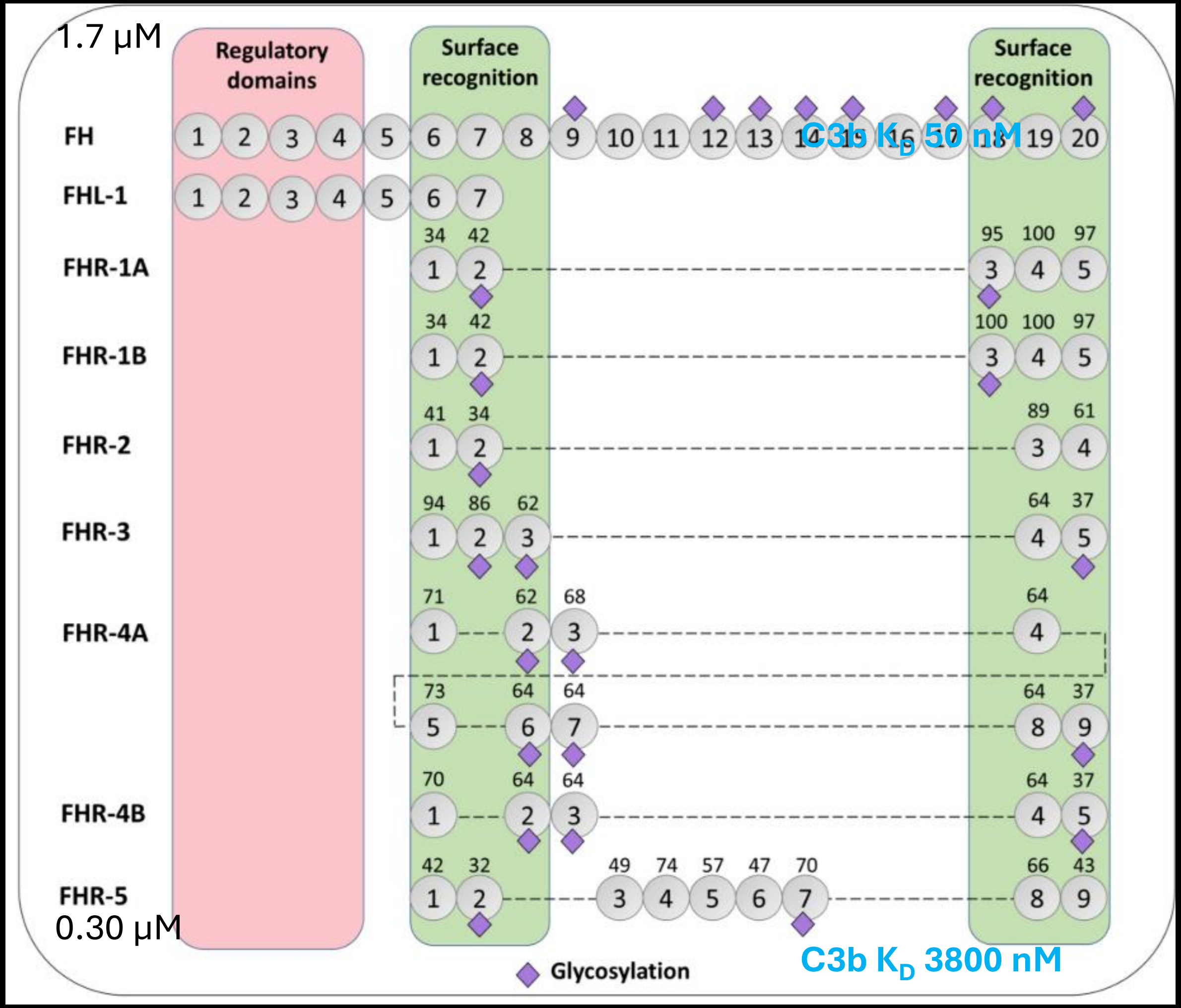
- Conversely, **cases with low-C3 and high-C4 may indicate predominant AP activation (non-C4-mediated, *"altern-pathic"*), which seems to be associated with a poorer prognosis[8]**



Poppelaars F. et al., 2021, DOI: 10.3390/jcm10204715

FH has undergone large genomic duplications – the FH family





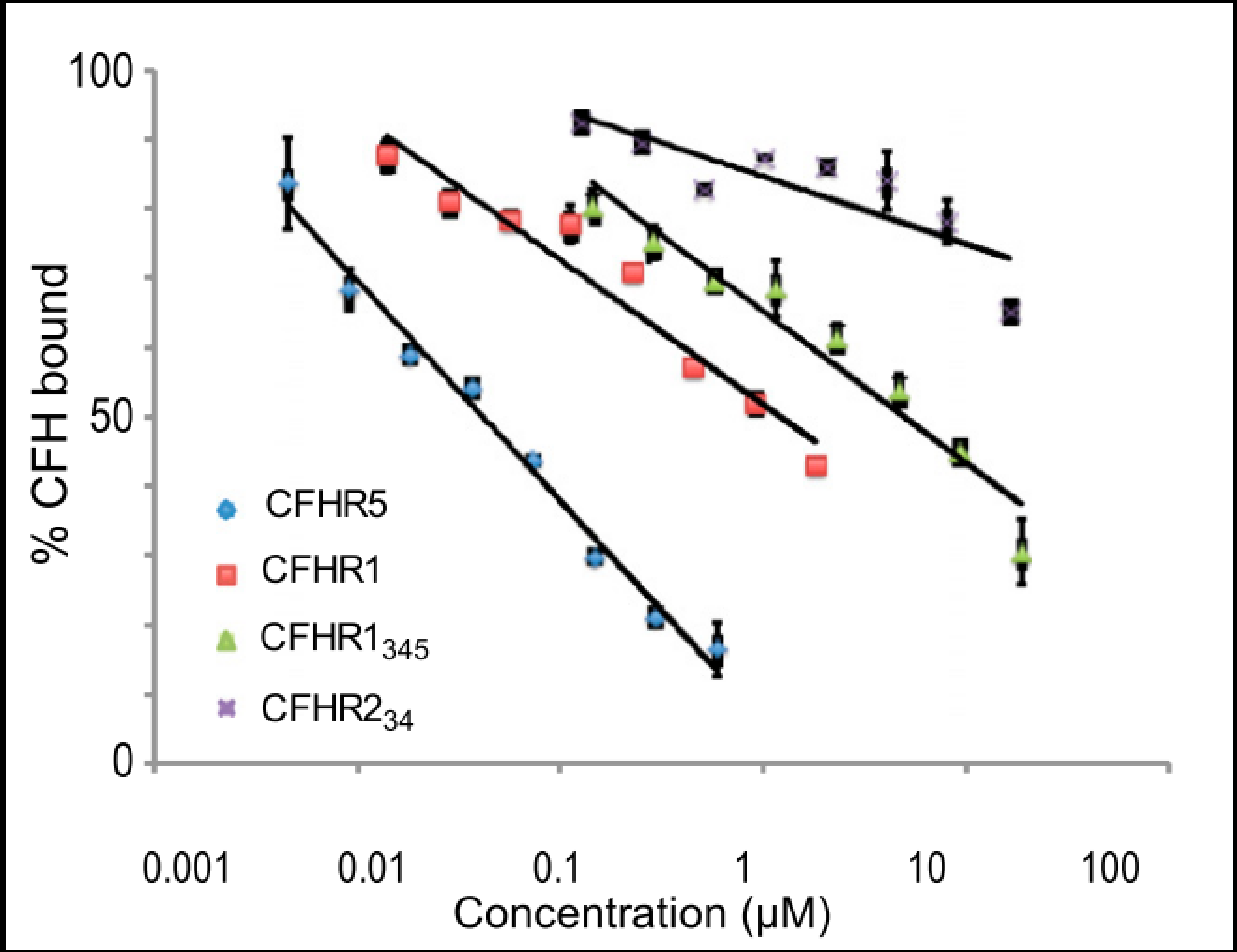
Complement factor H–related proteins in IgA nephropathy—sometimes a gentle nudge does the trick (Joshua M. Thurman and Jennifer Laskowski).

Complement activation probably contributes to glomerular inflammation and damage in IgA nephropathy. In this issue, 2 groups report that levels of factor H–related protein are elevated in patients with IgA nephropathy and correlate with disease progression.

These studies provide new evidence that the complement cascade is important to the pathogenesis of this disease. **These results also suggest that Factor H–related protein levels may be useful for identifying those patients at high risk of disease progression.**

Kidney International (2017) 92, 790–793; http://dx.doi.org/10.1016/j.kint.2017.05.025

Effects of CFHR & CFHR5 on Complement



Goicoechea De Jorge, E. *et al.* (2013) 'Dimerization of complement factor H-related proteins modulates complement activation in vivo', *Proceedings of the National Academy of Sciences of the United States of America*, 110(12), pp. 4685–4690. Available at: https://doi.org/10.1073/pnas.1219260110.

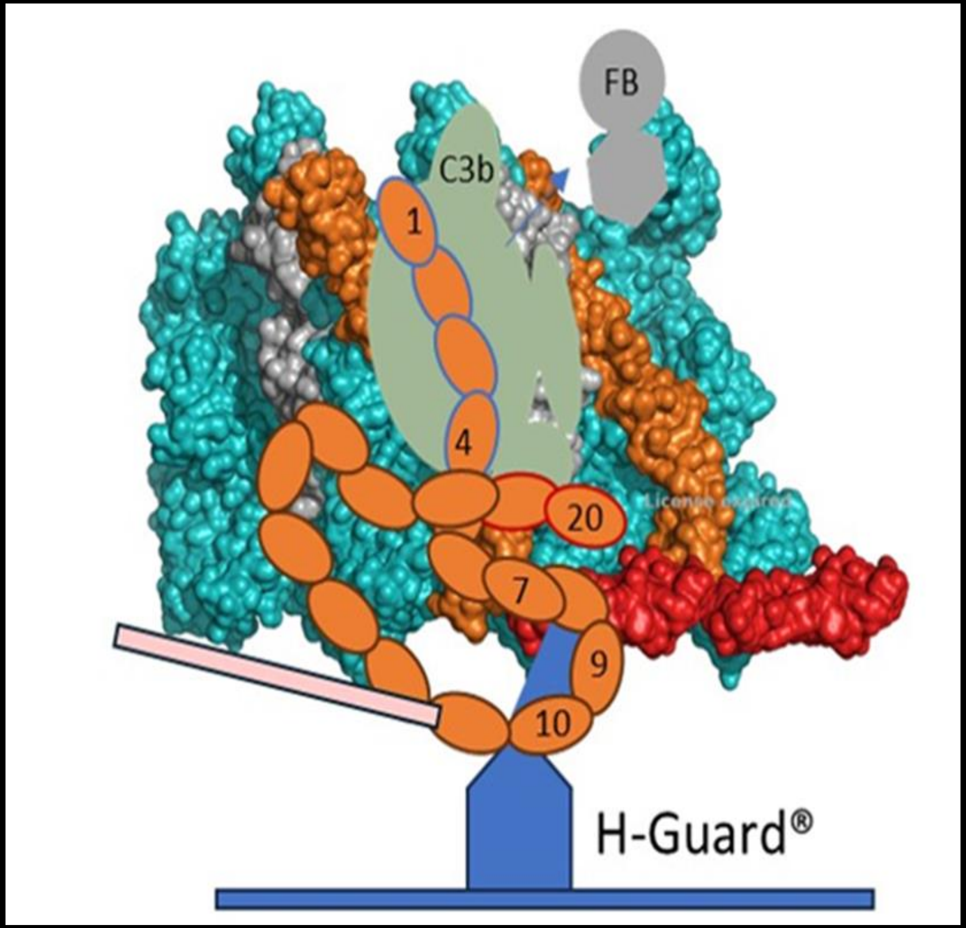
CFHR1 and CHFR5 deregulate complement activation by competitively inhibiting of the interaction of CFH with C3b.

(A) CFH binding to C3b is inhibited by either recombinant CFHR5 or serum-derived CFHR1. ELISA wells were coated with C3b and 0.07 μM CFH was incubated with increasing amounts of either CFHR1 (0.014–1.8 μM) or CFHR5 (0.005–0.6 μM).

(B) Both proteins reduced the CFH–C3b interaction in a dose-dependent manner. Similar results were obtained when recombinant CFHR1345 (0.14–18 μM) and CFHR234 (0.13–16 μM) were used. (B)

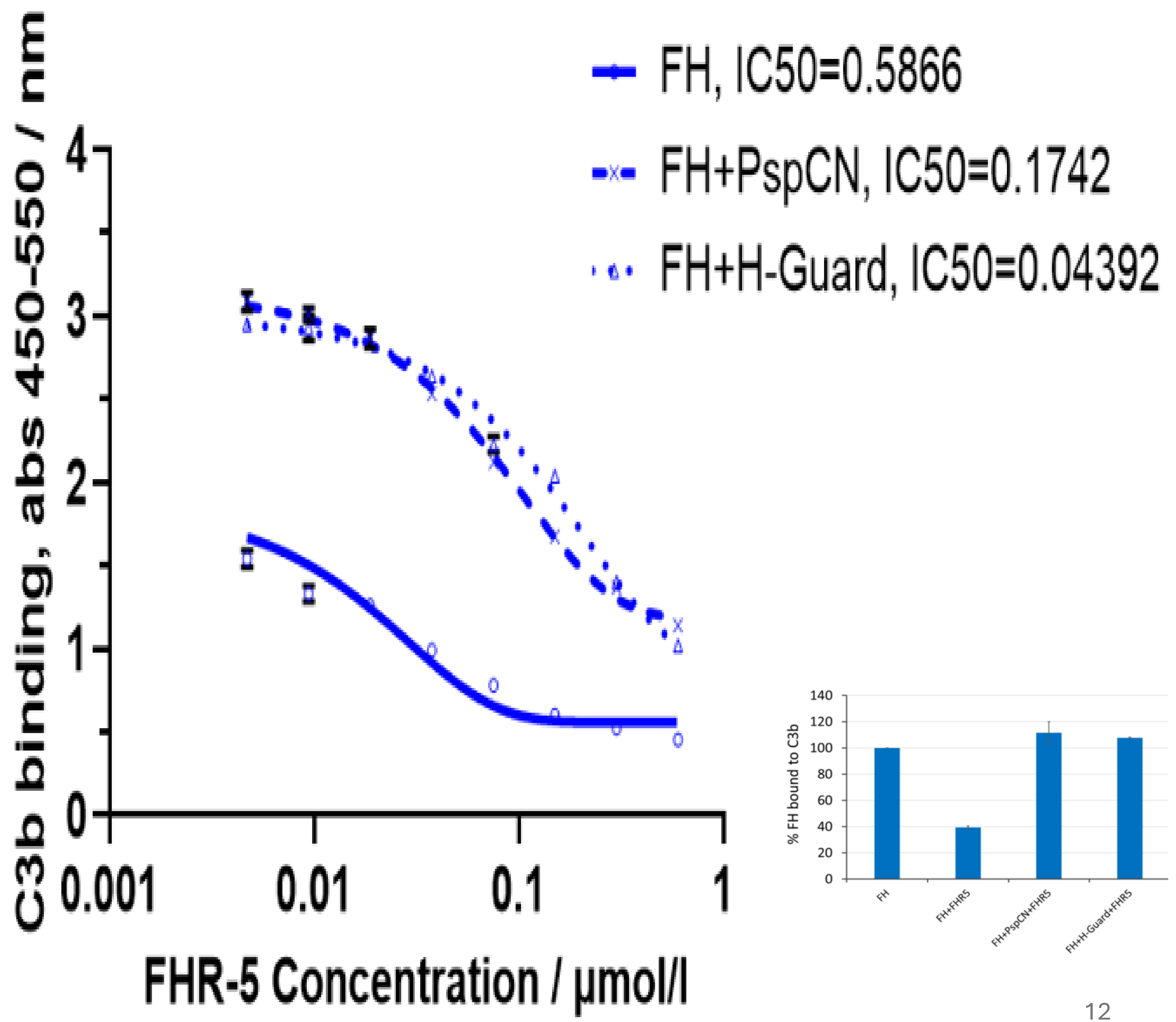
KD or not KD that is the question

- Factor H + H-Guard *Lite* binding to C3b KD 20nM.
- FHR-5 binds to C3bKD 3800nM
- FH+H-Guard *Lite* binding to C3d KD 130nM
    - This is because H-Guard Lite exposes the CCP19-CCP20 binding site on C3b
- Factor H alone does not bind to C3d.
- Kinetics/residence time (k-off/k-on) set how fast displacement happens.
- **Based on KD's H-Guard Lite should be effective at displacing FHR-5 and this was confirmed by our experimental data.**



H-Guard & H-Guard-Lite (PspCN mimic) significantly enhance Factor H potency.

- **H-Guard® enables FH to compete efficiently with FHR5 for C3b binding**
- Competition assays performed at Invizius[9,10]



[9]Nichols EM. et al., 2015, DOI: 10.1038/ki.2015.233
[10]Goicoechea de Jorge E. et al., 2013, DOI: 10.1073/pnas.1219260110

H-Guard® and H-Guard-*Lite* increase Factor H potency in the presence of FHR5

ELISA assay to demonstrate increase of FH potency by H-Guard® & PspCN on a C3b-FHR5 surface

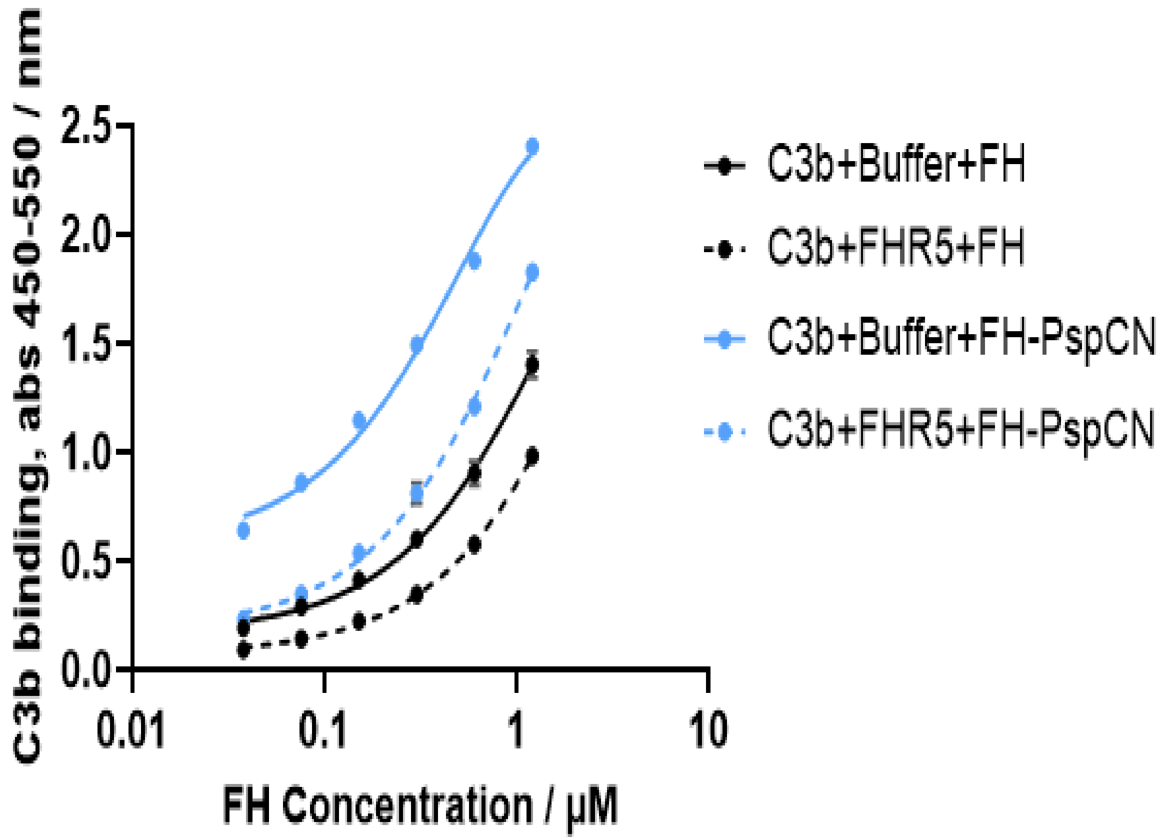
Coat wells with C3b

Incubation 1: Physiological levels FHR5 or buffer

Incubation 2: FH, FH+ PspCN or FH+H-Guard® dilution series

Incubations 3 & 4: Antibodies

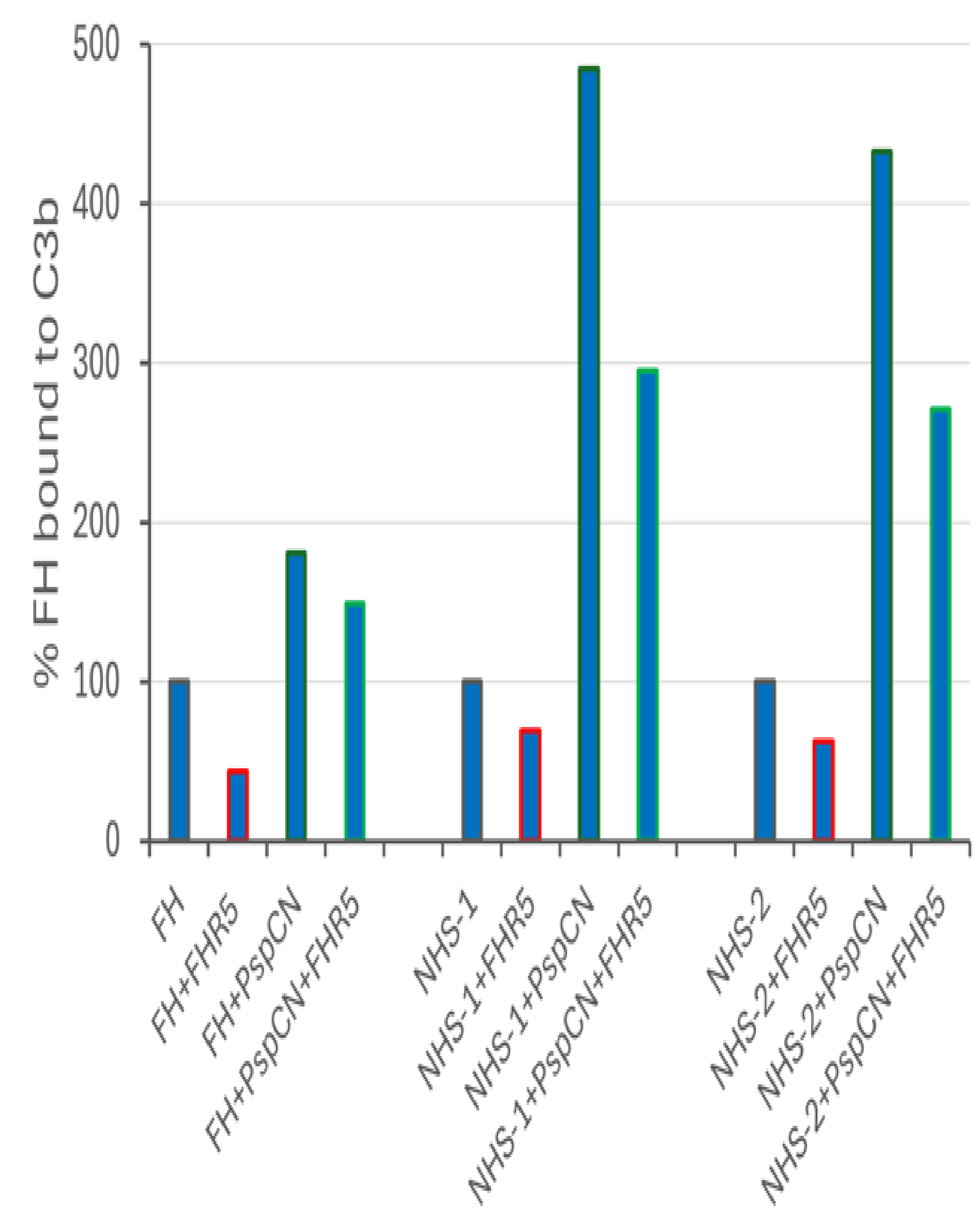
Detection system & read at 450-550 nm



H-Guard-*Lite*(PspCN) rescues FH binding to C3b from FHR5 in Normal Human Serum(NHS)

- Adding FHR5 to NHS reduces endogenous FH ability to bind to C3b by ~30-40 %

- **H-Guard-*Lite*(PspCN) added to NHS can rescue endogenous FH binding to C3b from FHR5**



FH: Plasma-purified FH

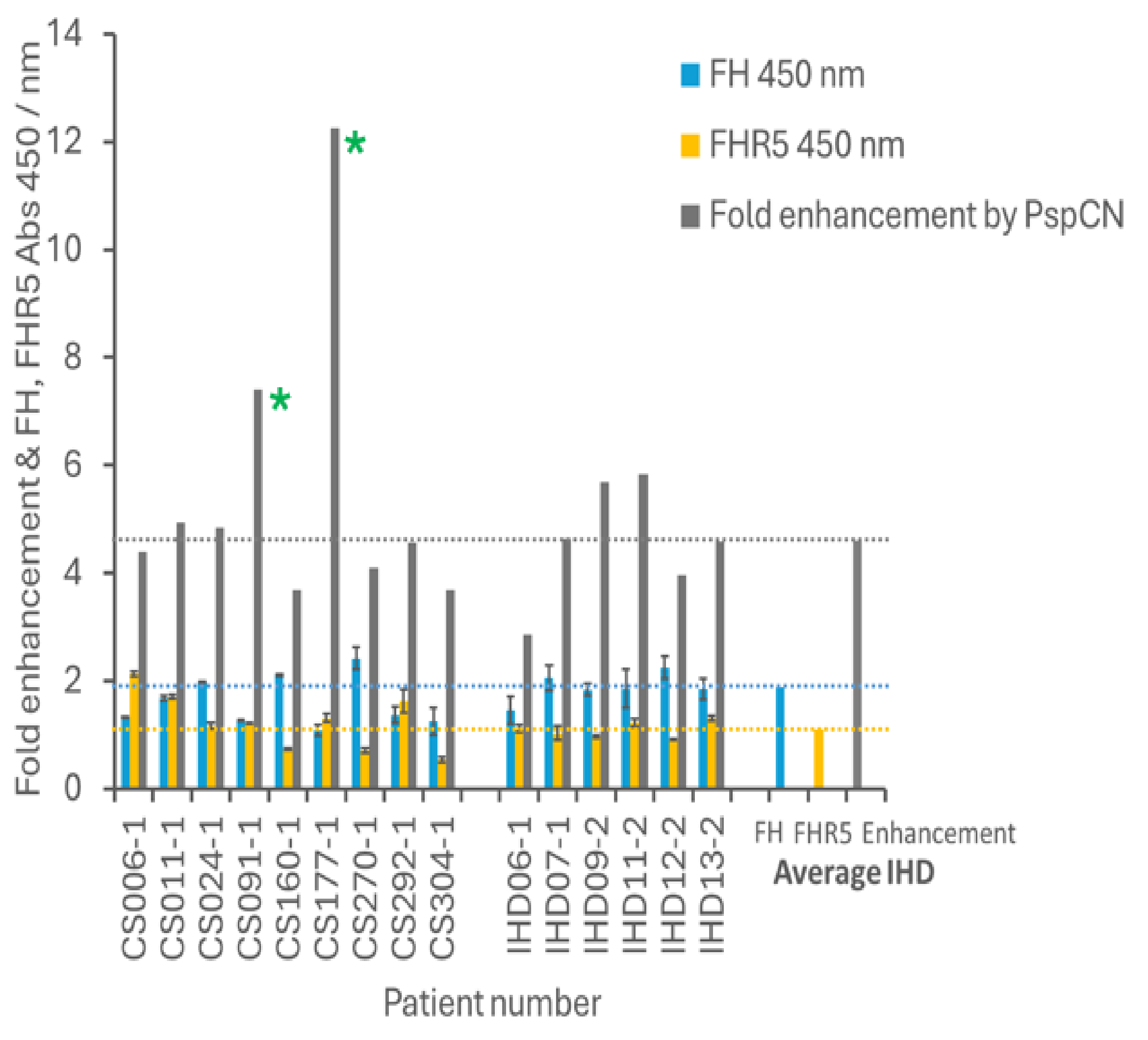
NHS-1: Normal Human Serum pool 1

NHS-2: Normal Human Serum pool 2

H-Guard *Lite* rescues FH binding to C3b in all IgAN patient blood samples

- In all IgAN patients & Invizius Healthy Donors (IHD) endogenous Factor H activity is enhanced by PspCN (H-Guard-*Lite*)

- **2 IgAN patients: CS091 & CS177 *→ "super-responders"**



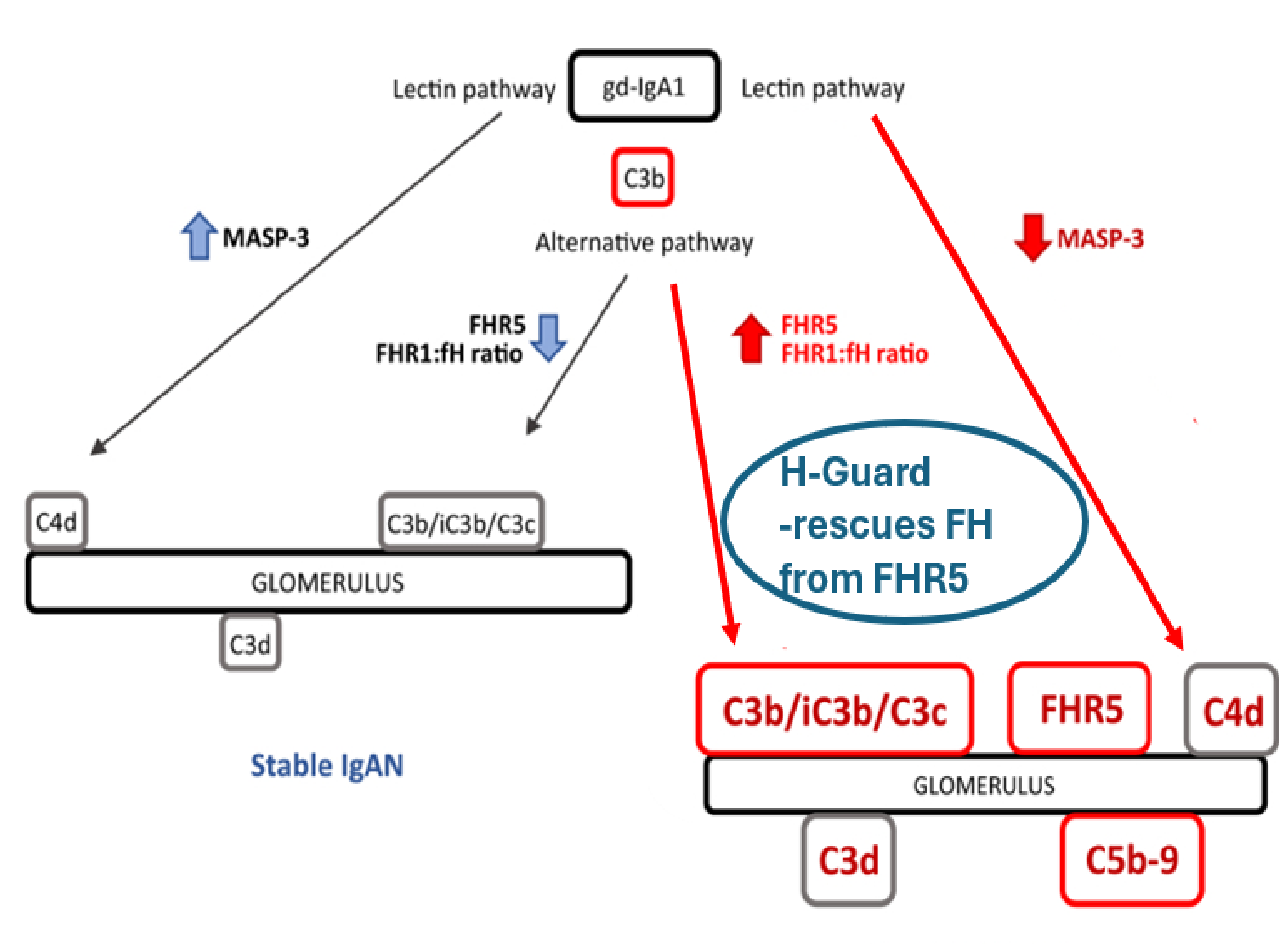
H-Guard *Lite* has the potential of reducing FHR 1& 5 Complement driven damage in Progressive IgA Nephropathy

- **Complement activation through the alternative pathway is a key driver of pathology in IgAN.**

- H-Guard- *Lite* technology enhances the body's own Factor H, and regulates the alternative pathway by targeting C3b & the C3 and C5 convertases

- **H-Guard *Lite* technology significantly increases FH potency in the presence of FHR5, a powerful de-regulator of Complement**

- H-Guard *Lite* technology is a negative modulator and not a direct inhibitor of the Complement cascade



Adapted from: Medjeral-Thomas NR. et al., 2017, DOI: 10.1016/j.ekir.2017.11.015

Summary

- IgA nephropathy is the most prevalent primary glomerulonephritis worldwide, with an incidence of 1 to 2.5 cases per 100,000 populations.
- The current therapeutic market for IgAN is $2b and is expected to double by 2035.
- H Guard and H-Guard *Lite* enhance Factor H binding to C3b and accelerate decay of the C3 convertase
- H-Guard *Lite* technology enhances the body's own Factor H activity by 500%
- In IgAN, FHRs compete with FH for C3b binding increasing complement activation and disease progression
- H Guard *Lite*-enhanced FH will be more effective at displacing FHRs from C3b

- Displacing FHRs with enhanced H-Guard *Lite* FH will down-regulate complement activation and should improve protection of kidney function and thereby prevent/delay IgAN mediated kidney damage.

Acknowledgements:

Dr Joe Conner (Invizius)
Dr Eliza Makou (Invizius)
Prof Sandip Mitra (Manchester University Hospital Trust)

Thank you for your attention

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