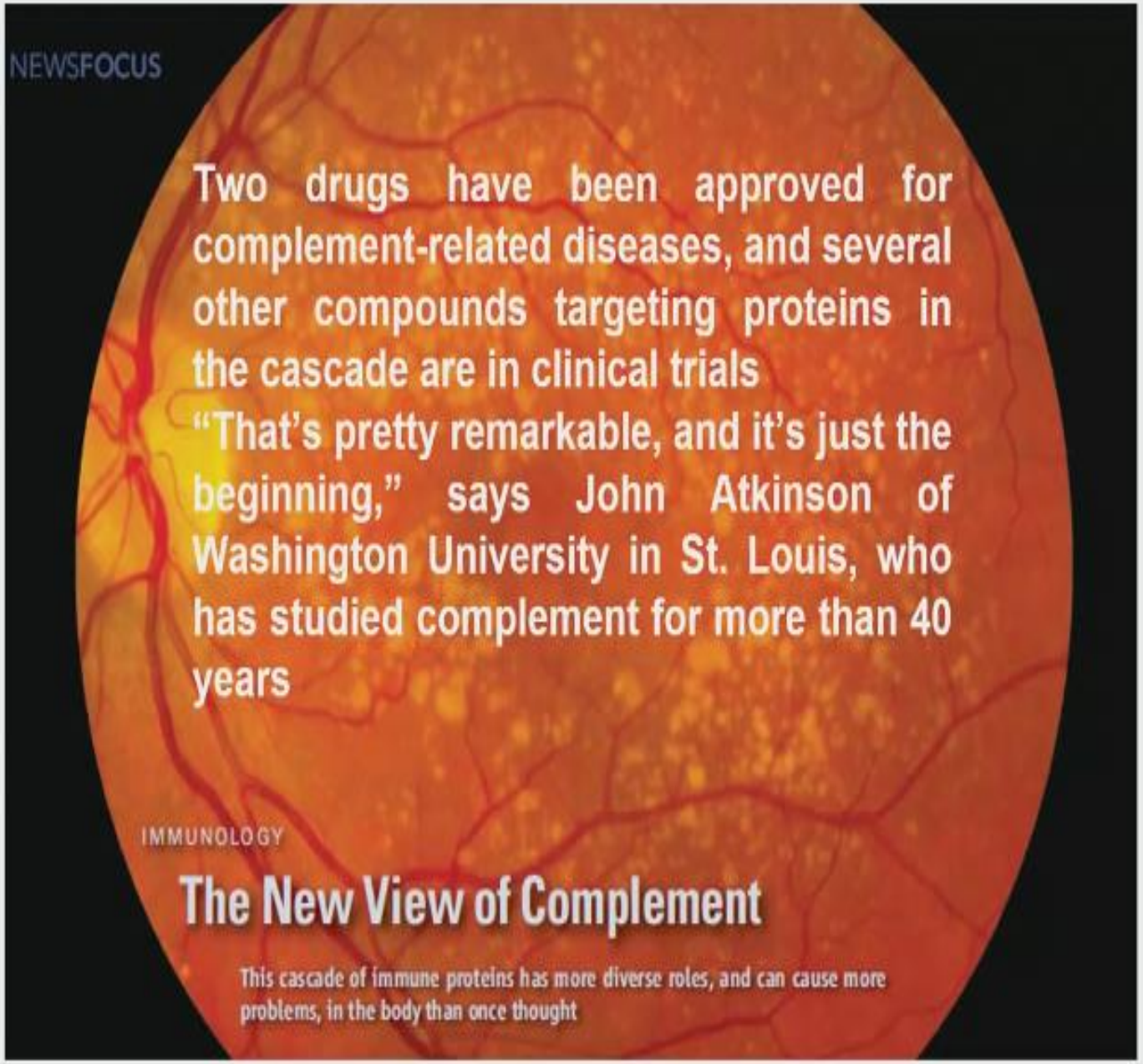
F_Yassari
SBMU

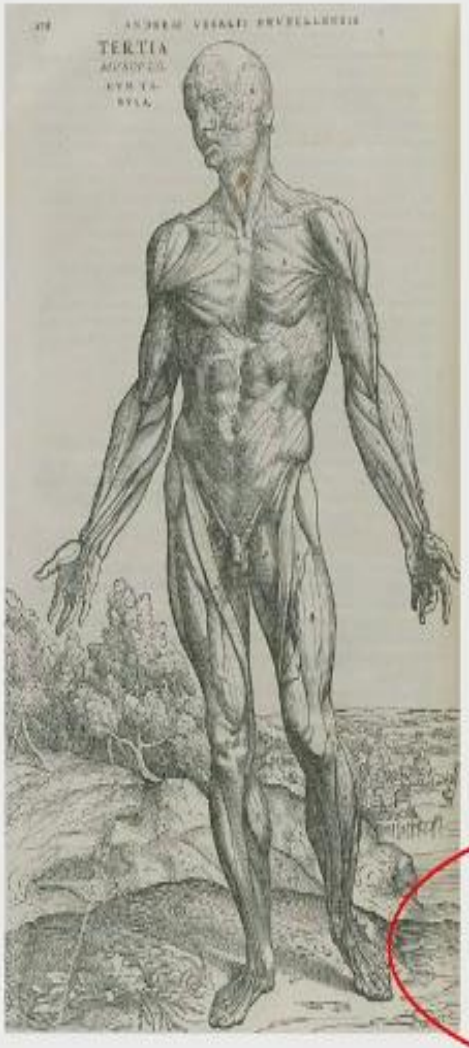

NEWSFOCUS
Two drugs have been approved for complement-related diseases, and several other compounds targeting proteins in the cascade are in clinical trials
"That's pretty remarkable, and it's just the beginning," says John Atkinson of Washington University in St. Louis, who has studied complement for more than 40 years
IMMUNOLOGY
The New View of Complement
This cascade of immune proteins has more diverse roles, and can cause more problems, in the body than once thought

Complement was also one of the earliest defenses to evolve, it belongs to the innate arm and is always ready for action

"It's an amazing first responder. It can lyse a bug in 30 seconds"

John Atkinson, University School of Medicine, St Louis

DISEASES ASSOCIATED WITH COMPLEMENT ACTIVATION



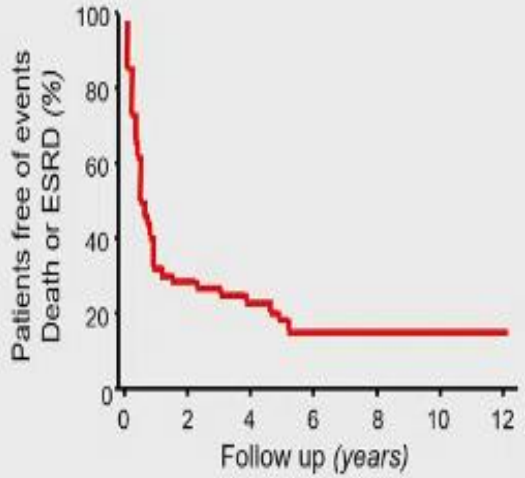
- Stroke
- Alzheimer's diseases
- Age-related macular degeneration
- Asthma
- Myocardial infarction
- Crohn's disease
- Rheumatoid arthritis
- Membranous nephropathy
- IgA nephropathy
- SLE
- ANCA vasculitis
- **Hemolytic uremic syndrome**
- **MPGN /C3 glomerulopathy**
- **COVID-19**

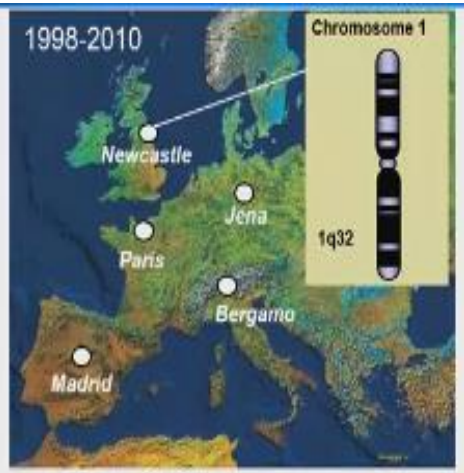
HEMOLYTIC UREMIC SYNDROME

A multisystem disease of microangiopathic hemolytic anemia and thrombocytopenia with predominant but not exclusive renal involvement

aHUS
Incidence:
0.5-2/1,000,000/year

Sex: no difference

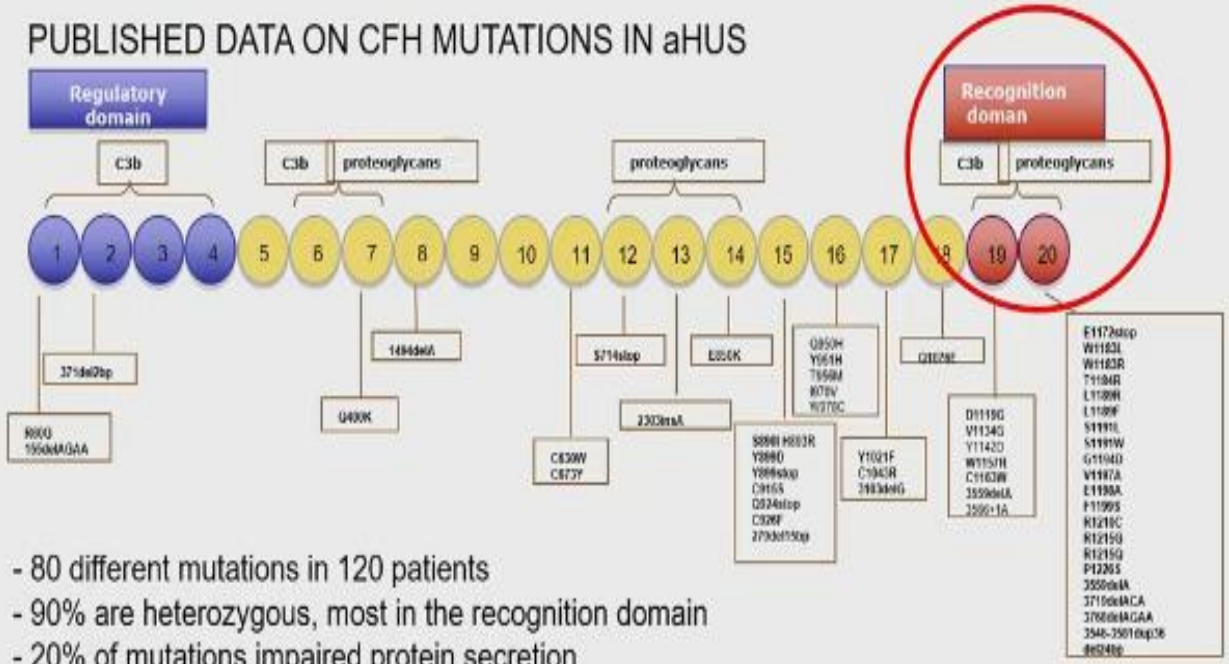




In three large families with HUS an area on chromosome 1q32 where factor H gene is mapped segregated with the disease

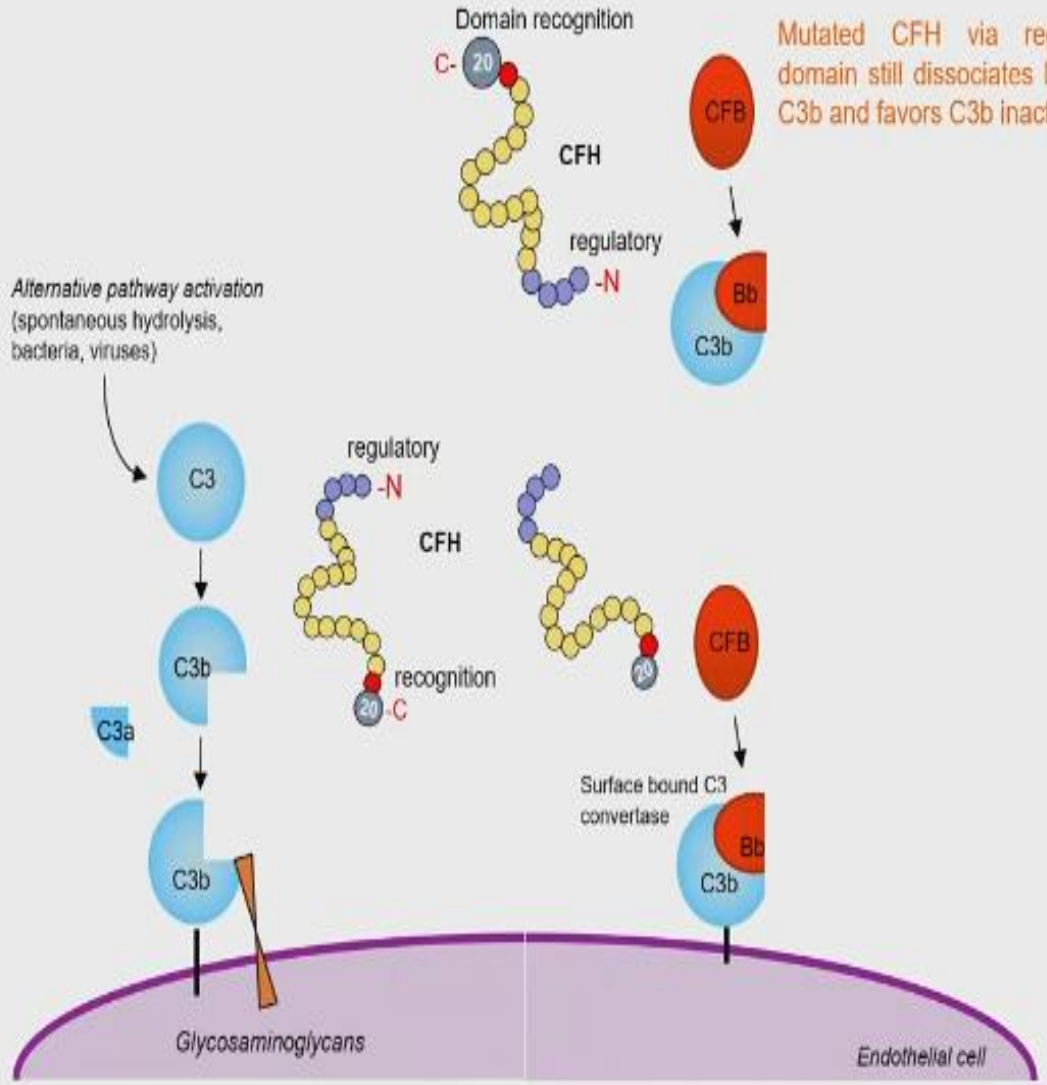
Warwicker and Goodship, Kidney Int, 1998

PUBLISHED DATA ON CFH MUTATIONS IN aHUS

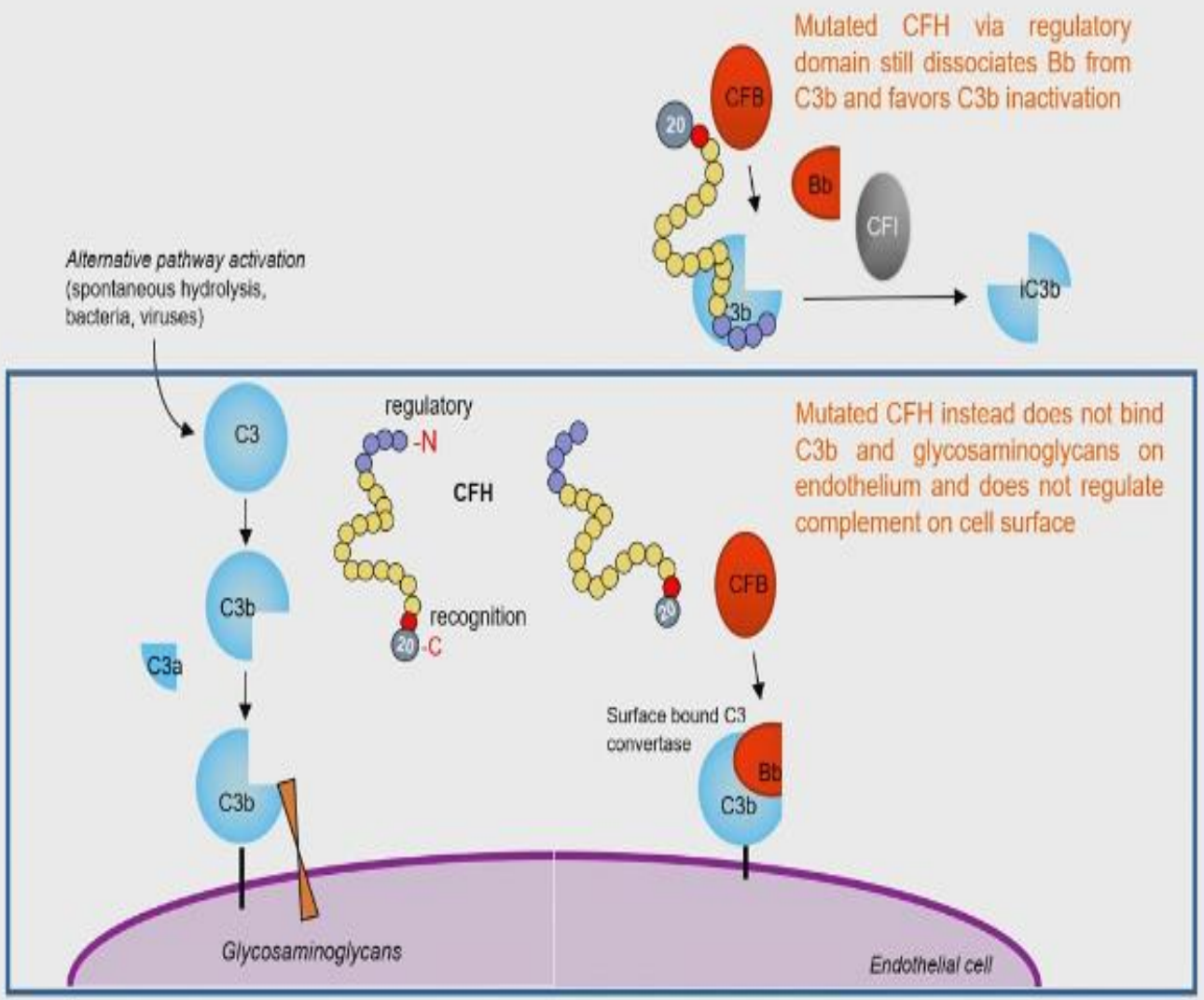


- 80 different mutations in 120 patients
- 90% are heterozygous, most in the recognition domain
- 20% of mutations impaired protein secretion with low plasma CFH

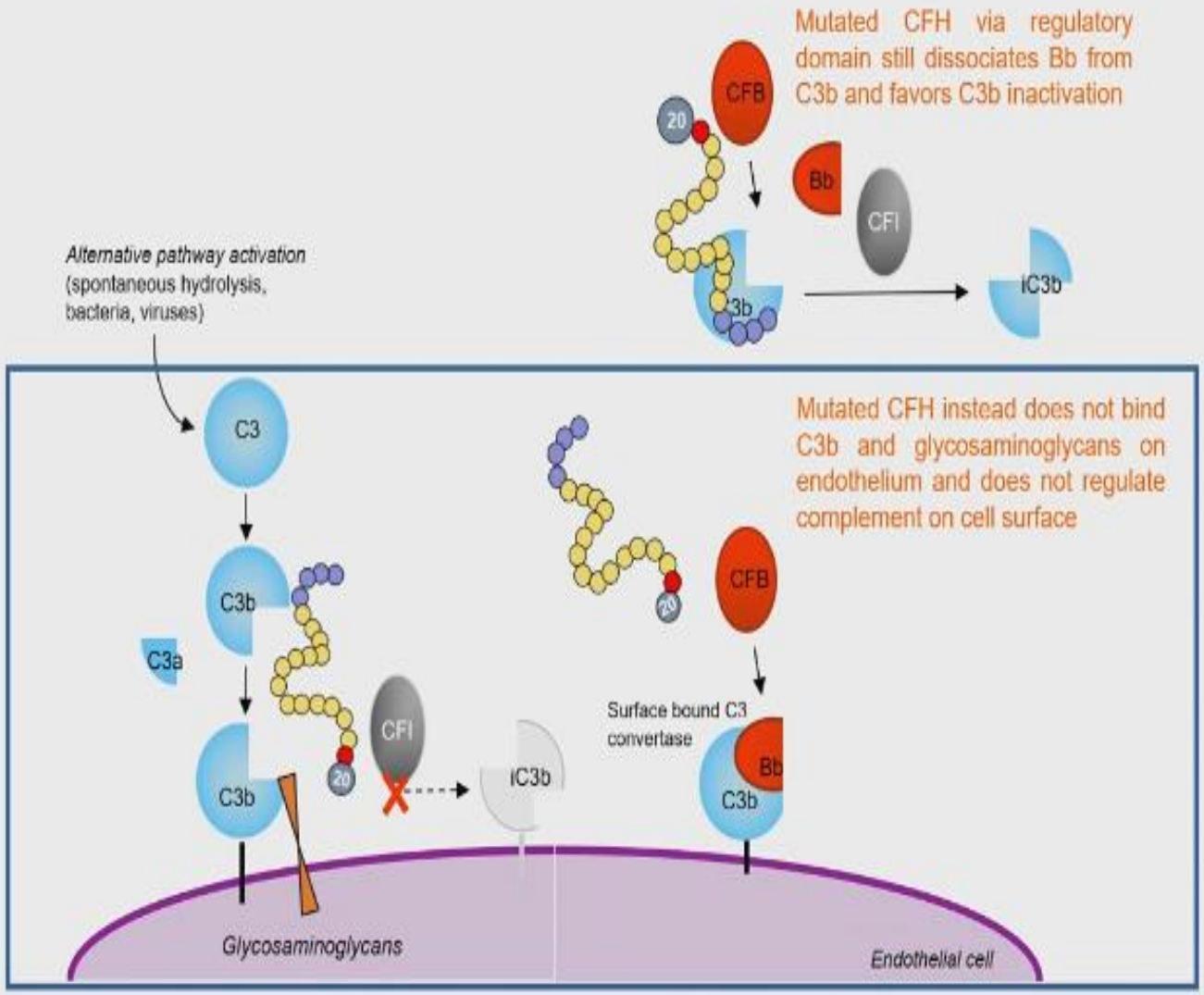
SOLID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN aHUS



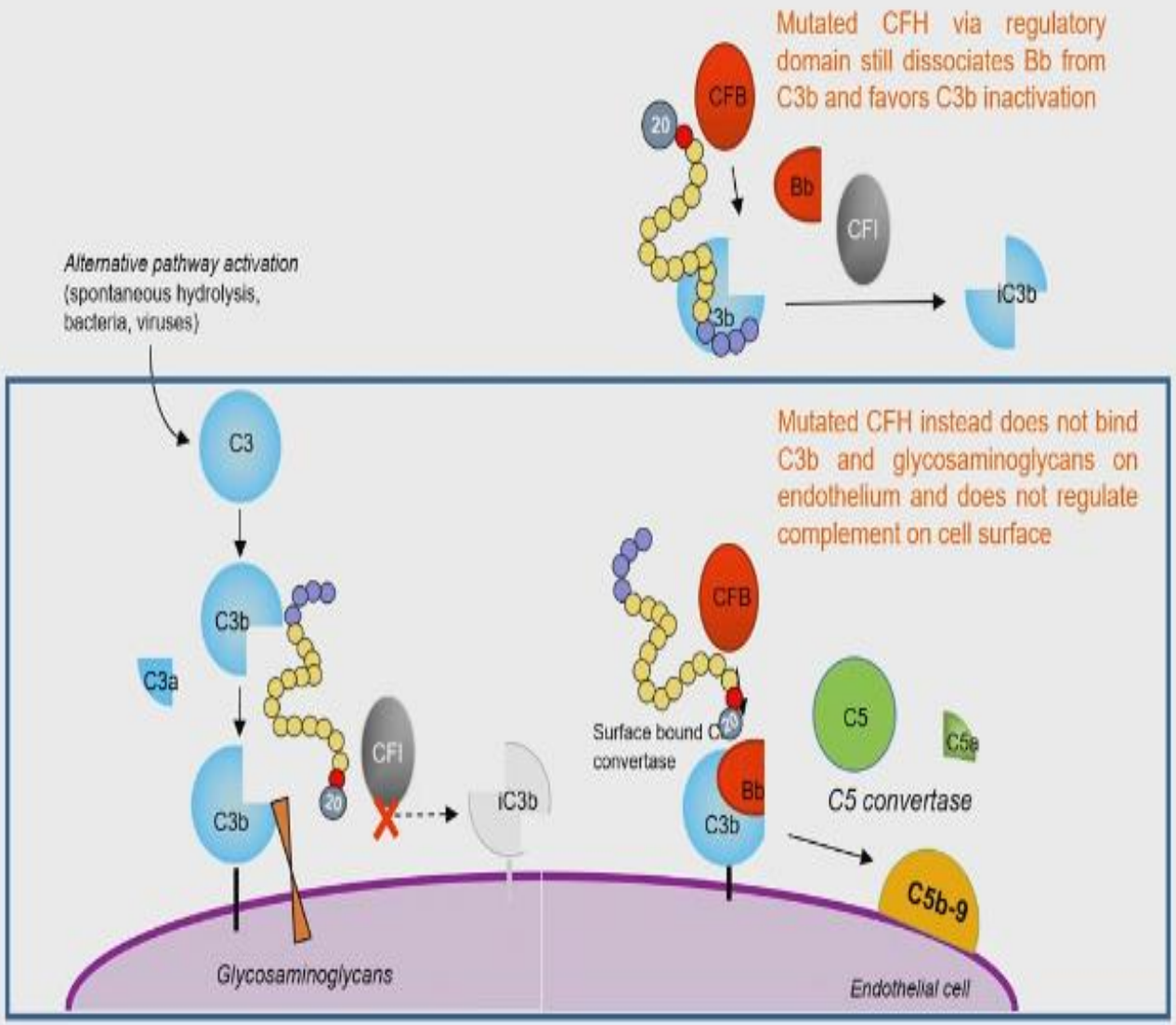
SOLID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN aHUS



SOLID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN aHUS

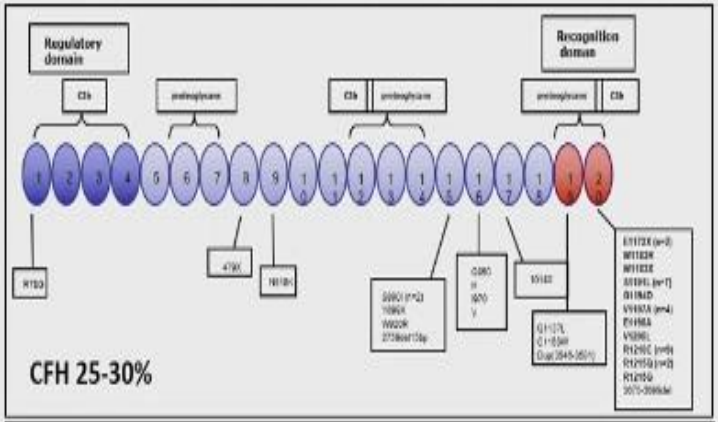


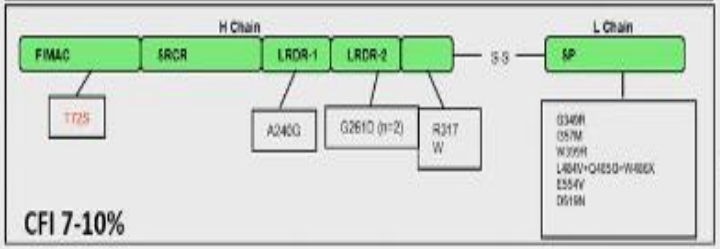
SOLID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN aHUS

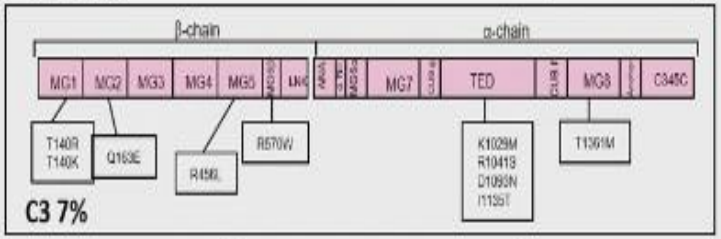


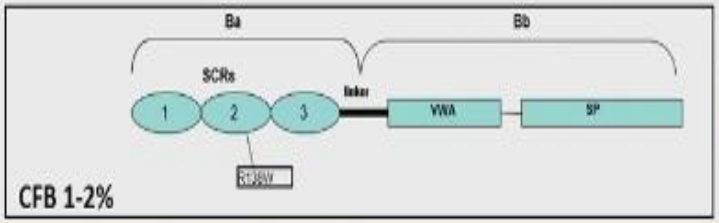
COMPLEMENT GENE MUTATION IN aHUS PATIENTS

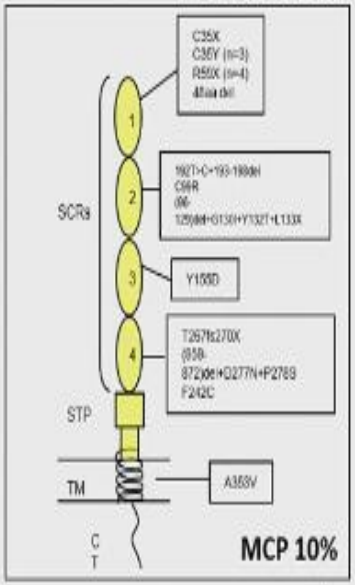
Published data

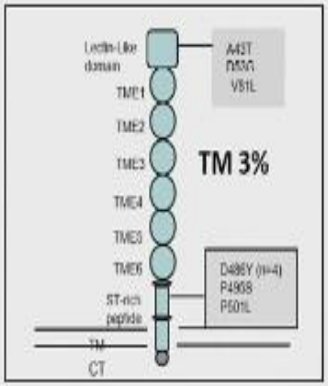


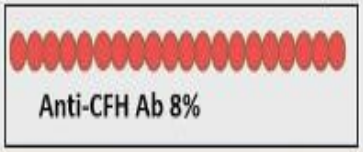






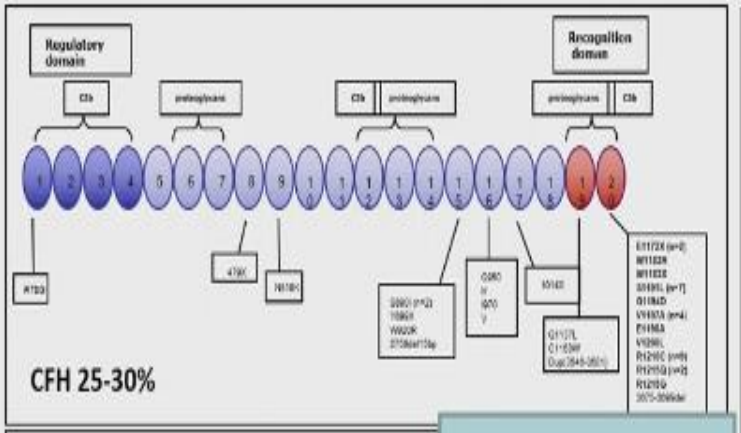




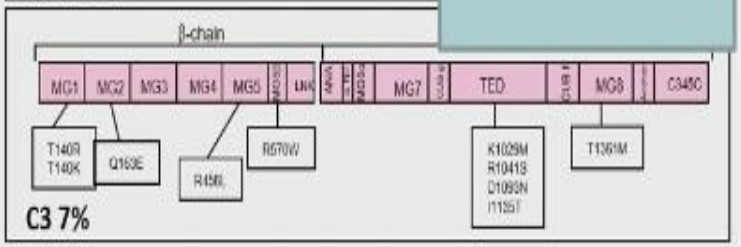


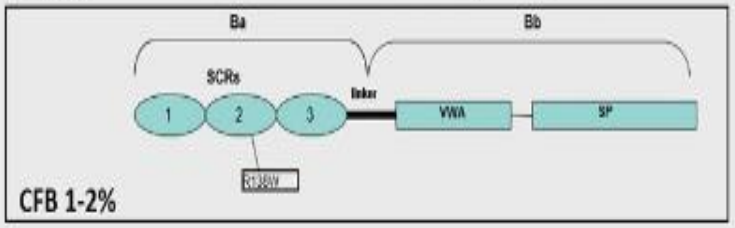
COMPLEMENT GENE MUTATION IN aHUS PATIENTS

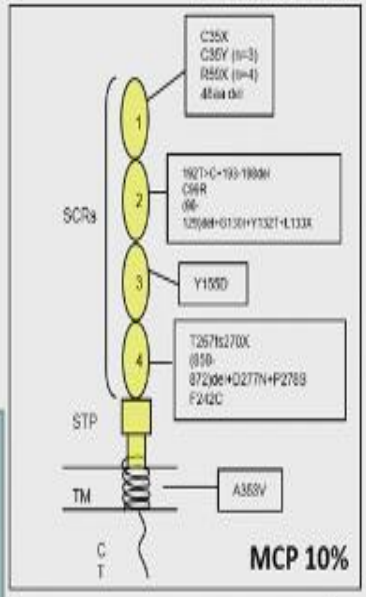
Published data

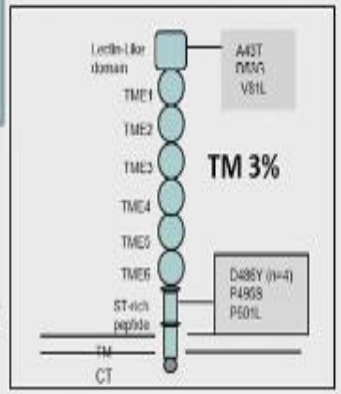


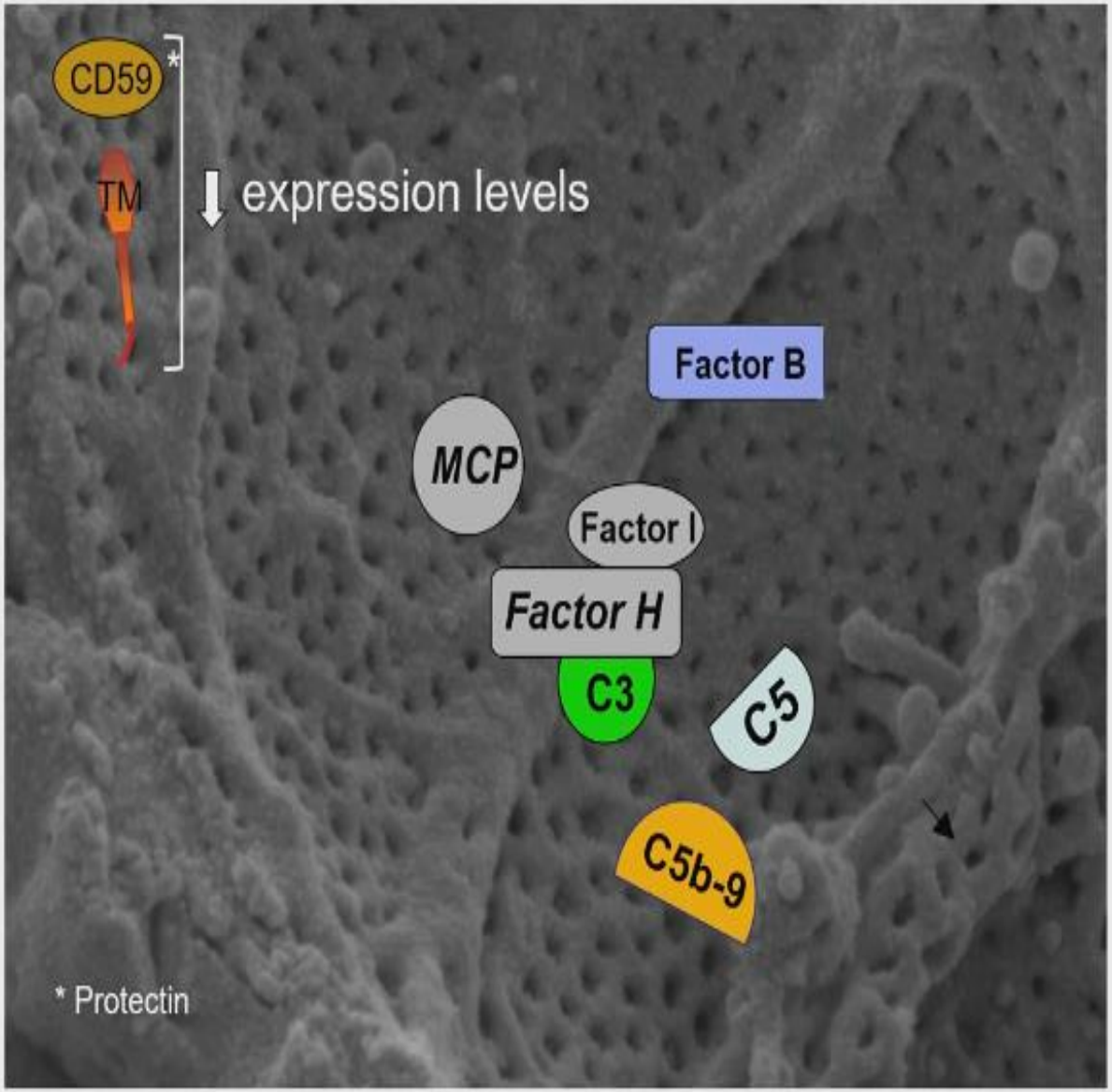
50-60%

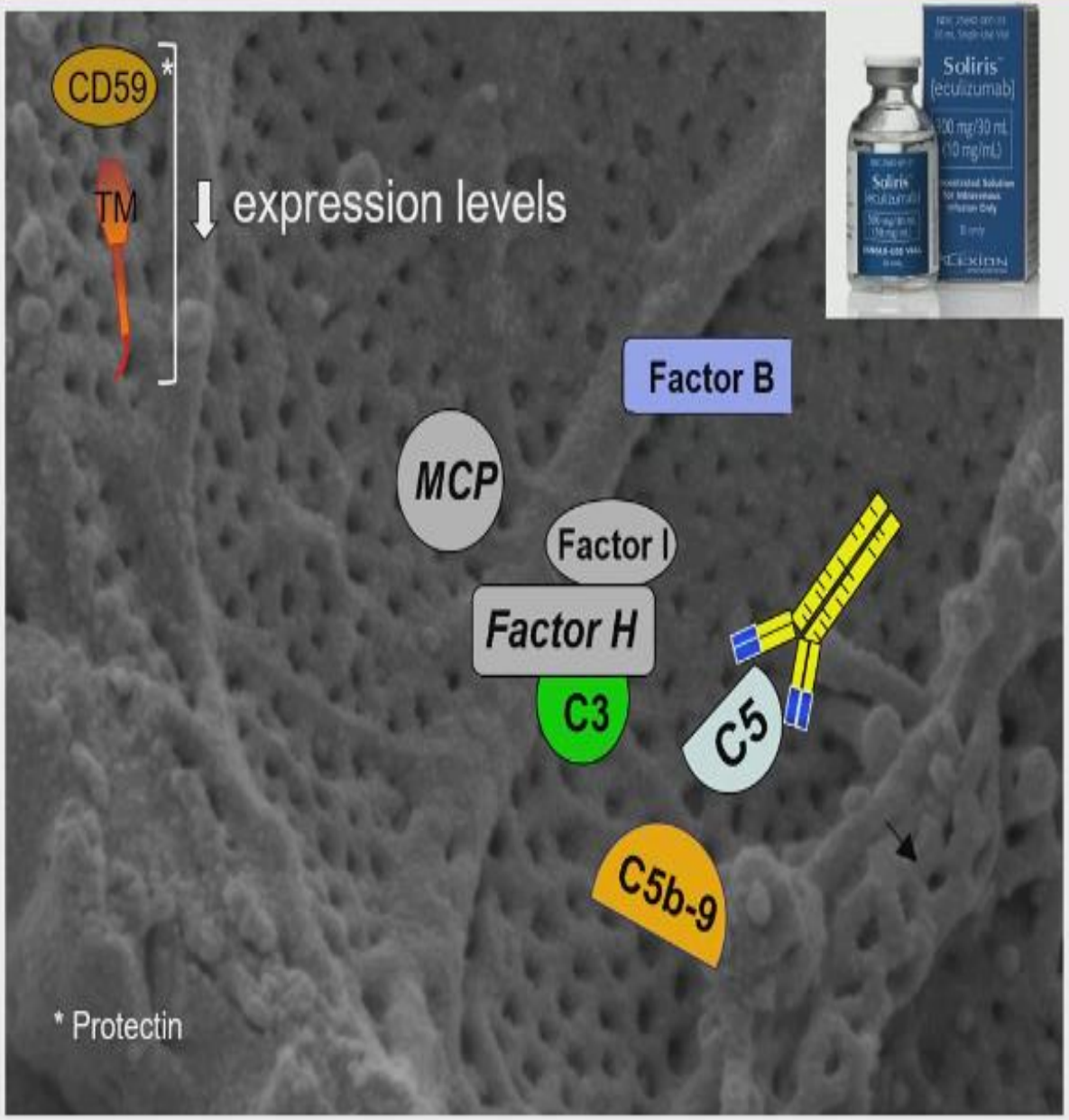






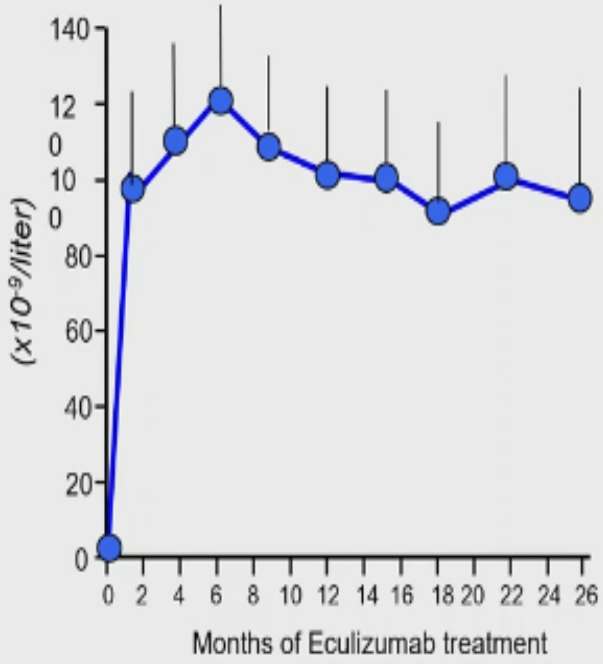



CD59
*
TM
expression levels
Factor B
MCP
Factor I
Factor H
C3
C5
C5b-9
* Protectin


CD59
*
TM
expression levels
Soliris
(eculizumab)
Factor B
MCP
Factor I
Factor H
C3
C5
C5b-9
* Protectin

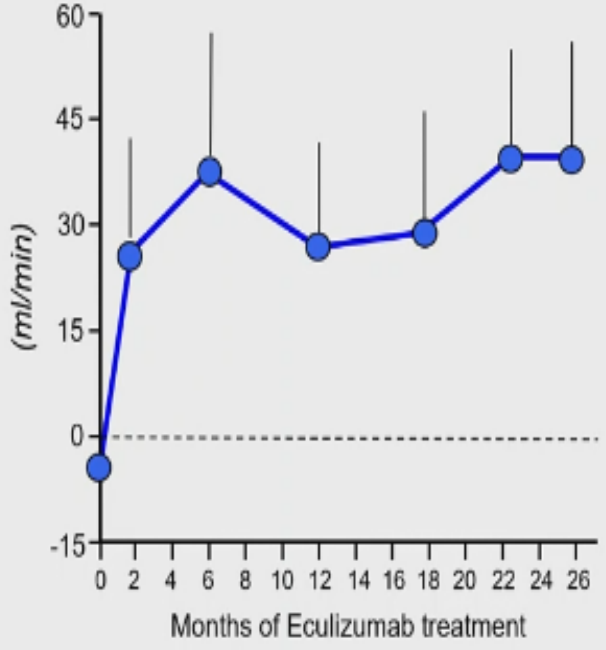
Platelet count

mean change from baseline



Estimated GFR

mean change from baseline



Treatment effect was sustained for up to 26 months

Licht et al., *Kidney Int*, 2015

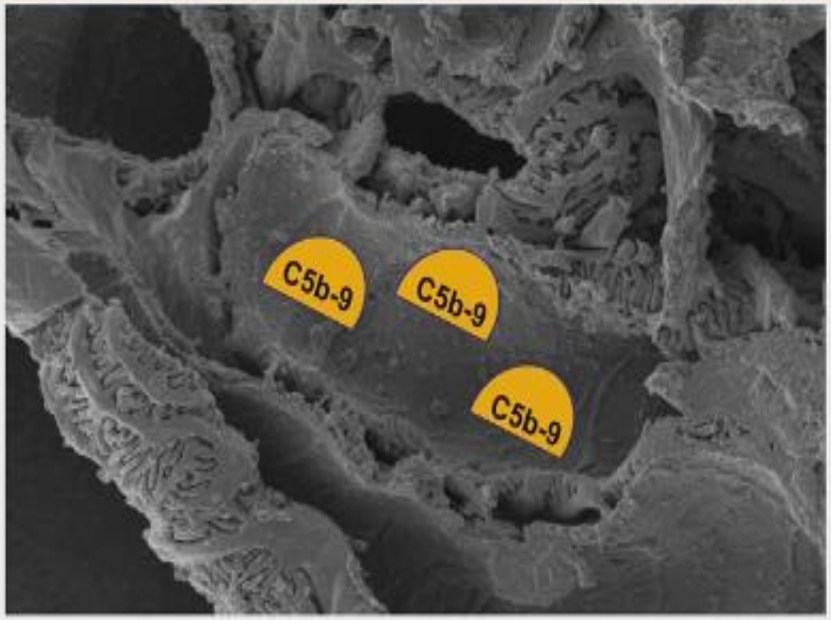
330.000

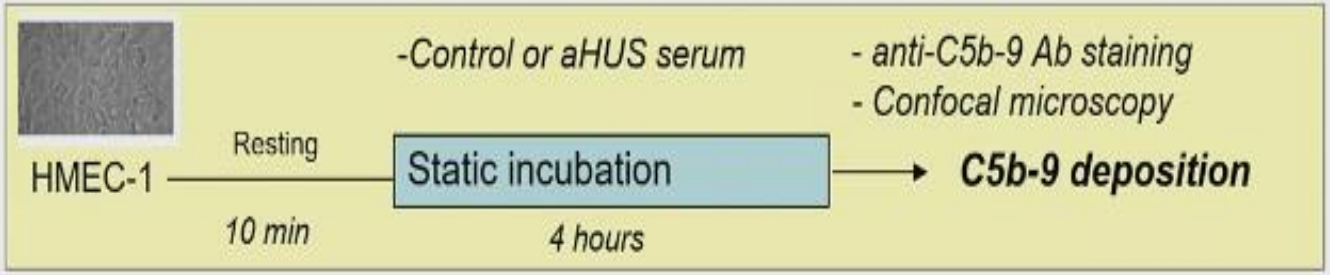
euro per patient per year

(in child)

Every 15 days forever?

How to monitor and possibly tapering?





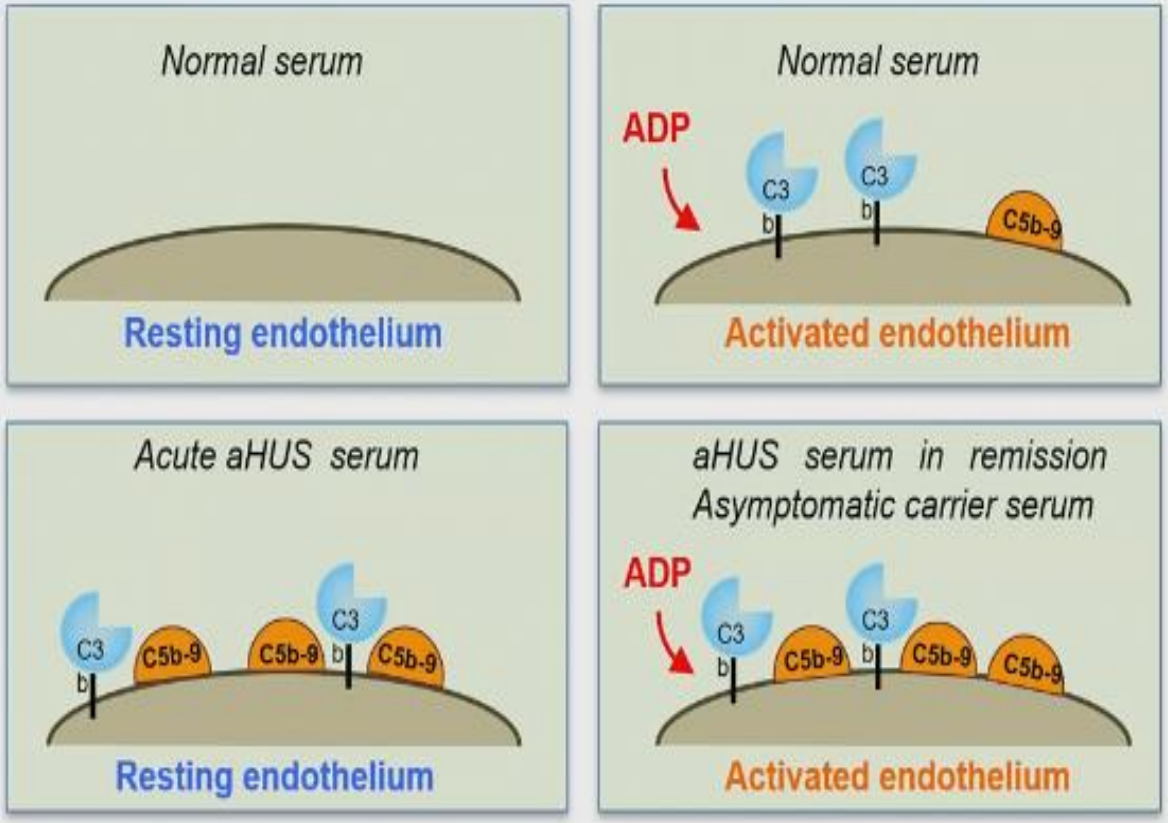
Noris et al., *Blood*, 2014

BLOOD, 11 SEPTEMBER 2014 • VOLUME 124, NUMBER 11

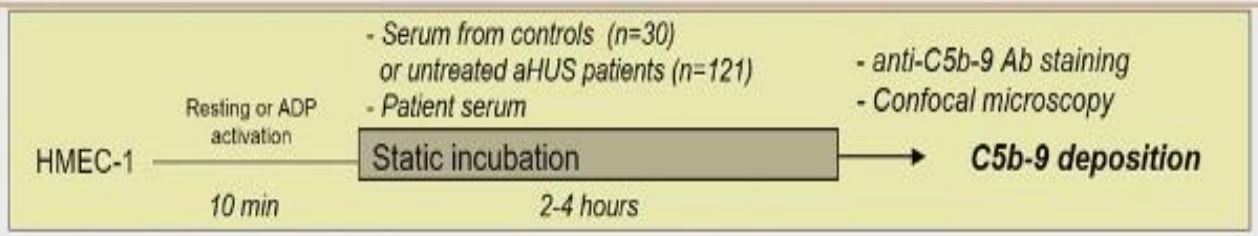
Comment on Noris et al, page 1715

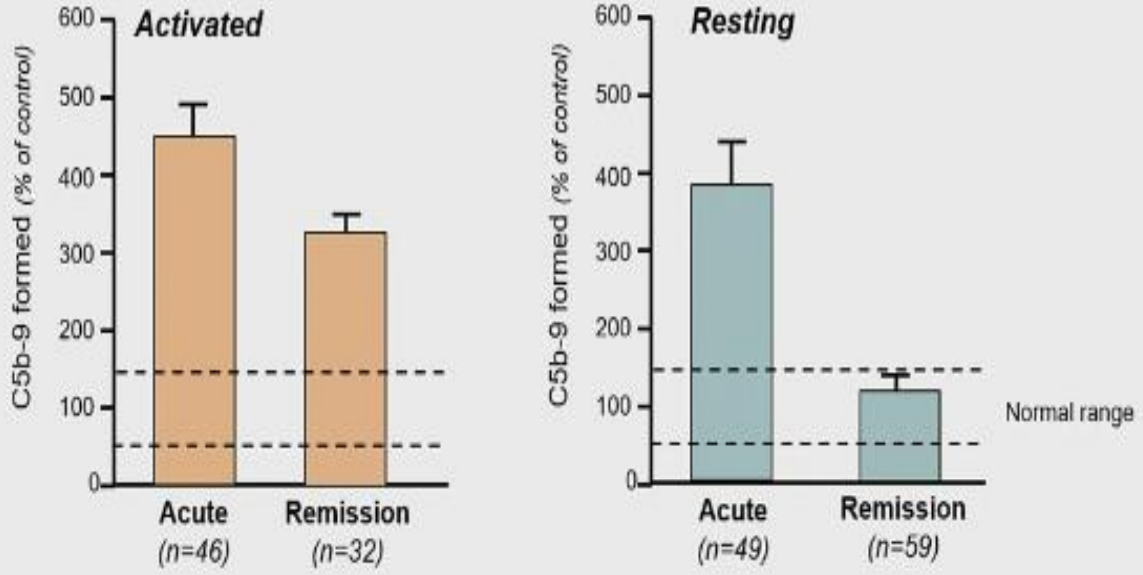
COMPLEMENTing the diagnosis of aHUS

Vahid Afshar-Kharghan THE UNIVERSITY OF TEXAS MD ANDERSON CANCER CENTER



EX VIVO C5b-9 ENDOTHELIAL DEPOSITION IN A LARGE COHORT OF aHUS PATIENTS



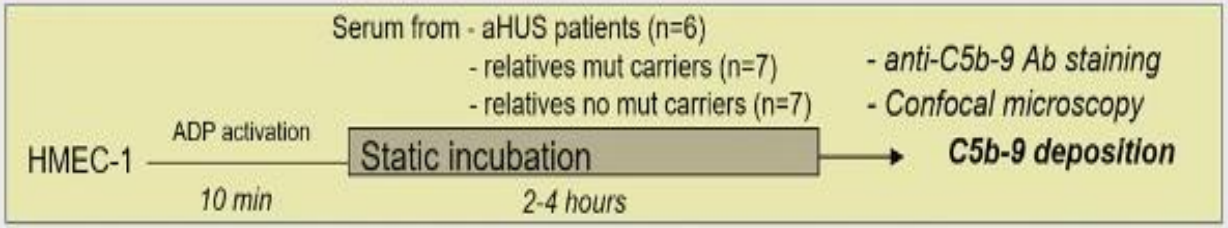


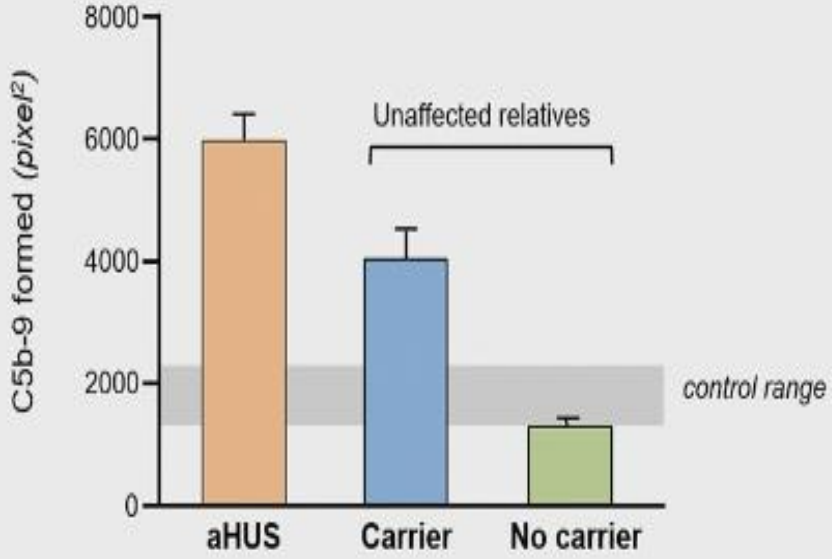
Activated: complement dysregulation (sensitivity 100 %)

Resting : disease activity (sensitivity 100%, specificity 97%)

Galbusera et al, *Am J Kidney Dis*, 2019

SERUM FROM UNAFFECTED CARRIERS OF COMPLEMENT GENE VARIANTS CAUSES THE FORMATION OF C5b-9 ON *ACTIVATED* HMEC-1

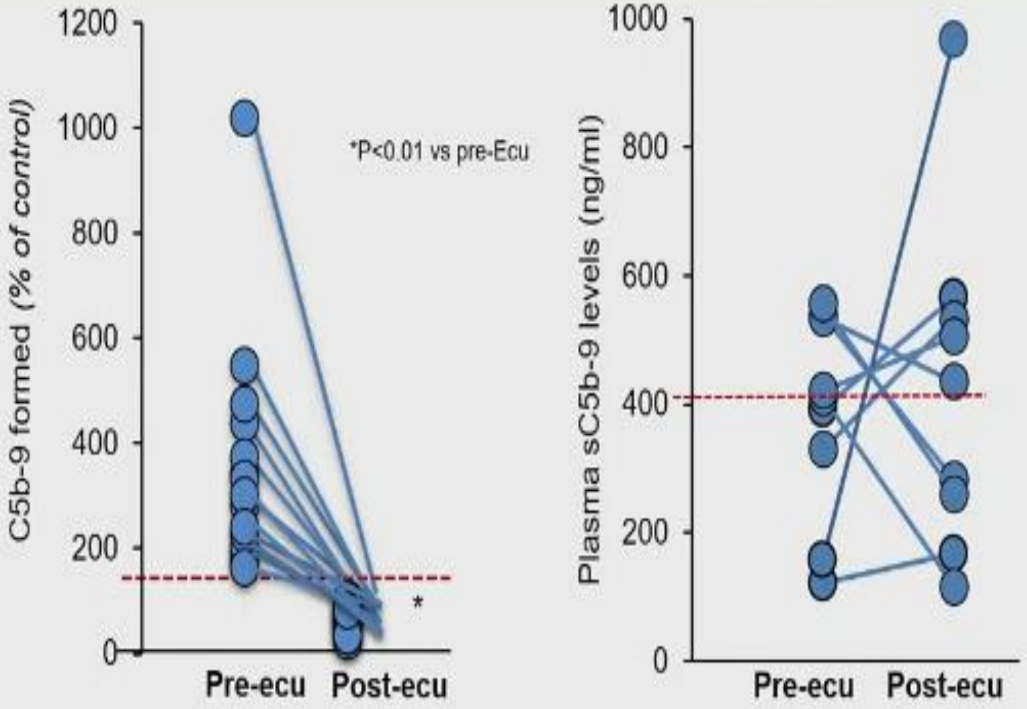




aHUS: with CFH (n=4) or C3 (n=1) or CFI (n=1) mutations
Carrier: with CFH (n=5), or C3 (n=1) or CFI (n=1) mutations
No carrier: without mutations

Noris et al., *Blood*, 2014

ECULIZUMAB TREATMENT IN aHUS PATIENTS FULLY NORMALIZED EX VIVO SERUM-INDUCED FORMATION OF C5b-9 ON *ACTIVATED* HMEC-1



- *In 20 aHUS cases treated with Eculizumab, serum-induced C5b-9 deposits on ADP-activated HMEC-1 ex-vivo normalized after treatment*
- *No significant change was observed in pre- and post-Eculizumab plasma sC5b-9 levels*

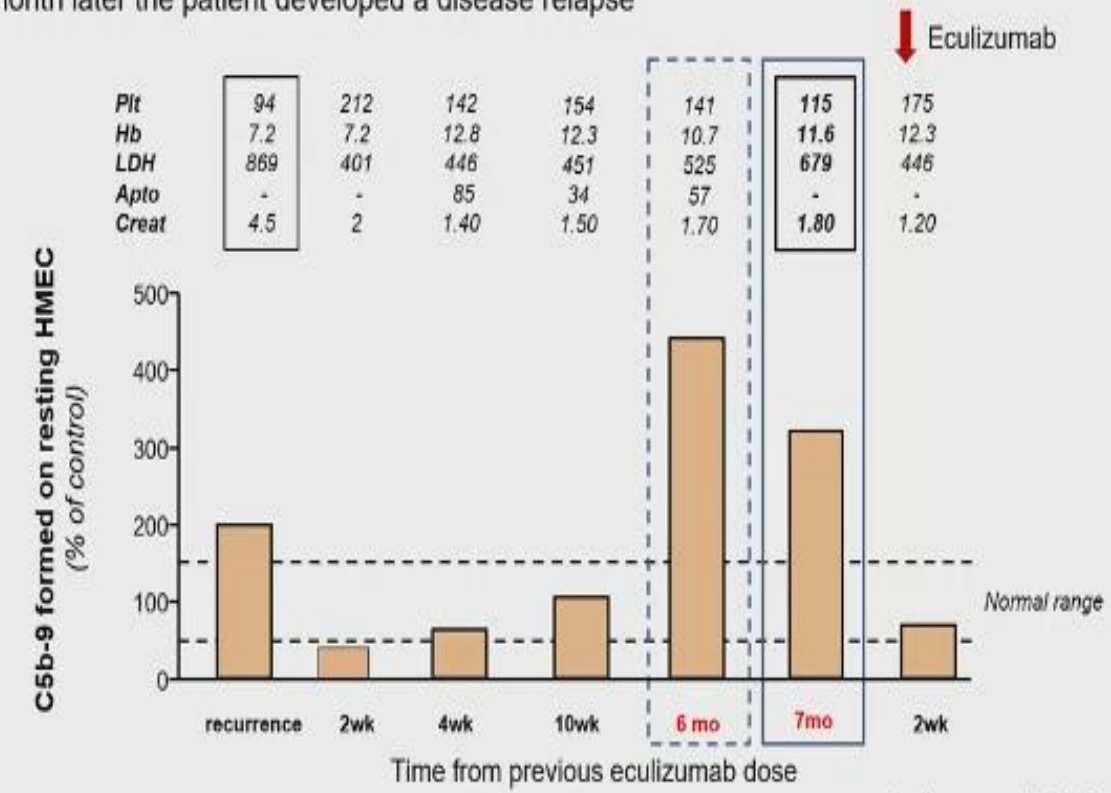
Galbusera et al., *Am J Kidney Dis*, 2019

42 year old woman

- recurrent plasma-dependent aHUS (onset at 34 years of age)

- heterozygous CFI mutation (p.R187Q)

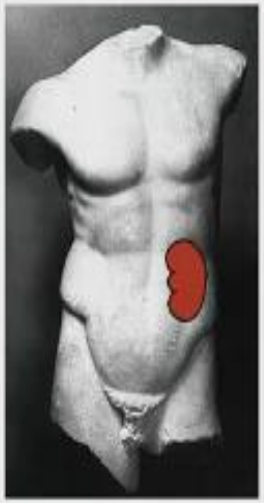
- During a recurrence the patient was treated with eculizumab resulting in disease remission
- After 7 months of eculizumab every 2 weeks, the interval between doses was increased till discontinuation
- Six month after eculizumab cessation, C5b-9 deposits on resting HMEC-1 rose above normal levels
- One month later the patient developed a disease relapse

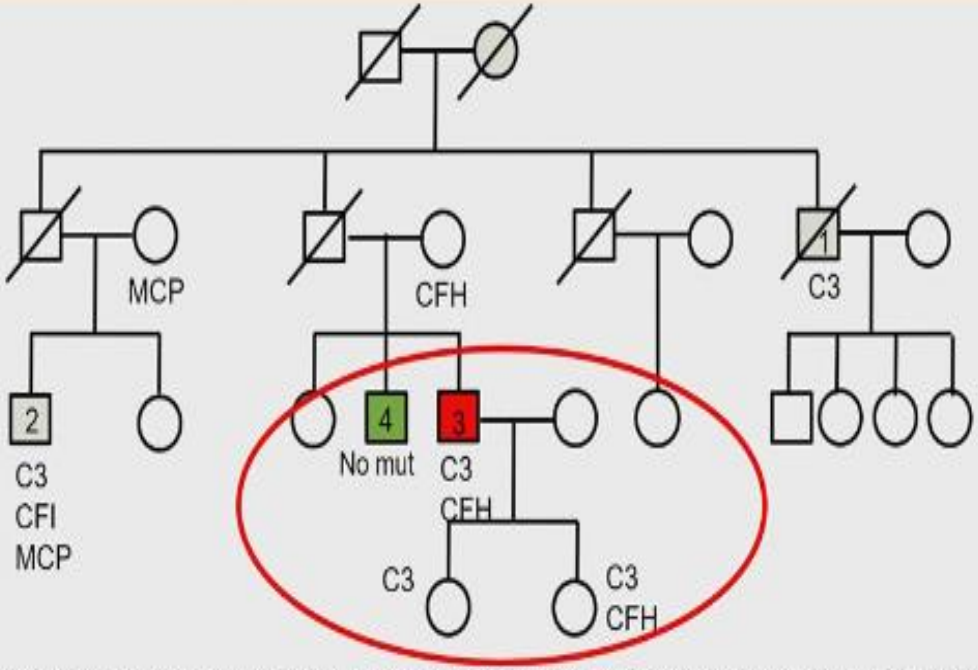


Galbusera et al., *Am J Kidney Dis*, 2019

A CASE OF FAMILIAL aHUS FROM VIETNAM

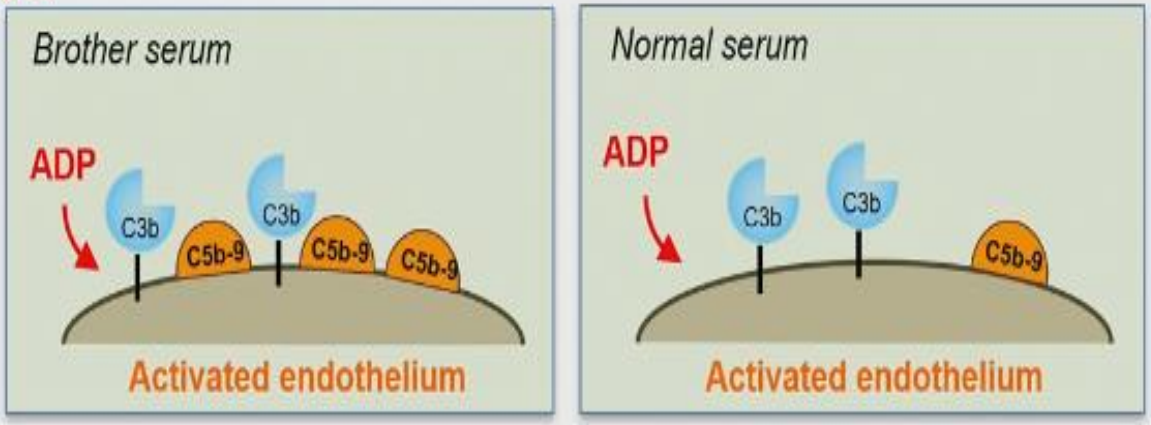
A CASE OF FAMILIAL aHUS FROM VIETNAM



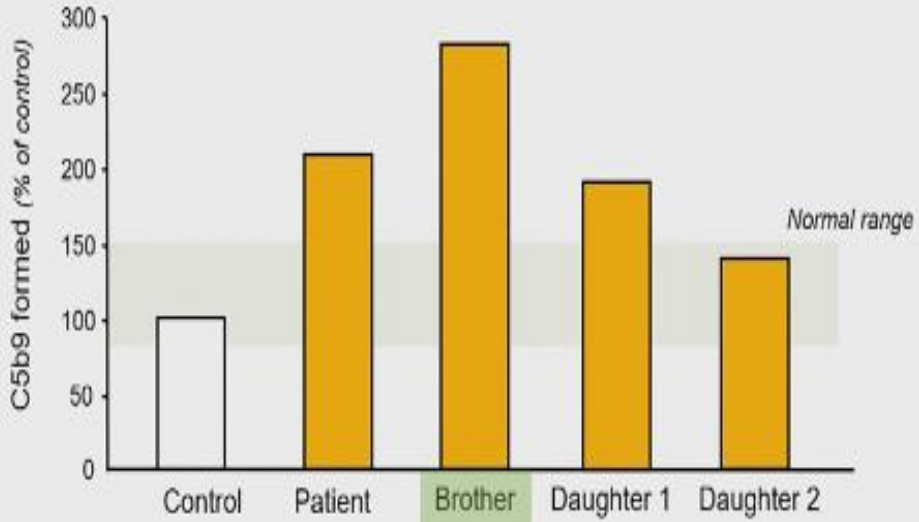


- A p.V1636A C3 heterozygous mutation was found in the proband (patient 3) and in his affected relatives (patients 1 and 2)
- A rare polymorphism in CFH (p.Q950H) was found in the proband, and a proband's daughter carries both the p.V1636A C3 mutation, the other has both the C3 mutation and the CFH variant
- The proband's unaffected brother (subject 4) does not carry the C3 mutation nor the CFH variant

2015

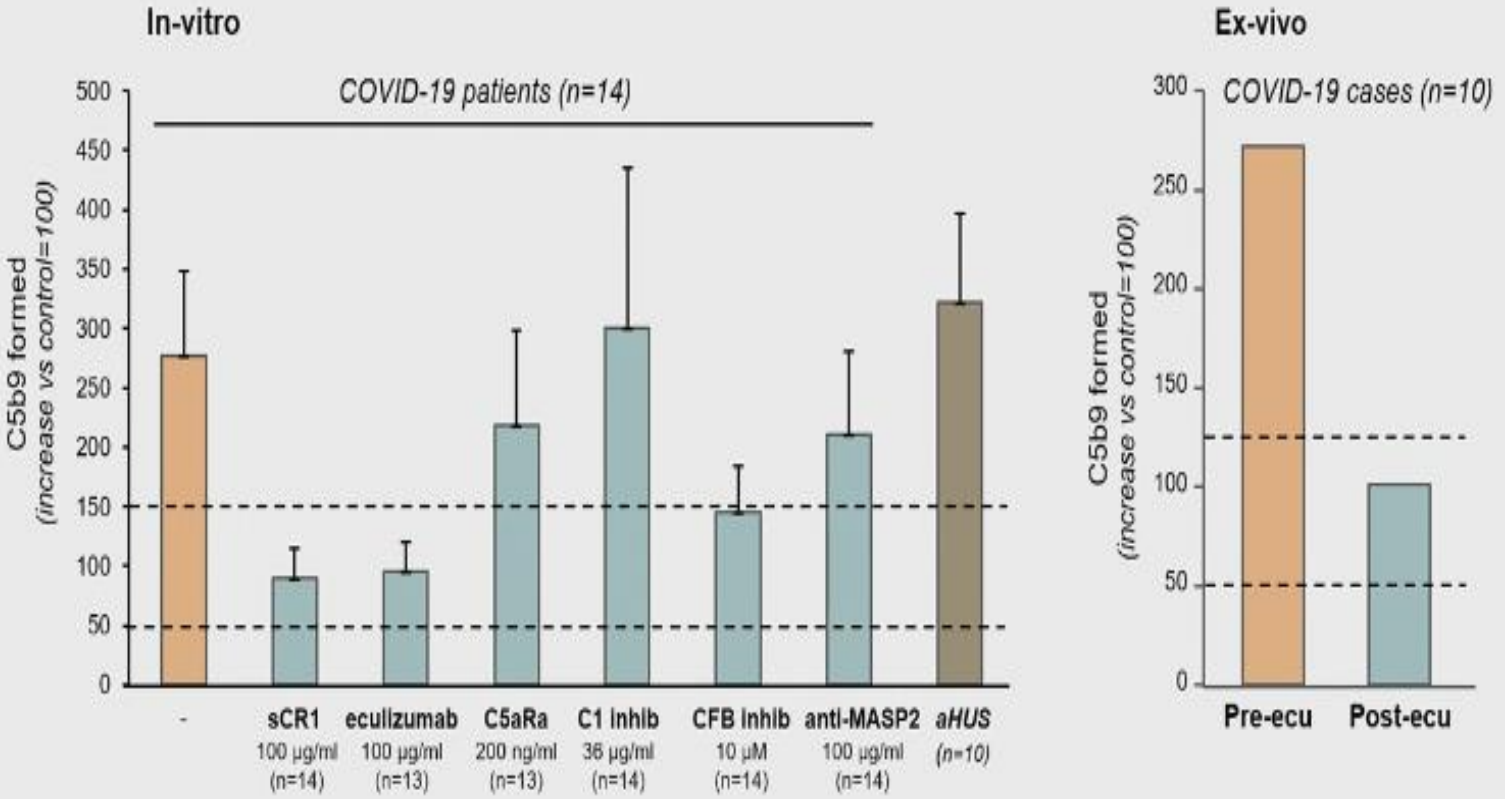


Noris et al., *Blood*, 2014



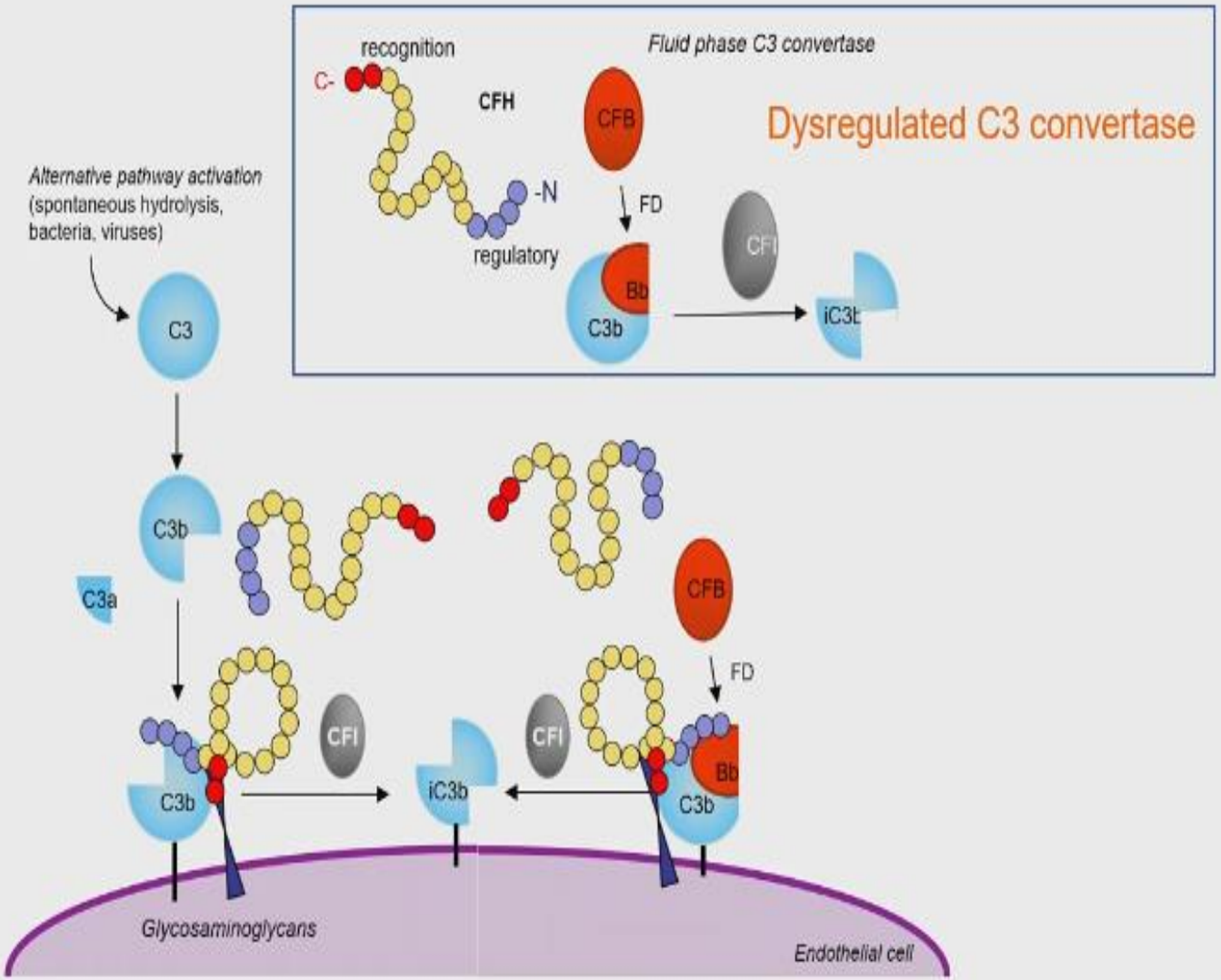
	Control	Patient	Brother	Daughter 1	Daughter 2
C3 mutation		p.V1636A	No mut	p.V1636A	p.V1636A
CFH variant		p.Q950H	No var	p.Q950H	No var
			?		

EVIDENCE OF COMPLEMENT ACTIVATION ON ENDOTHELIUM IN SEVERE COVID-19 PATIENTS



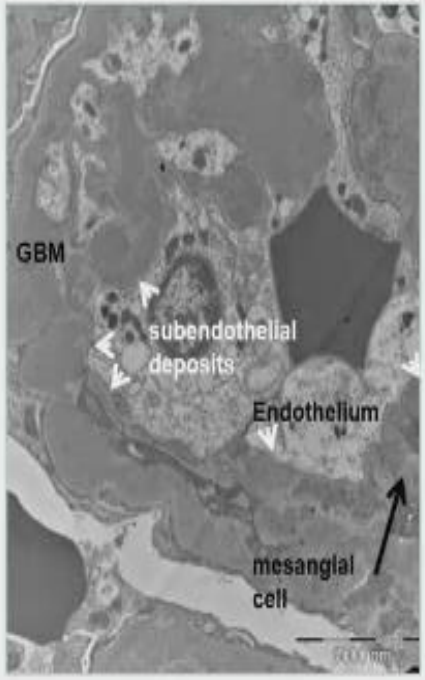
Galbusera, *Personal Communication*

FLUID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN MPGN



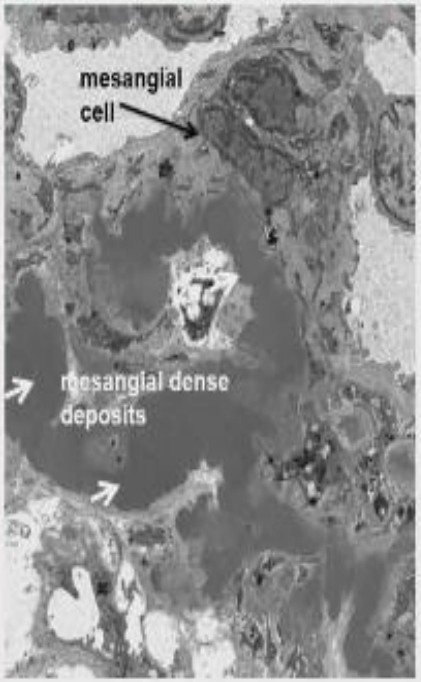
A RARE FORM OF CHRONIC NEPHRITIS THAT OCCURS PRIMARILY IN CHILDREN AND YOUNG ADULTS AND IS CHARACTERIZED BY DIFFUSE PROLIFERATIVE LESIONS AND WIDENING OF THE CAPILLARY LOOPS

MPGN I



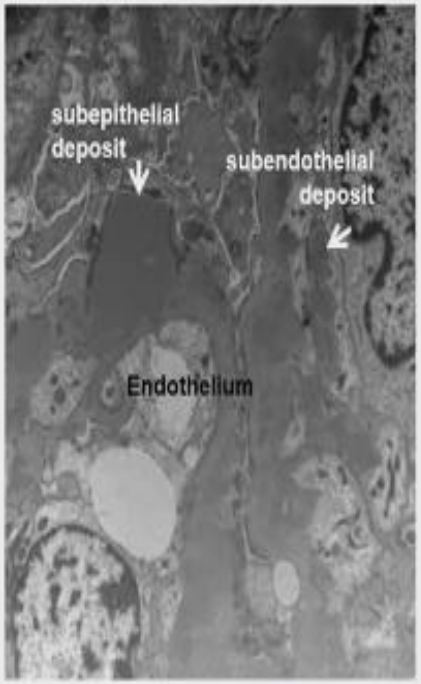
- Subendothelial deposits

MPGN II (Dense Deposit Disease)

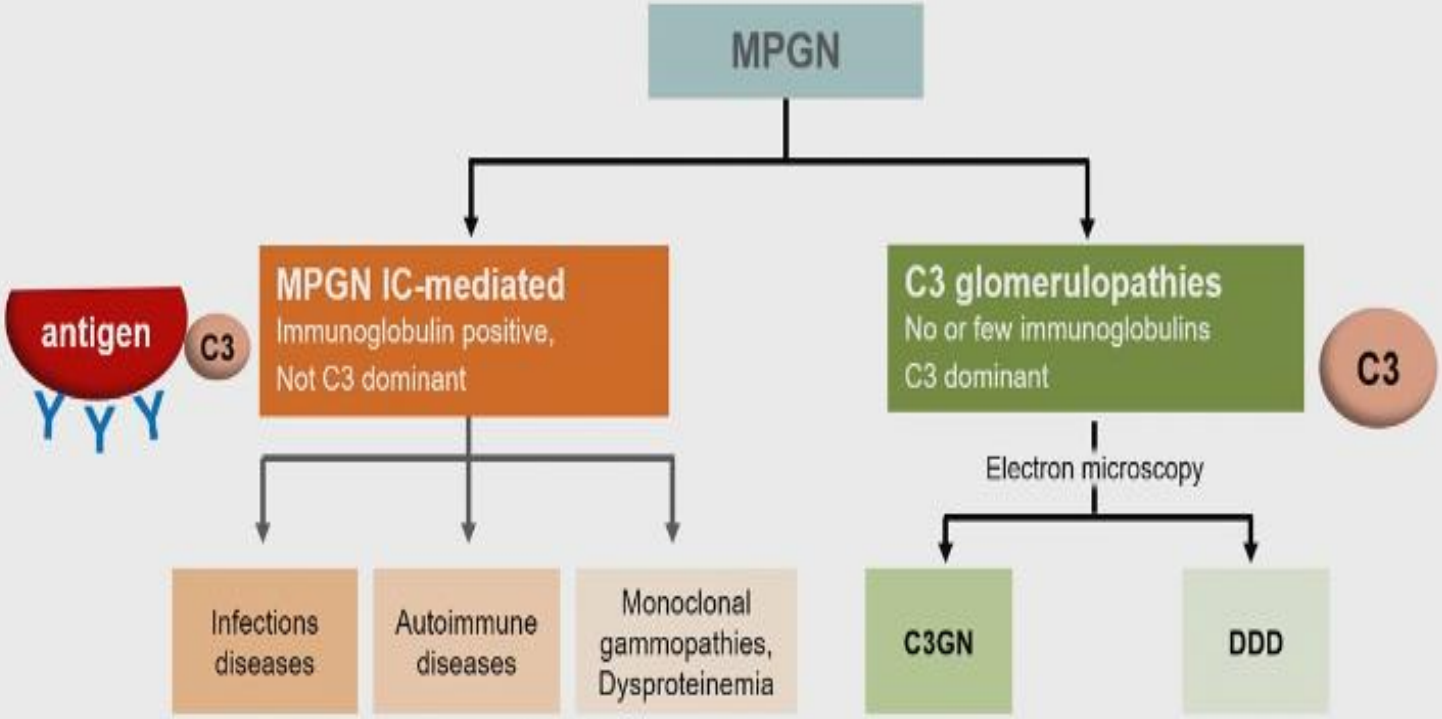


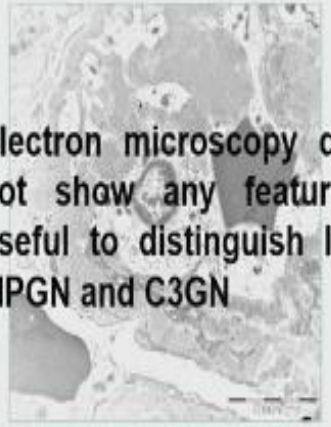
- Highly electron dense deposits in the GBM

MPGN III



- Subendothelial deposits
- Subepithelial deposits





Electron microscopy did not show any features useful to distinguish IC-MPGN and C3GN

- Ultra-rare
- Complement dysregulation
- Broad inter-individual variability
- Classification challenges and complications
- Incurable ?

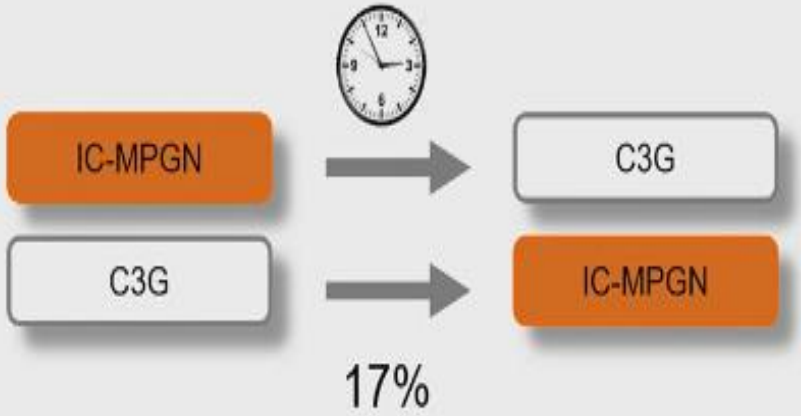
Sethi et al., *N Engl J Med*, 2012

- Low serum C3 and normal serum C4 levels were found in the large majority of patients with IC-MPGN or with C3G, indicating activation of the alternative pathway of complement

- The prevalence of patients with low C3 and normal C4 levels did not differ between IC-MPGN and C3G and between the three histology groups (IC-MPGN: 70%, C3GN: 74%, DDD: 84%)

- Up to 17% of patients shift from IC-MPGN to C3G and vice versa when a kidney biopsy is repeated

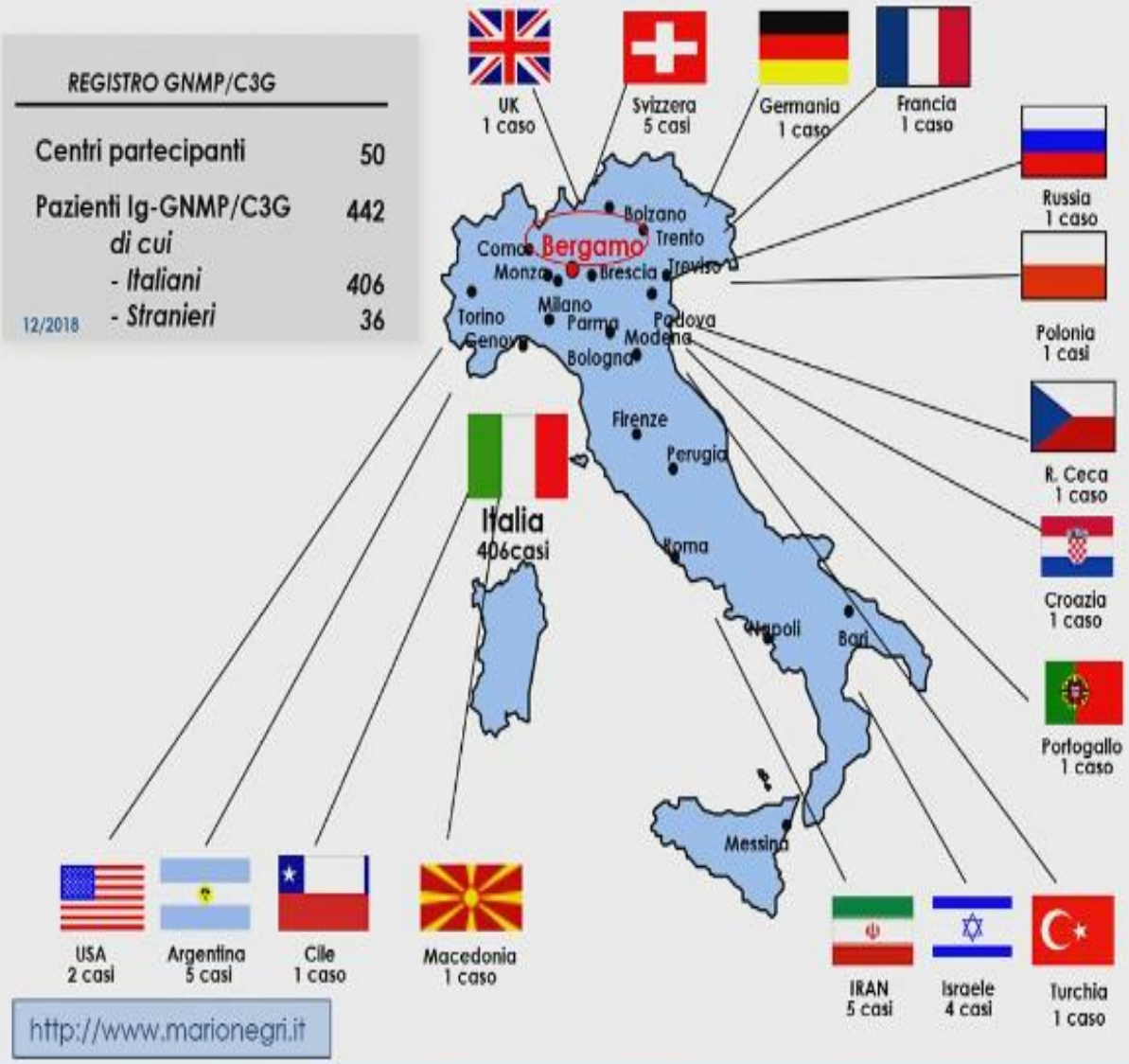
Iatropoulos et al, *JASN*, 2018



REGISTRO DELLA GLOMERULONEFRITE MEMBRANOPROLIFERATIVA/C3G

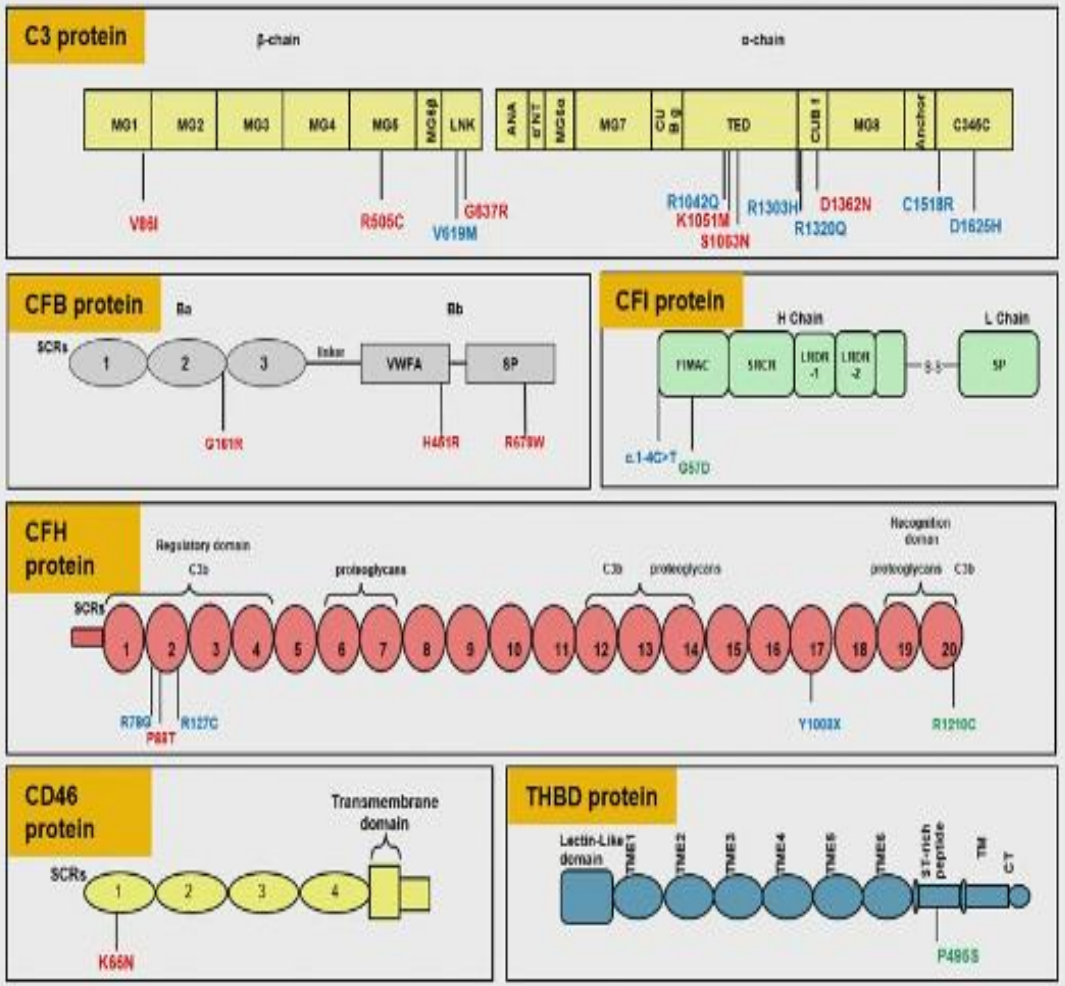
REGISTRO GNMP/C3G	
Centri partecipanti	50
Pazienti Ig-GNMP/C3G	442
di cui	
- *Italiani*	406
- *Stranieri*	36

12/2018



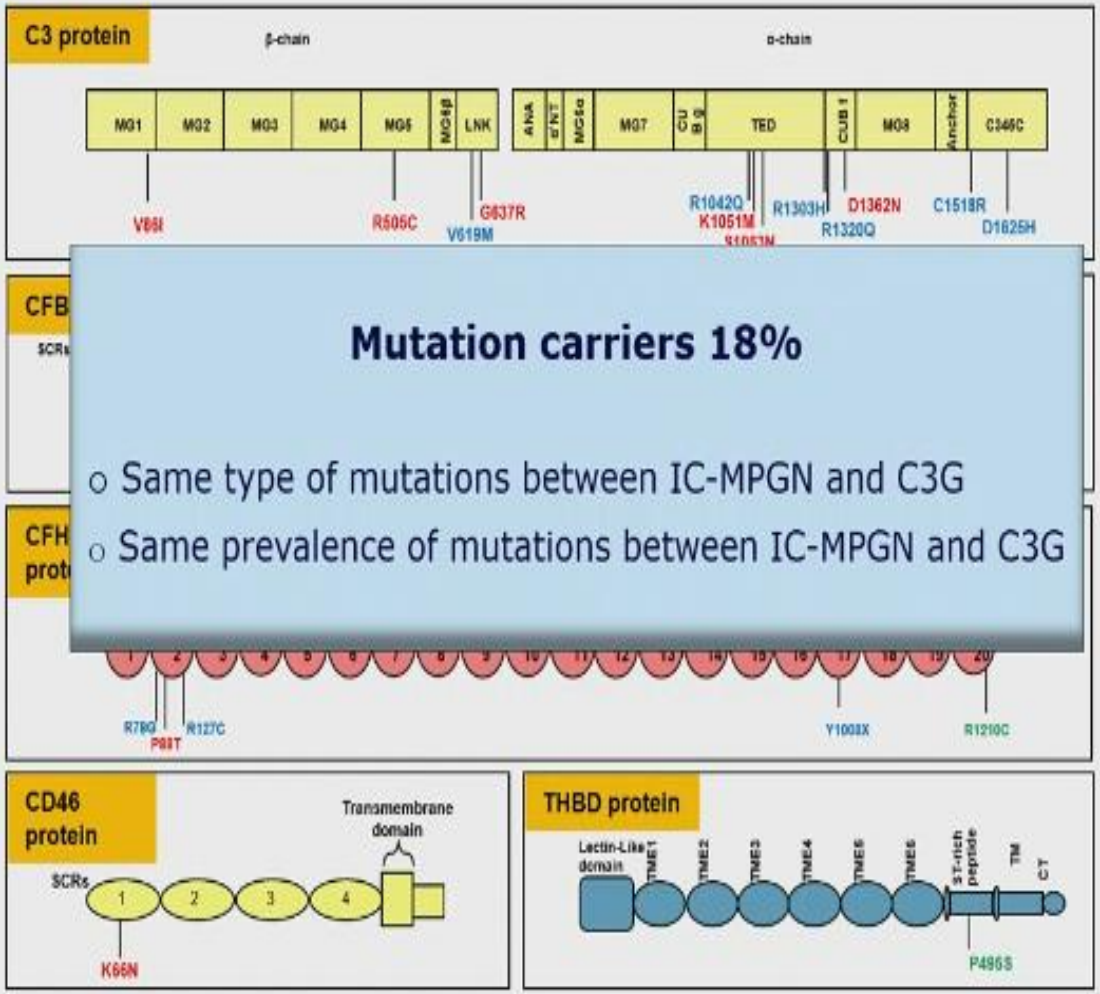
http://www.marionegri.it

COMPLEMENT GENE MUTATIONS IN IC-MPGN C3GN DDD



Iatropoulos et al, *Mol Immunol*, 2016

COMPLEMENT GENE MUTATIONS IN IC-MPGN C3GN DDD



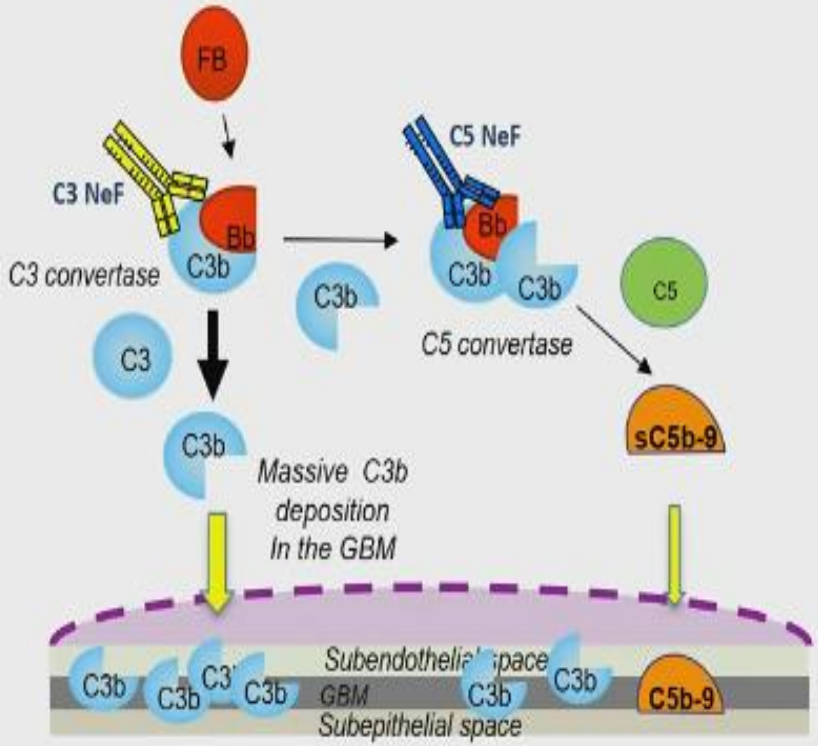
Mutation carriers 18%

- Same type of mutations between IC-MPGN and C3G
- Same prevalence of mutations between IC-MPGN and C3G

Iatropoulos et al, *Mol Immunol*, 2016

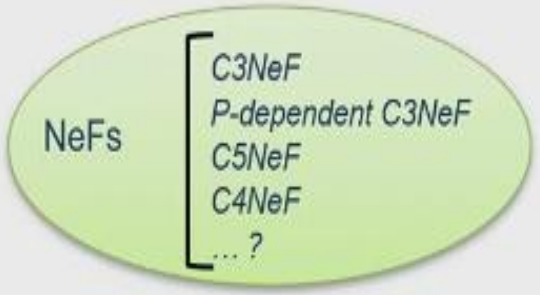

LONDON NEWS
JACK THE RIPPER
AND THE EAST END


C3Nef?
LONDON NEWS
JACK THE RIPPER AND THE EAST END



Patients tested		C3NeF+
- DDD	n = 21	78 %
- C3GN	n = 73	44 %
- IC-MPGN	n = 67	45 %

Iatropoulos P. et al. 2016



- C3NeFs belong to an heterogenous family of autoantibodies (NeFs) that stabilize the convertases complexes

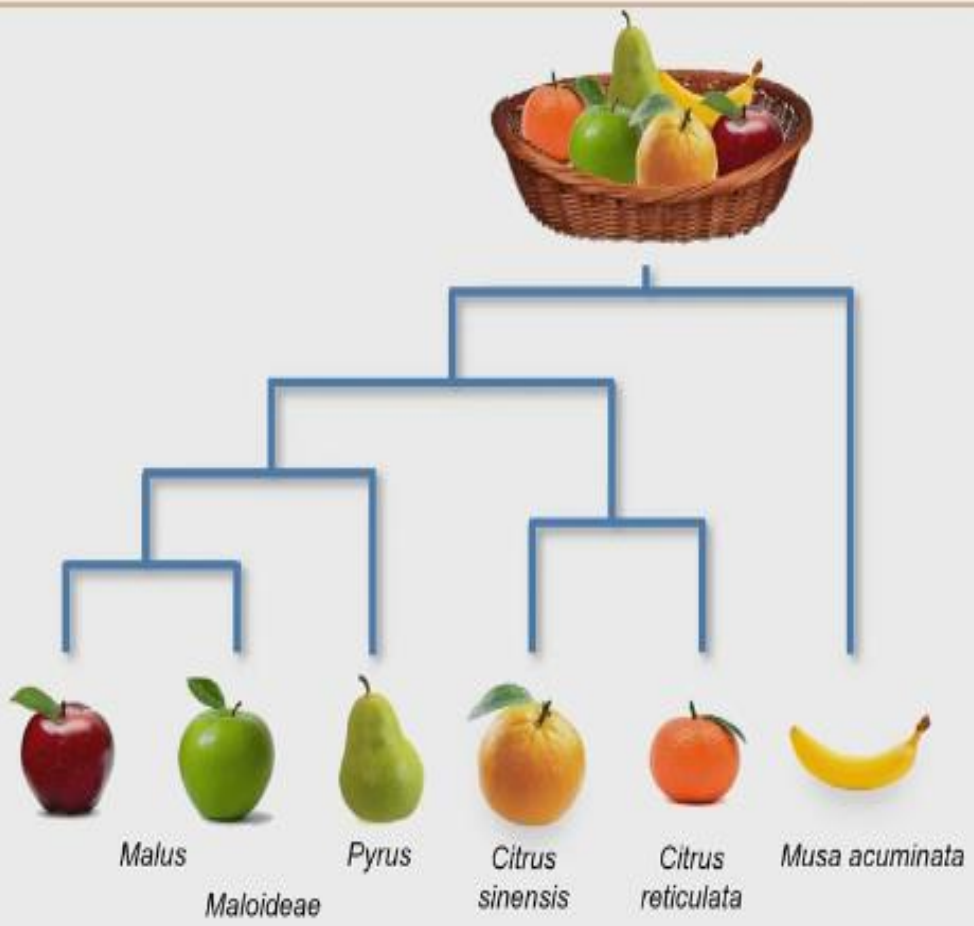
- C3NeFs bind to the assembled C3 convertase and prevents its spontaneous and FH- mediated decay

Daha et al., *Immunology*, 1981

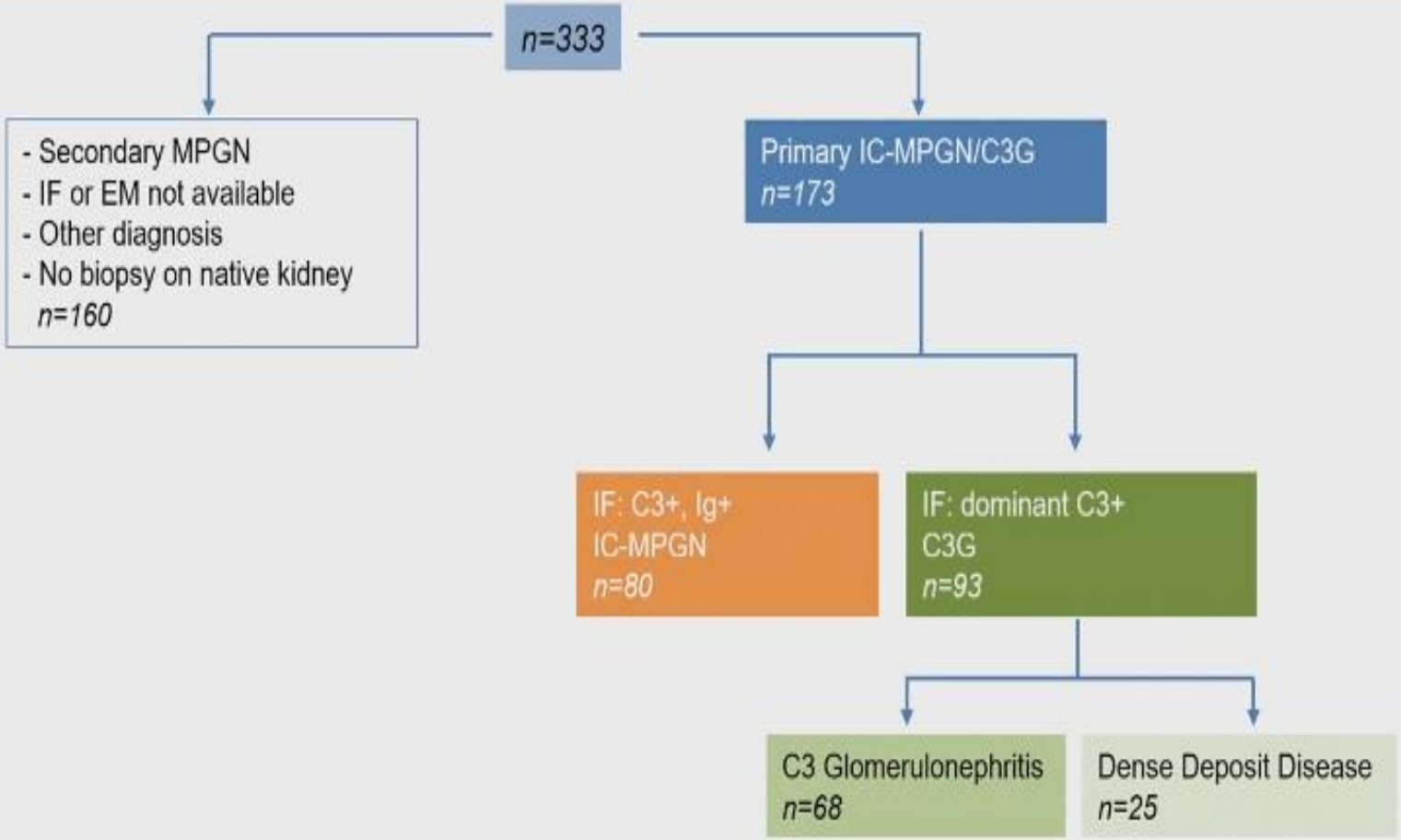
- C5NeFs bind to the assembled C5 convertase and prevent its spontaneous and FH-mediated decay

Marinozzi et al, Kidney Int, 2017

EXAMPLE OF CLUSTER ANALYSIS
Subdivide and group the fruit



SELECTION OF PATIENTS FOR CLUSTER ANALYSIS



Iatropoulos P et al., *JASN*, 2018

Histologic, biochemical, genetic and clinical variables in 173 patients with primary IC-MPGN/C3G

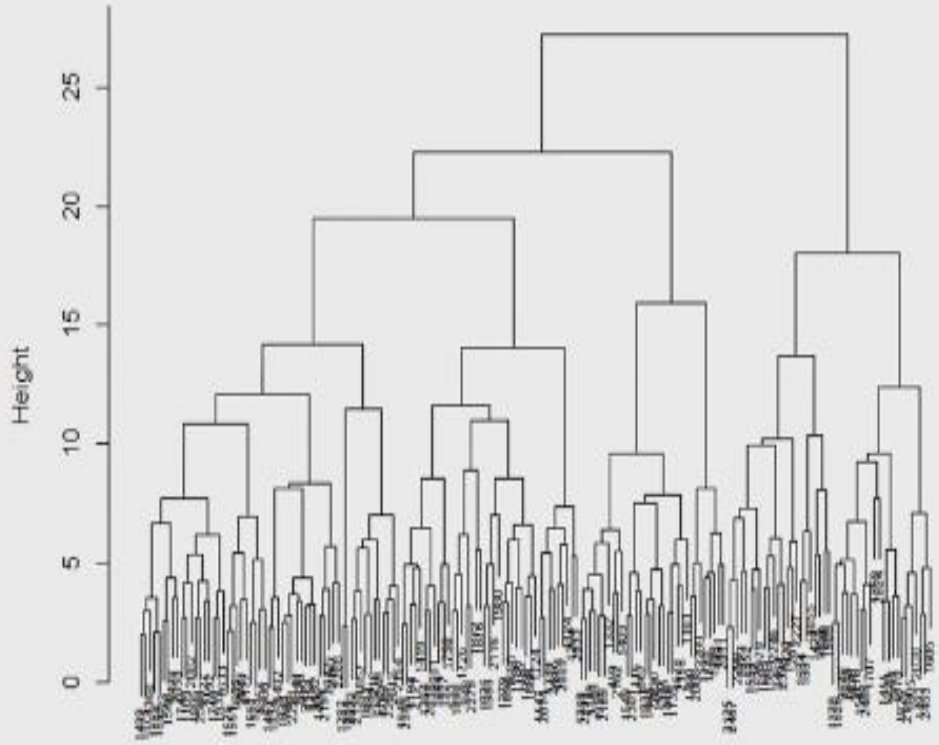
Examples of variables included in cluster analysis

Clinical features (*n*=7)	Histology findings (*n*=17)	Complement profile (*n*=4)	Genetic data (*n*=7)
Age (onset)	C3 staining on IF	Serum C3	N° of AP complement gene mutations*
Familiarity for nephropathy	IgG staining on IF	Serum C4	CFH p.V62I
Micro-/Gross hematuria at onset	Intramembranous electron dense deposits	Plasma sC5b-9	CFH p.H402Y
Proteinuria/Nephrotic syndrome at onset	Subendothelial deposits	Presence of C3NeF*	CD46 c.-366A>G
Renal impairment/ESRD at onset	% of sclerotic glomeruli		CFB p.Q/W32R
Sex	Degree of mesangial proliferation		C3 p.R102G
Trigger	…		THBD p.A473V

*used as a single composite variable 'N° of AP abnormalities'.

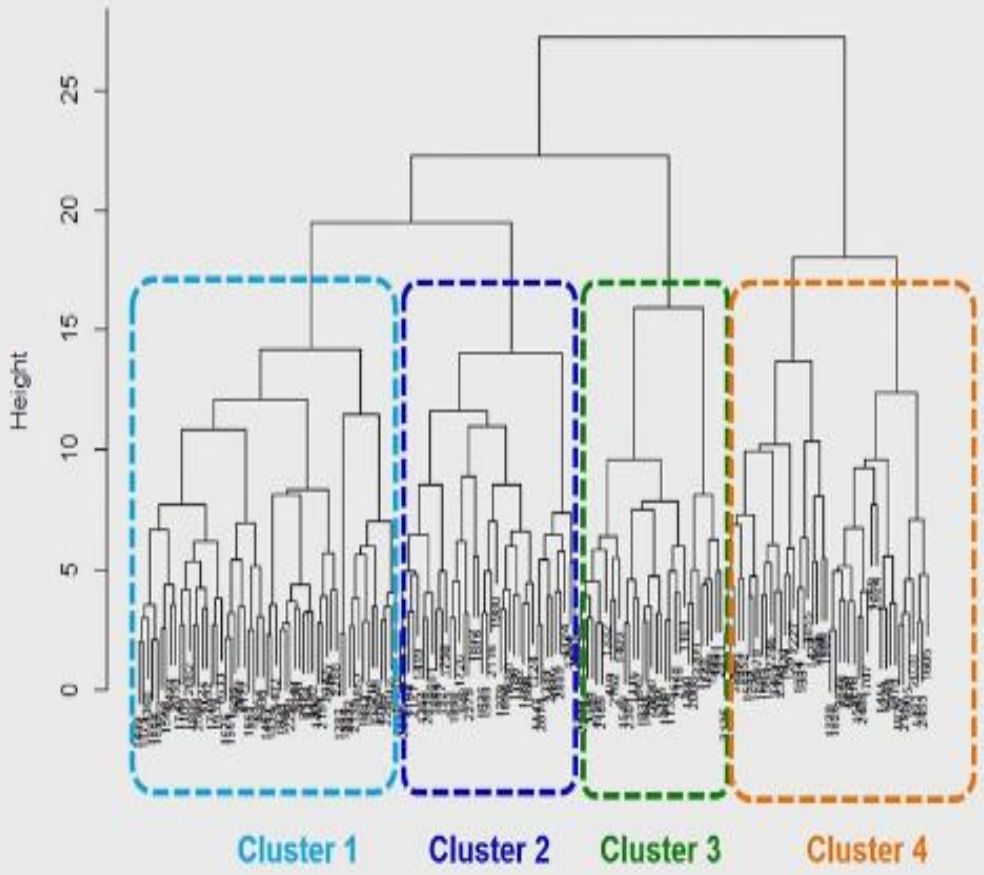
Iatropoulos P et al., *JASN*, 2018

CLUSTER ANALYSIS IN PATIENTS WITH IC-MPGN AND C3G DEMONSTRATES THE PRESENCE OF FOUR GROUPS



Iatropoulos P et al., *JASN*, 2018

CLUSTER ANALYSIS IN PATIENTS WITH IC-MPGN AND C3G DEMONSTRATES THE PRESENCE OF FOUR GROUPS

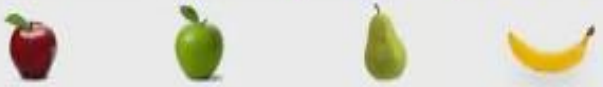


Iatropoulos P et al., *JASN*, 2018

CLUSTER ANALYSIS IDENTIFIED FOUR DISTINCT GROUPS OF PATIENTS

		Cluster 1	Cluster 2	Cluster 3	Cluster 4
Glomerular C3	score	2.7	2.7	2.8	2.5
Mutation or C3NeFs	%	75	63	79	14
Serum C3	mg/dl	⇊	⇊	⇊	N
Plasma sC5b-9	ng/ml	⇈	⇈	N / ↑	N
Glomerular IgG	score	0.4	2.0	0.5	1.0
Glomerular C1q	score	0.3	1.6	0.3	0.6
Highly electron dense deposits	%	7	0	73	0

N: normal



Cluster 1: Fluid-phase C3 and C5 convertase activation

Cluster 2: Fluid-phase C3 and C5 convertase activation+ classical pathway activation

Cluster 3: Fluid-phase C3 convertase activation only

Cluster 4: Solid-phase complement activation

Iatropoulos P et al., *JASN*, 2018

Data from 33 newly recruited patients are in line with observations in the cluster cohort

CLUSTER ANALYSIS IDENTIFIED FOUR DISTINCT GROUPS OF PATIENTS

Clusters 1-3:
Fluid–phase complement activation

Cluster 1
Low serum C3 and high plasma sC5b-9 levels

Cluster 2:
Low serum C3 and high plasma sC5b-9 levels
Ig and C1q staining on IF

Cluster 3:
Low serum C3 and mostly normal plasma sC5b-9 levels
Very dense deposits on EM

Cluster 4:
Solid–phase complement activation
Normal serum C3 levels, normal plasma C5b-9
Intense C3 staining on IF

Iatropoulos P et al., *JASN*, 2018

DIFFERENT HISTOLOGIC GROUPS FALL IN TWO DIFFERENT CLUSTERS

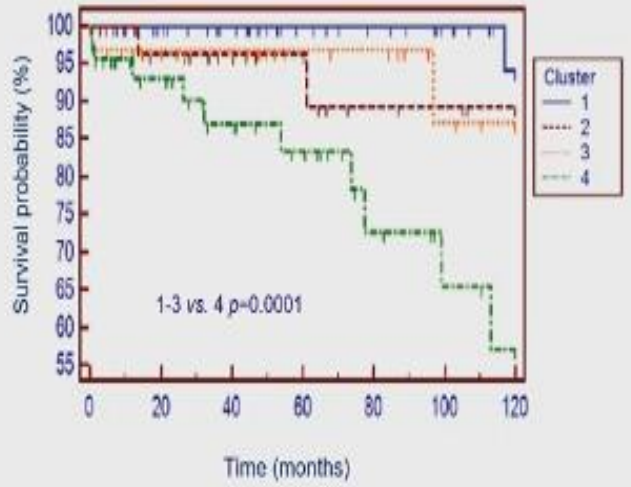
Histologic diagnosis	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Overall p-value*
IC-MPGN	16	30	8	26	
C3GN	42	2	4	20	
DDD	4	0	21	0	< 0.001

**p*-value was calculated with Fisher Exact Test

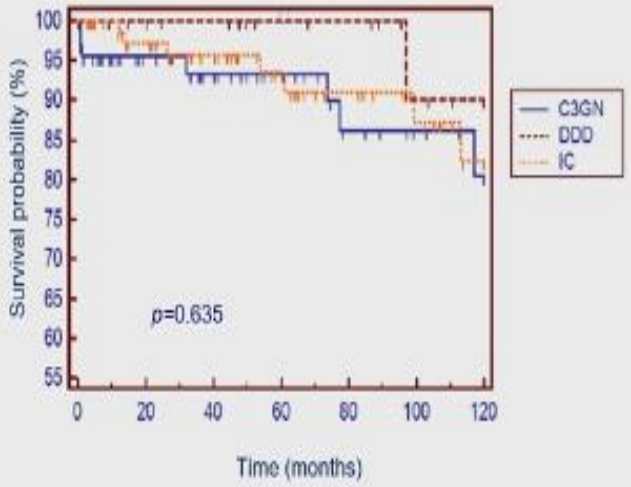
Iatropoulos P et al., *JASN*, 2018

CLUSTER ANALYSIS HAD PROGNOSTIC VALUE WHILE HISTOLOGY CLASSIFICATION DID NOT

ESRD according to clusters



ESRD according to histologic groups

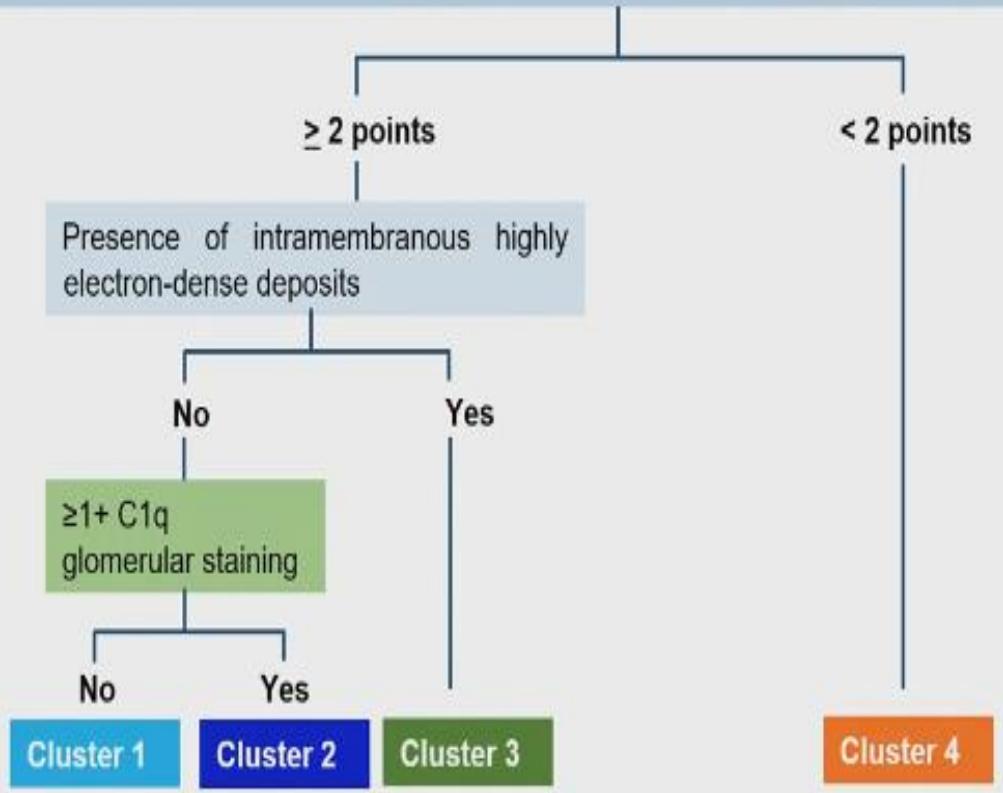


- Kaplan-Meier analyses showed that patients in the cluster 4 have a higher risk of ESRD during follow-up compared to the other three clusters
- No difference in development of ESRD was found between the histologic groups

Iatropoulos P et al., *JASN*, 2018

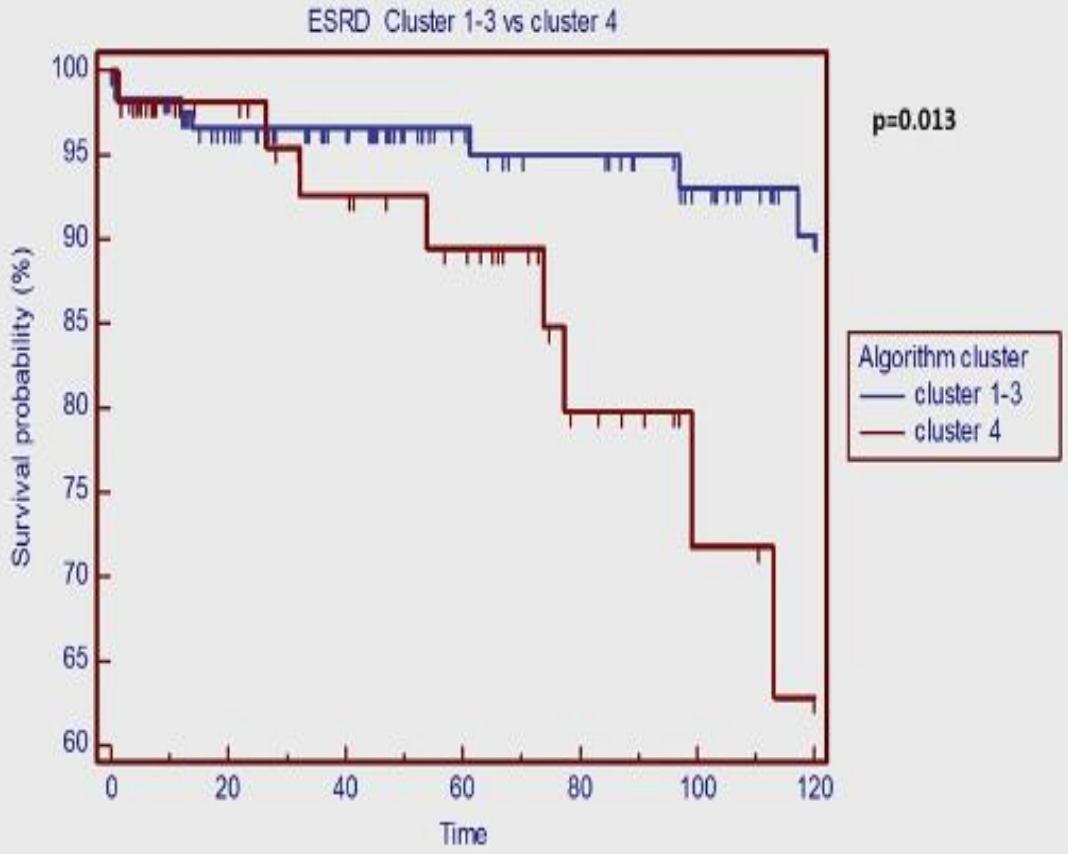
ALGORITHM TO ASSIGN PATIENTS TO CLUSTERS

- For each allele with mutation	1 point
- Presence of C3NeF	1 point
- Serum C3 ≤ 50mg/dl	2 points
- Serum C3 > 50 & <90mg/dl	1 point
- Presence of intramembranous highly electron-dense deposits	2 points



Iatropoulos P et al., *JASN*, 2018

PROGNOSTIC VALUES OF ALGORITHM CLUSTER ON THE EXTENDED COHORT



At variance with conventional classification the cluster analysis allows to identify:

- patients with fluid-phase versus solid-phase complement alternative pathway activation
- patients with early fluid-phase alternative pathway complement activation
- patients with both early and terminal fluid-phase alternative pathway complement activation

and also to predict response to therapy and renal survival

J Am Soc Nephrol, 2018

EDITORIAL

Clusters Not Classifications: Making Sense of Complement-Mediated Kidney Injury

H.Terence Cook and Matthew C. Pickering
Centre for Complement and Inflammation Research, Imperial College of London, London, United Kingdom

"Mathematics trump pathology"

- Tool to integrate clinical parameters, complement protein measurements, complement genetics, autoantibody results, and a renal biopsy
- Most importantly, their cluster analysis did better at predicting renal survival than the division into MPGN, DDD, and C3GN

"To come"

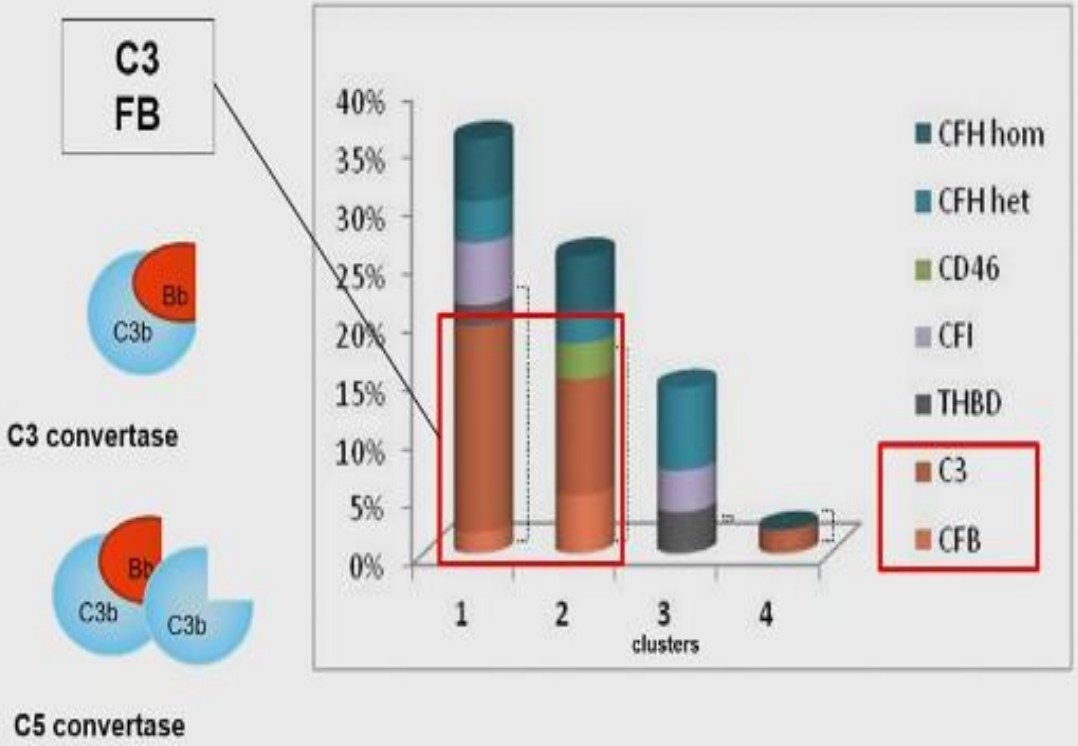
- Must be validated
- The ultimate test of the utility of this approach in the clinic will be if it can also identify groups of patients who will benefit from distinct complement-modulating therapies

Validation of pathogenic patterns in a novel cohort of patients with membranoproliferative glomerulonephritis by cluster analysis

"Our results confirm the main findings of the original cluster analysis and indicate that the observed distinct pathogenic patterns replicate in our cohort"

Garam et al., *Clinic Kidney J*, 2020

C3 AND CFB MUTATIONS ARE MOSTLY PRESENT IN CLUSTERS 1 AND 2



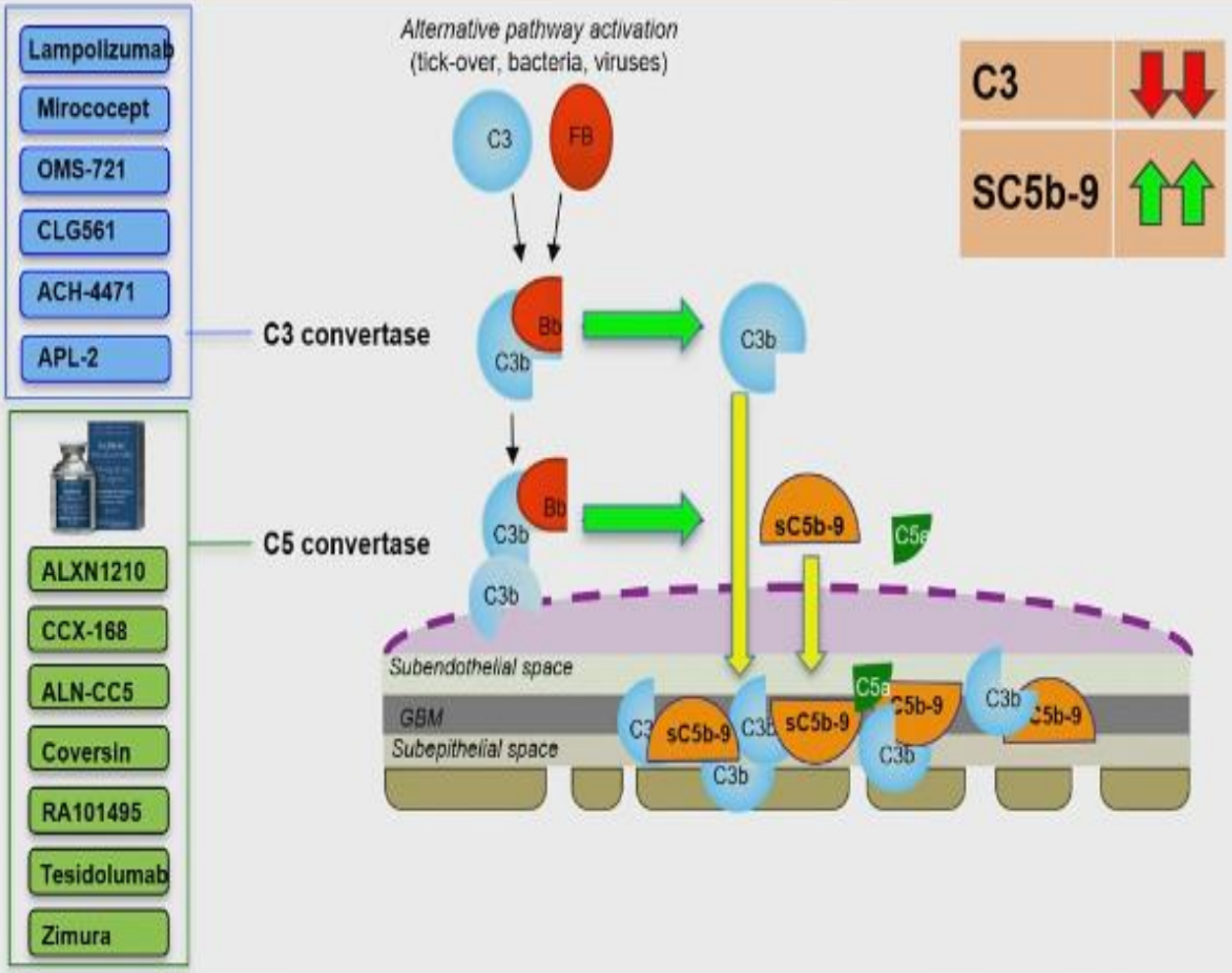
Clusters 1 and 2

C3	↓↓
SC5b-9	↑↑

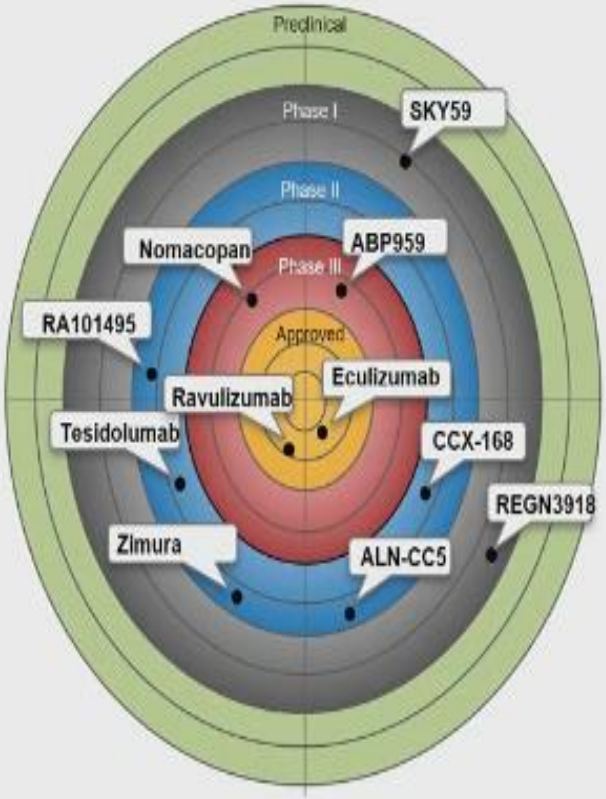
C3 and C5 convertases are both dysregulated in fluid phase

Iatropoulos P *et al.*, *JASN*, 2018

IN CLUSTERS 1 AND 2, C3 AND C5 ALTERNATIVE PATHWAY CONVERTASES ARE BOTH DYSREGULATED



Terminal complement inhibitors



6 complement-targeted drug candidates in preclinical

Eculizumab: anti-C5 Ab blocks C5 cleavage
(Alexion) **aHUS, PNH, C3G, AMR, Myastenia gravis**

Ravulizumab: anti-C5 Ab blocks C5 cleavage long half-life
ALXN1210 *(Alexion)* **PNH**

ABP959: Anti-C5 antibody
(Amgen) **PNH, aHUS**

Avacopan: C5aR antagonist (CCX-168),
(Chemocentrix) **aHUS, Vasculitis, C3GN**

Nomacopan: Small C5 inhibitor,
Coversin *(Akari)* **PNH, aHUS, *Post-BMT TMA***

ALN-CC5: siRNA Targeting C5
(Alnylam) **PNH, aHUS**

RA101495: cyclic peptide, C5 inhibitor
(RaPharma) **PNH, AMD, TAM, Uveitis**

Tesidolumab: Anti-C5 antibody
(Novartis) **PNH, AMD, TAM, Uveitis**

Zimura: aptamer-based C5 inhibitor
(Ophthotech) **IPCV, AMD**

REGN3918: Anti-C5 antibody
(Regeneron) **PNH**

SKY59: Anti-C5 antibody
(Roche) **PNH**

EAGLE Study

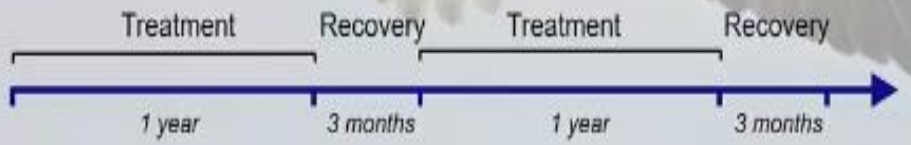
Evaluating the Morphofunctional Effects of Eculizumab* Therapy in Primary Membranoproliferative Glomerulonephritis:

A Pilot, Single Arm Study in 10 Patients with Persistent Heavy Proteinuria and low C3 levels and high sC5b9 levels (>1000 ng/ml)

Clusters 1 and 2

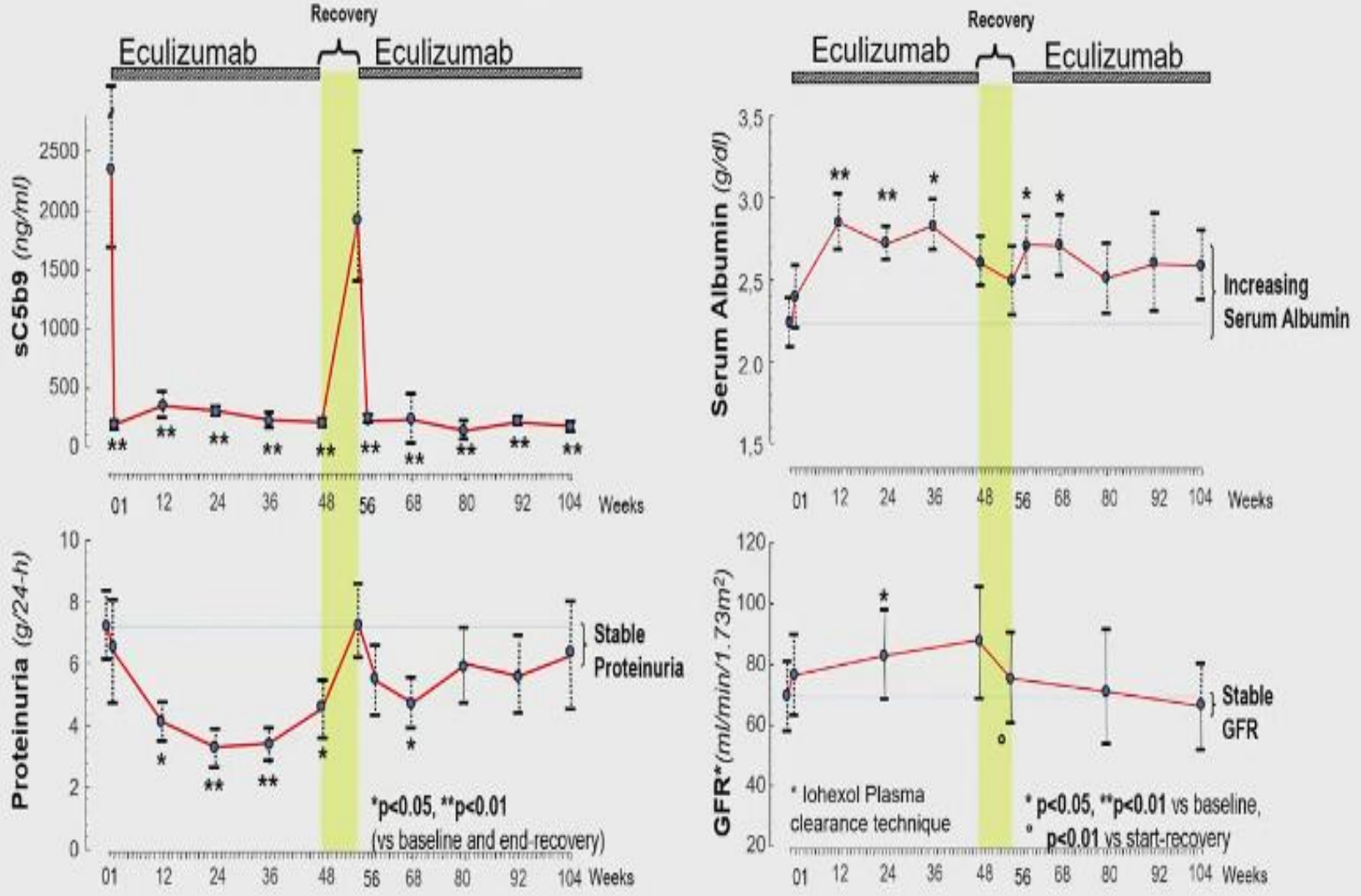
Elena Mondo, Piero Ruggenenti, Erica Daina, Marina Noris, Elena Bresin and Giuseppe Remuzzi

Unit of Nephrology, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy
Clinical Research Center for Rare Diseases "Aldo e Cele Daccò",
Mario Negri Institute for Pharmacological Research, Bergamo, Italy



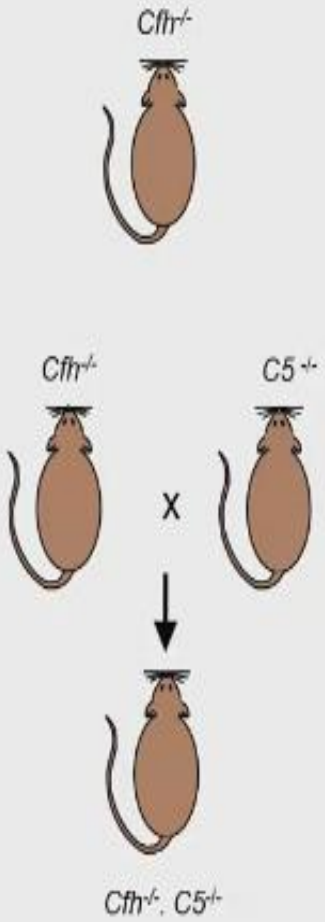
* 900 mg weekly for four infusion and maintenance phase 1200 mg at week 5; then 1200 mg every 2 weeks for 1 years

IN PATIENTS OF CLUSTERS 1 AND 2 ECULIZUMAB DECREASES DISEASE SEVERITY BUT DOES NOT ABOLISH GLOMERULAR C3 DEPOSITION



Ruggenenti et al., *Am J Kidney Dis*, 2019

IN CFH$^{-/-}$ MICE C5 DEFICIENCY DECREASES DISEASE SEVERITY BUT DOES NOT ABOLISH GLOMERULAR C3 DEPOSITION



Murine model of spontaneous C3G

Cfh$^{-/-}$ mice have:

- low plasma C3 levels,
- glomerular C3 and C5b-9 deposits
- electron-dense GBM changes
- glomerular inflammation
- albuminuria

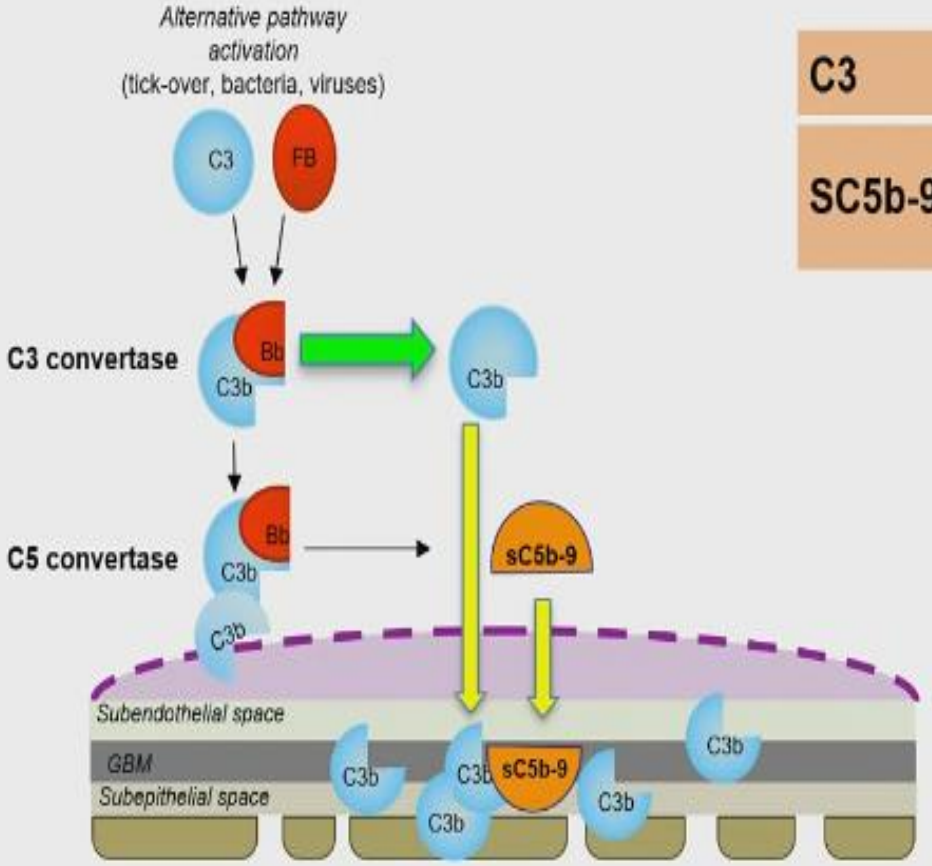
In *Cfh$^{-/-}$* mice deficiency of C5 was associated with:

- ↓ glomerular cellularity
- ↓ mortality
- ↓ albuminuria

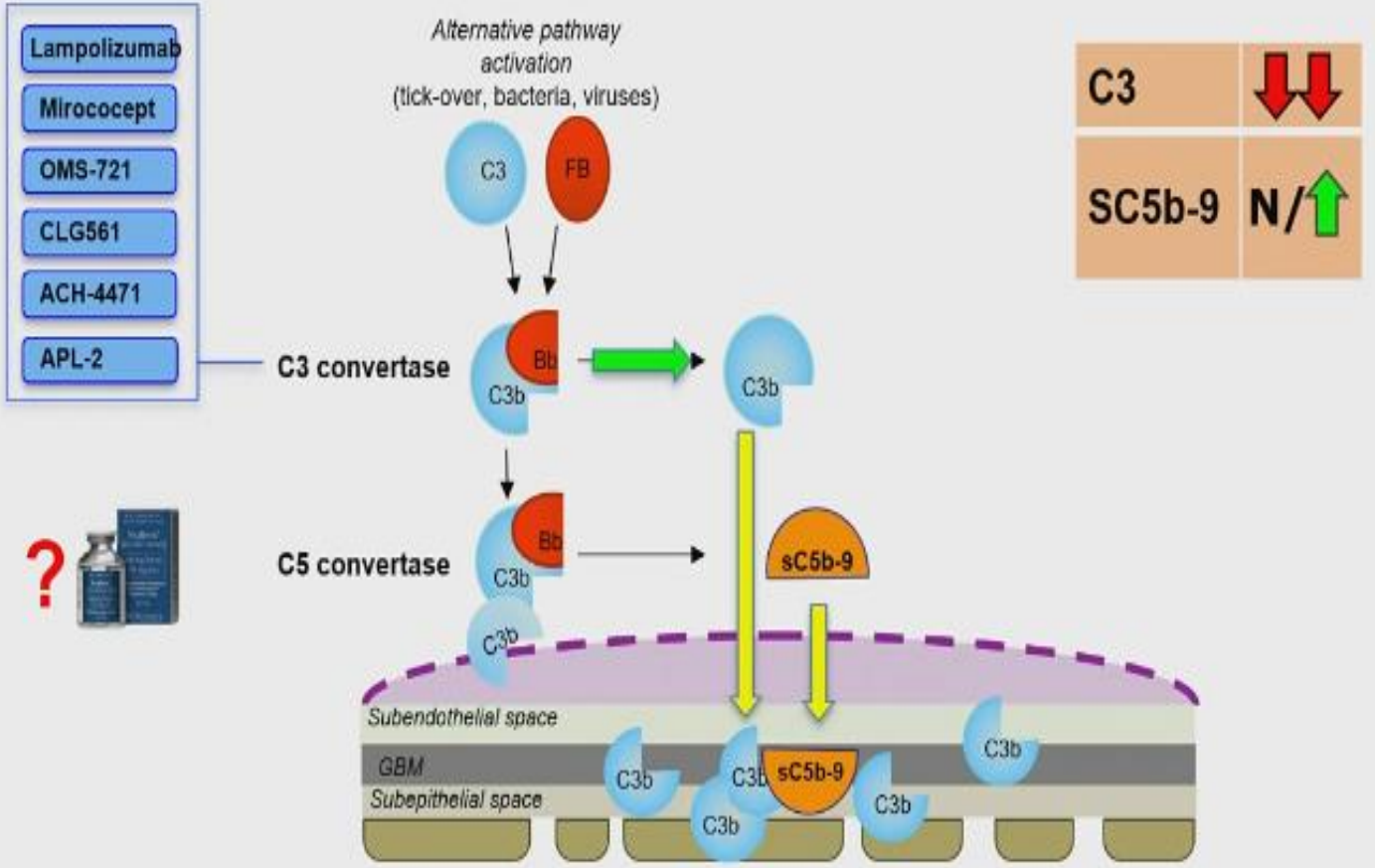
compared to *Cfh$^{-/-}$* mice

Pickering, *PNAS*, 2006

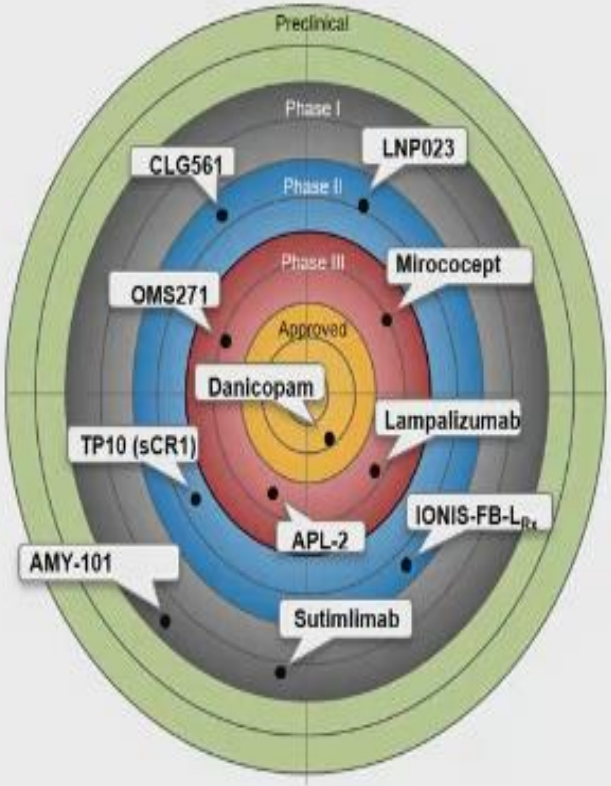
IN CLUSTER 3 THERE IS A PREVALENT C3 CONVERTASE FORMATION THEN STABILIZED BY HIGH FREQUENCY OF C3NEF



IN CLUSTER 3 THERE IS A PREVALENT C3 CONVERTASE FORMATION THEN STABILIZED BY HIGH FREQUENCY OF C3NEF



Upstream complement inhibition



5 complement-targeted drug candidates in pre-clinical

Danicopan Small FD inhibitor, (ACH-4471) + eculizumab
(Achillion) **PNH**

Narsoplimab: anti-MASP2 antibody
OMS271 *(Omeros)* **aHUS, post-BMT TMA**

Mirococept: membrane localized inhibitor derived from CR1: blocks C3 convertase
(Inflazyme Pharmaceutical) **I/R injury**

Lampalizumab: Anti-FD antibody
(Roche) **Ocular diseases**

APL-2 cyclic peptide inhibitor of C3
(Apellis) **PNH, G3G**

TP10: soluble CR1 (blocks C3 convertase)
(Avant Immunotherapeutics) **MI**

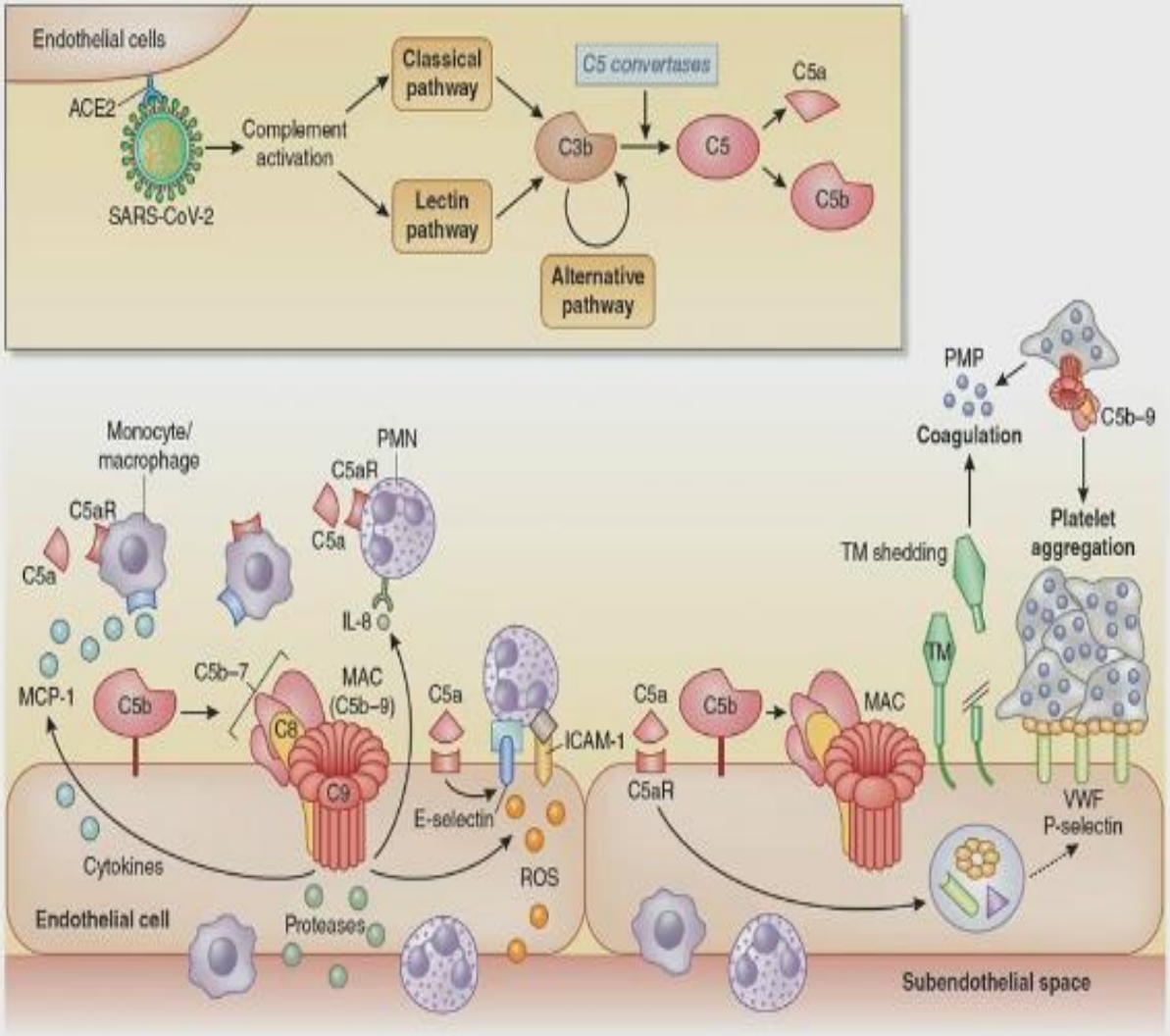
LNP023: anti-FB antibody, blocks the alternative pathway C3 convertase
(Novartis) **PNH**

CLG561: anti-properdin antibody, reduces the formation of the alternative pathway C3 and C5 convertases
(Novartis) **Ocular diseases**

IONIS-FB-L$_{Rx}$: antisense oligonucleotide targeting hepatic expression of FB
(Ionis/Roche) **Ocular diseases**

AMY-101: peptide, blocks C3 activation
(Amyndas) **PNH, C3G**

Sutimlimab: anti-C1s antibody
(Sanofi) **Cold Agglutinin Disease**



Noris et al., The case of Complement Activation in COVID-19 multiorgan impact, *Kidney Int*, in press