ERKNet
The European Rare Kidney Disease Reference Network

european society for paediatric nephrology

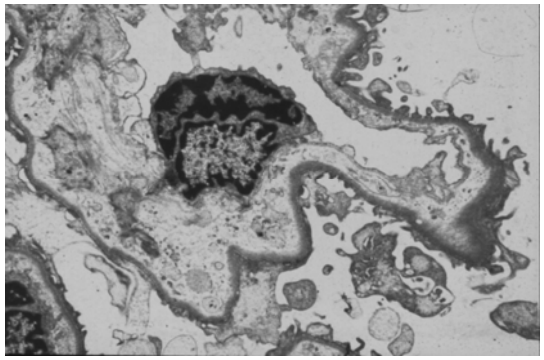
WELCOME TO

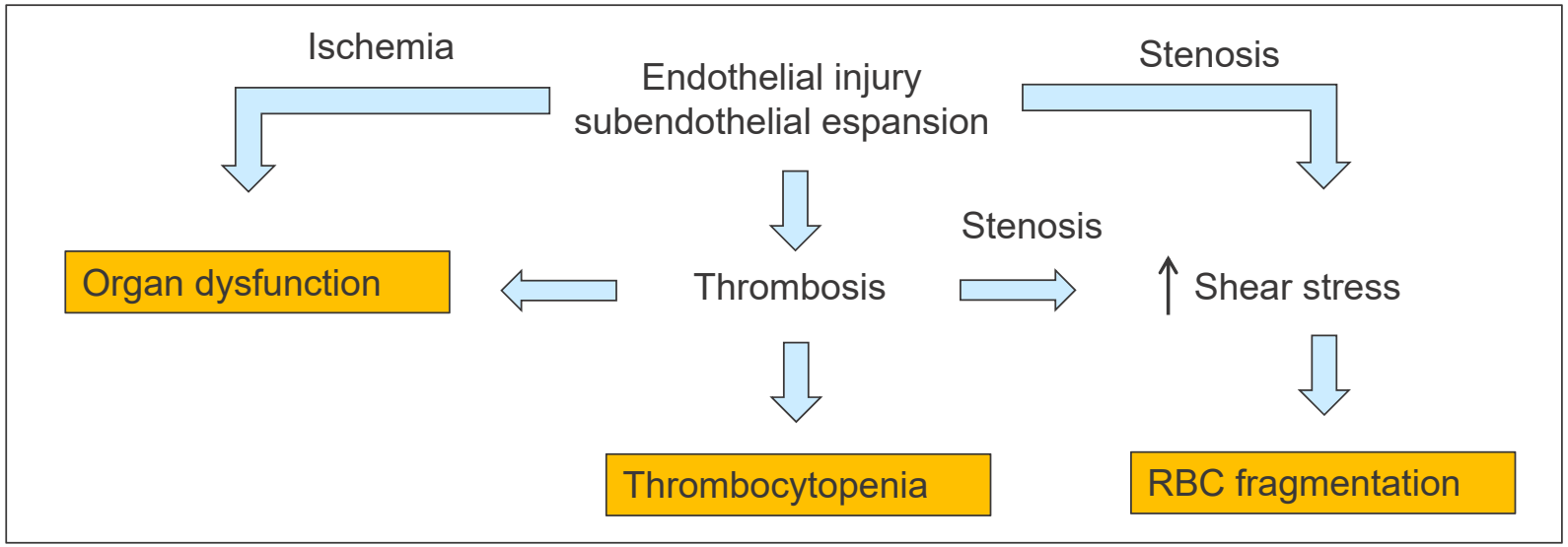
ESPN/ERKNet Educational Webinars on Pediatric Nephrology & Rare Kidney Diseases

- **Date:** 01 June 2021
- **Topic:** Atypical Hemolytic Uremic Syndrome
- **Speaker:** Marina Noris
- **Moderator:** Elena Levtchenko

HEMOLYTIC UREMIC SYNDROME

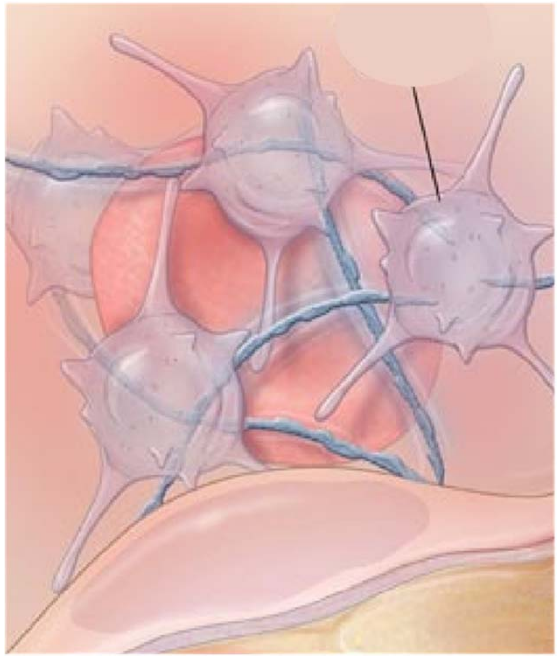
Histology lesions: Swelling and detachment of endothelial cells, accumulation of fluffy material in the subendothelium, thrombi and obstruction of the vessel lumina.





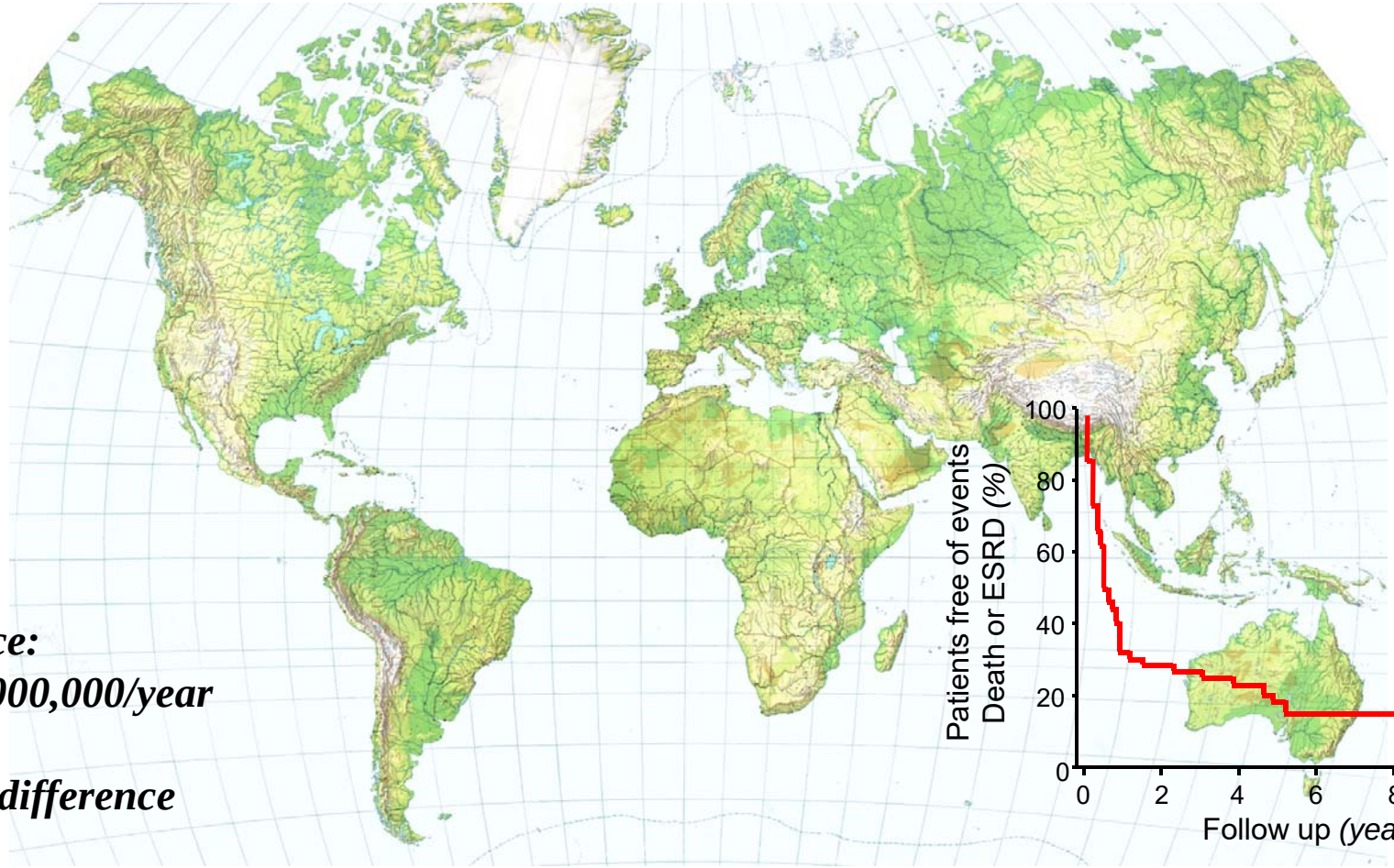
Ruggenenti and Remuzzi, Kidney Int 2001

dHUS	**STEC**	*Adhesion molecules and P-selectin up-regulation*
aHUS	**Comp Gene mutation**	*Defective complement regulation*
TTP	**ADAMTS13**	*UL-VWF multimers*
sHUS	**Autoimmune or systemic diseases, tx, infections glomerulopathies,**	*Complement activating conditions*



Microthrombi

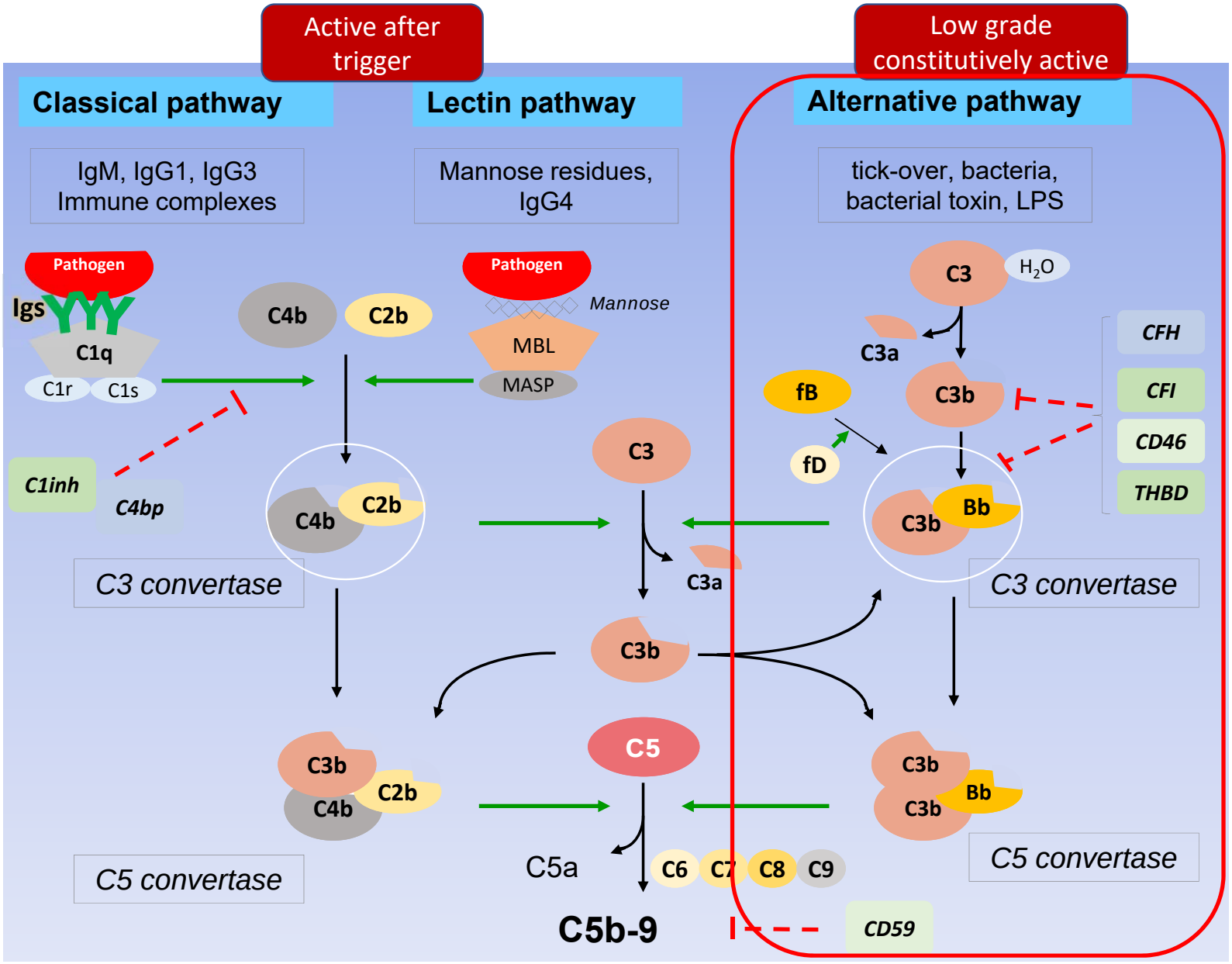
ATYPICAL HEMOLYTIC UREMIC SYNDROME



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

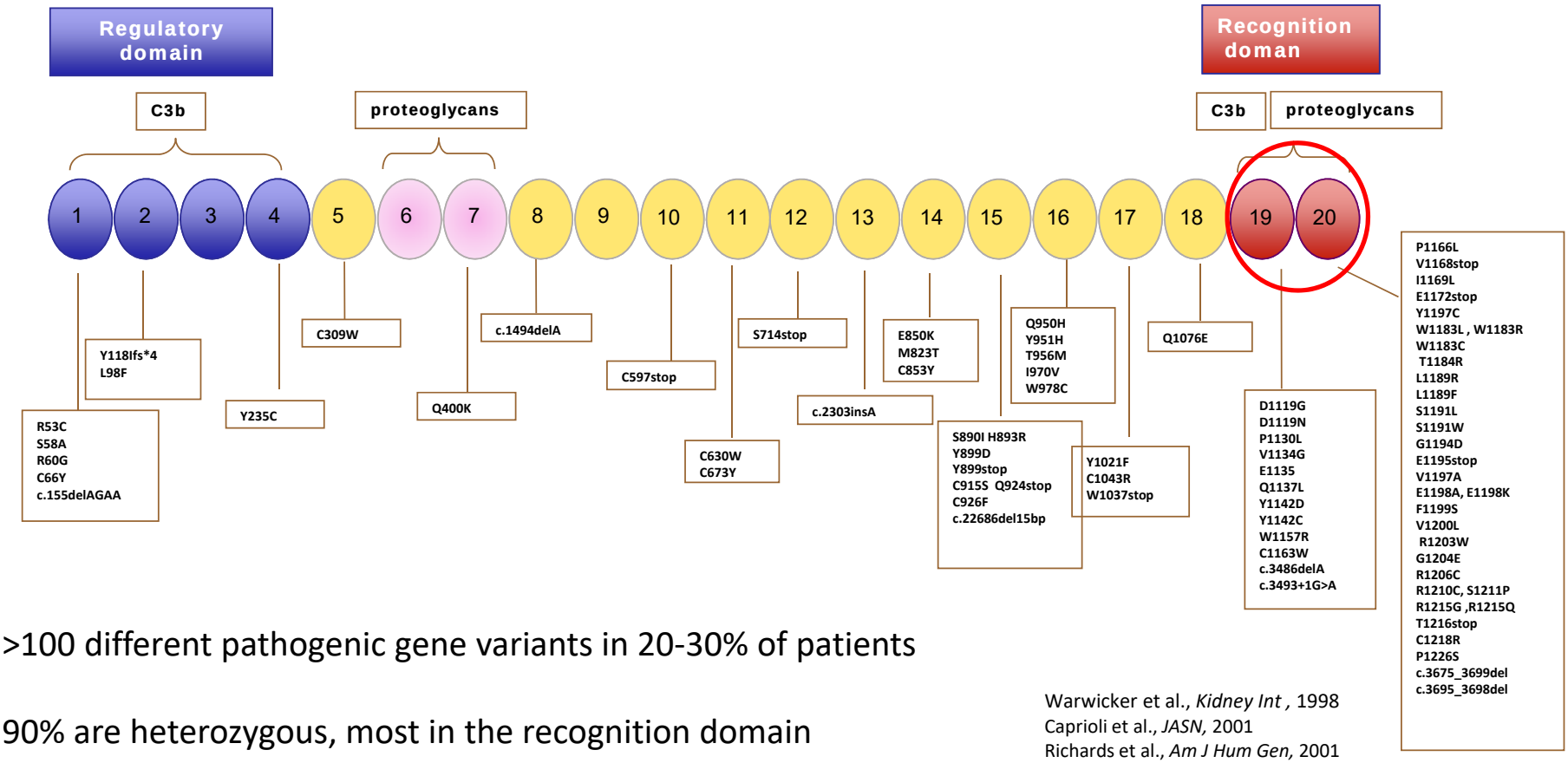
Sex: no difference

A life threatening multisystem disease of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure with predominant but not exclusive renal involvement.


Active after trigger
Low grade constitutively active
Classical pathway
Lectin pathway
Alternative pathway
IgM, IgG1, IgG3 Immune complexes
Mannose residues, IgG4
tick-over, bacteria, bacterial toxin, LPS
Pathogen
Igs
C1q
C1r
C1s
C4b
C2b
Pathogen
Mannose
MBL
MASP
C3
H2O
C3a
fB
fD
C3b
Bb
CFH
CFI
CD46
THBD
C1inh
C4bp
C4b
C2b
C3 convertase
C3
C3a
C3b
C3b
Bb
C3 convertase
C5
C3b
C4b
C2b
C5a
C6
C7
C8
C9
C5b-9
C3b
C3b
Bb
C5 convertase
C5 convertase
CD59

Factor H plays a pivotal role in the regulation of the alternative pathway of complement activation

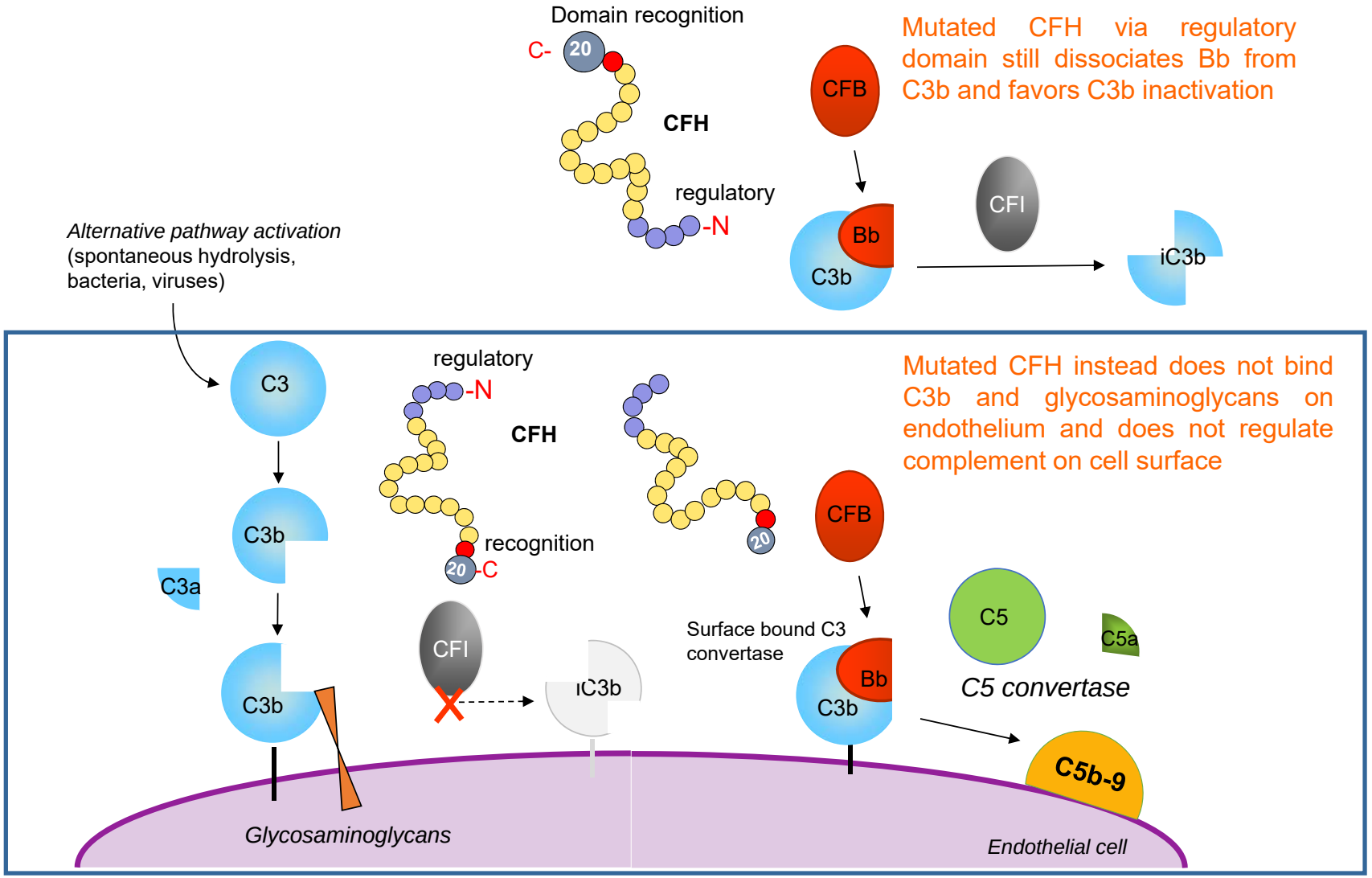
Produced mainly in the liver as a single peptide glycoprotein, factor H circulates in plasma at a concentration of 50 mg/dl



- >100 different pathogenic gene variants in 20-30% of patients
- 90% are heterozygous, most in the recognition domain

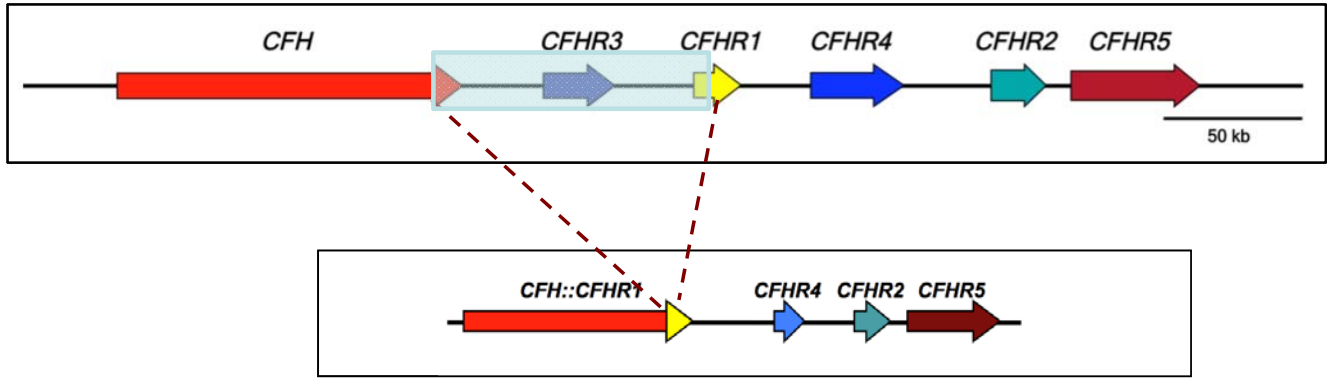
Warwicker et al., *Kidney Int* , 1998
Caprioli et al., *JASN,* 2001
Richards et al., *Am J Hum Gen,* 2001
Perez-Caballero et al., *Am J Hum Gen,* 2001
Dragon-Durey et al., *J Am Soc Nephrol,* 2004
Caprioli et al., *Blood*, 2006
Noris et al, CJASN 2010

SOLID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN aHUS



Manuelian et al., *JCI,* 2003
Sanchez-Corral et al., *Am J Hum Gen,* 2002
Heinen et al., *JASN,* 2007
Noris et al, Blood 2014
Merinero HM et al, Kidney Int 2018

HIGH HOMOLOGY IN THE REGULATORS OF COMPLEMENT ACTIVATION GENE CLUSTER



***CFH/CFHR1* hybrid gene**

Venables et al., *Plos Medicine,* 2006

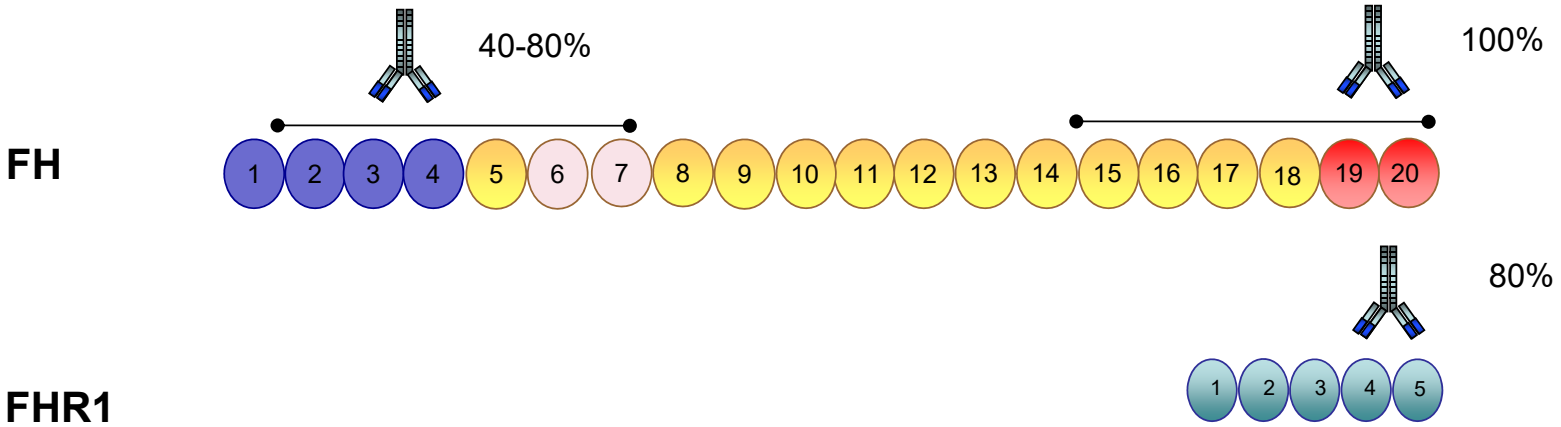
- High degree of sequence identity between the gene for factor H and the genes for the five factor H-related proteins (CFHR1 to 5) which favors non-allelic homologous recombinations giving rise to hybrid genes.
- Copy number variation assays (CGH arrays or MLPA) are required to detect hybrid genes
- Identified in 34/485 aHUS patients (7%).

Piras et al., *Frontiers in Medicine,* 2020

FH/FHR HYBRID PROTEINS FOUND IN PATIENTS WITH aHUS

In the FH/FHR hybrid molecules the C-terminal SCRs of CFH are substituted with those of FHR1 or with the entire FHR3, resulting in decreased complement regulatory activity on endothelial cell surface

ANTI-FH AUTOANTIBODIES IN AHUS



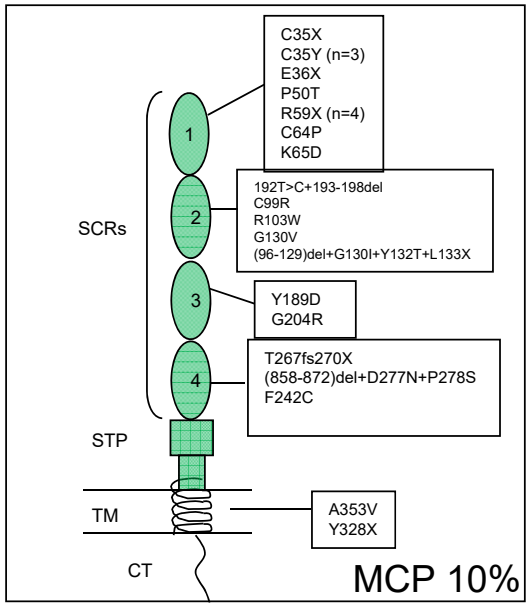
8-9% mostly children

Dragon-Durey et al JASN 2005
Blanc et al., *J Immunol,* 2012

- Anti-FH abs mainly target the FH C-terminus
- Most patients with aHUS and FH autoantibodies are homozygous for a deletion of genes encoding FH related proteins 1 and 3.
- *Failure of central and/or peripheral tolerance to FH related 1 and FH?*

Zipfel et al, *Plos Genetics,* 2007
Jozsi et al, *Blood,* 2008
Trojnar et al, *Front Immunol*, 2017

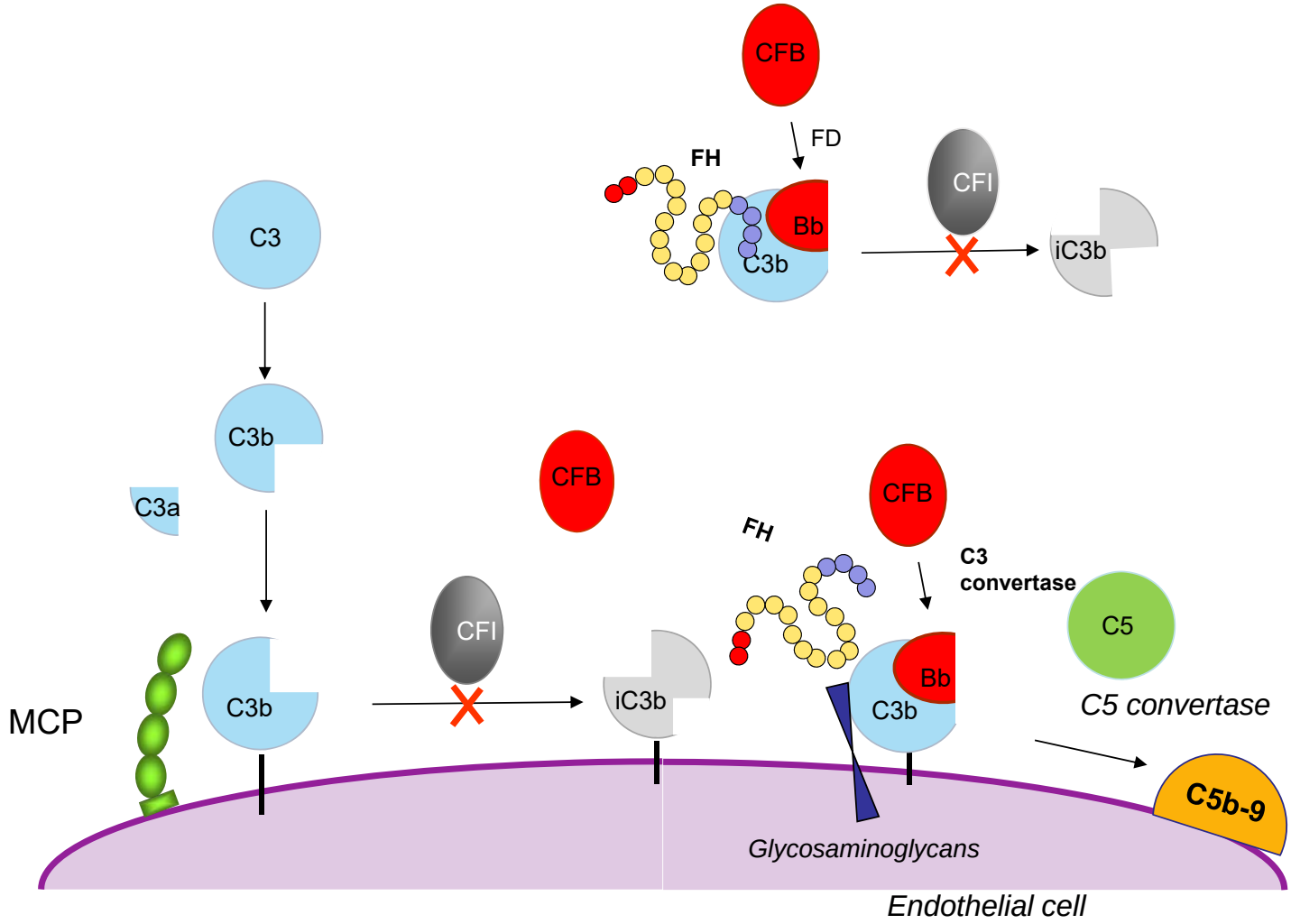
MCP VARIANTS IN aHUS



Loss of function heterozygous variants: low expression or reduced C3b binding and cofactor activity

Noris et al, Lancet 2003
Richards et al, PNAS 2003
Caprioli et al, Blood 2006

C3 AND CFB GAIN OF FUNCTION VARIANTS IN aHUS

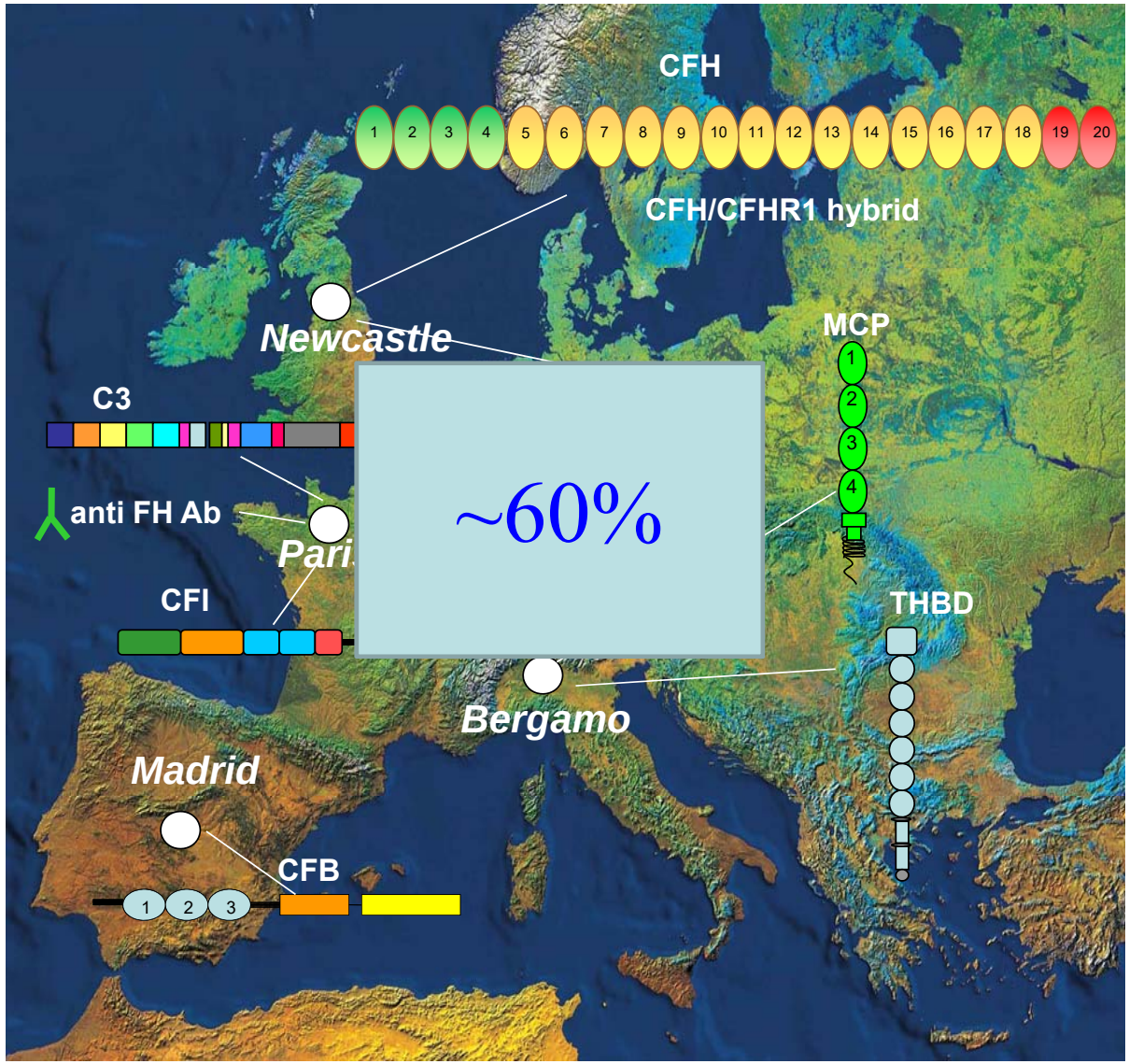


C3 7-10%

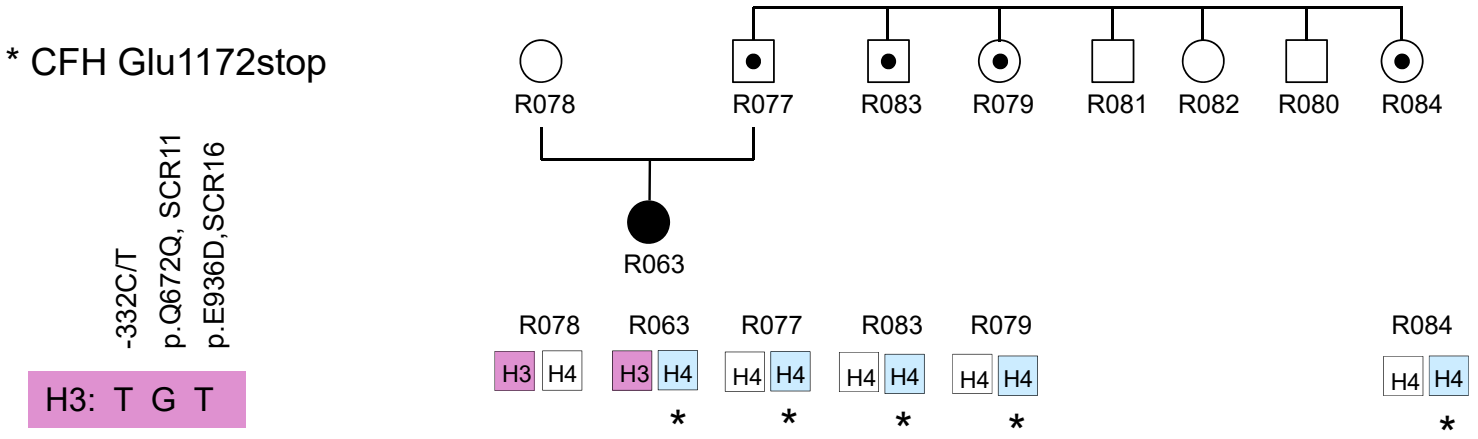
CFB: 1-2%

Goicoechea et al., *PNAS,* 2007
Roumenina et al., *Blood,* 2009
Fremeaux-Bacchi et al, Blood 2008

GENETICS OF AHUS: AN EUROPEAN DISCOVERY



THE CASE OF GENE POLYMORPHISMS IN CARRIERS OF PATHOGENETIC VARIANTS TO EXPLAIN INCOMPLETE DISEASE PENETRANCE



Caprioli et al., *Hum Molec Genet,* 2003

•Among 21 families with CFH pathogenetic variants (26 patients with aHUS and 47 unaffected carrier relatives) the H3 haplotype on wild-type allele was more prevalent in affected (69%) than in unaffected (17%) subjects.

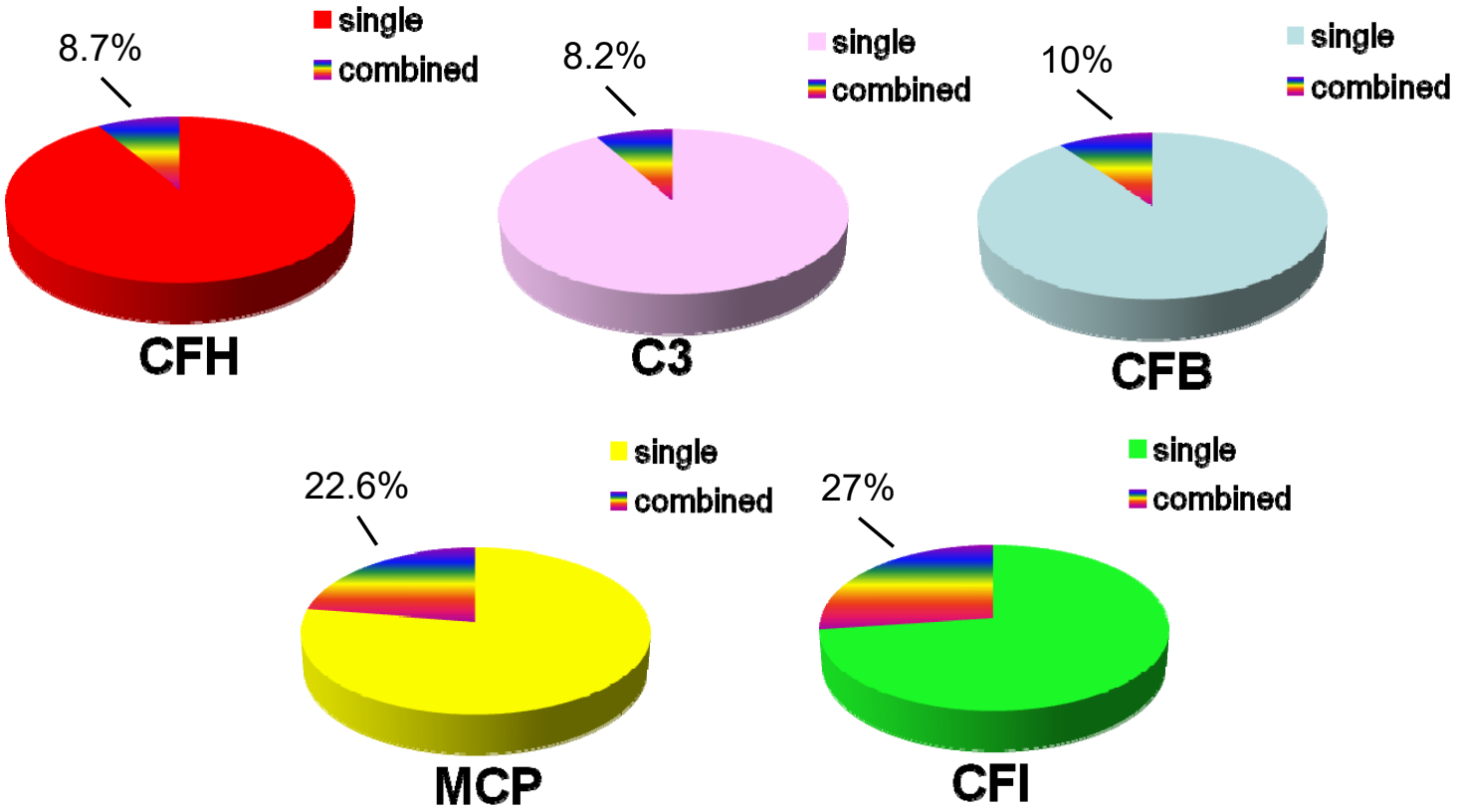
Breno M et al., *unpublished*

• In 80 pedigrees with a mutation in complement genes (CFH, or MCP or C3 or CFB) aHUS penetrance was 60% in subjects who also carried the CFH-H3 and the MCPggaac risk haplotypes, while only 18% of mutation carriers with neither risk haplotypes developed the disease.

Arjona E et al., *Blood 2020*

COMBINED COMPLEMENT GENE ABNORMALITIES IN aHUS

795 patients from 4 European cohorts



Combined complement gene abnormaties were found in around 10% of patients with CFH or C3 or CFB pathogenetic variants, and about 25% of patients with MCP or CFI pathogenetic variants.

Bresin et al, *JASN* 2013

Trigger

C3

C3b

C3a

CFB

CFH

CFI

C3 convertase

C3

C5 convertase

C5

C5a

C5b

C5b-9

TM

TM shedding

MCP

Bb

C3b

Bb

C3b

C3b

Eculizumab

A humanized monoclonal antibody that binds to C5

PMP

CD40L

Coagulation

Platelet aggregation

VWF

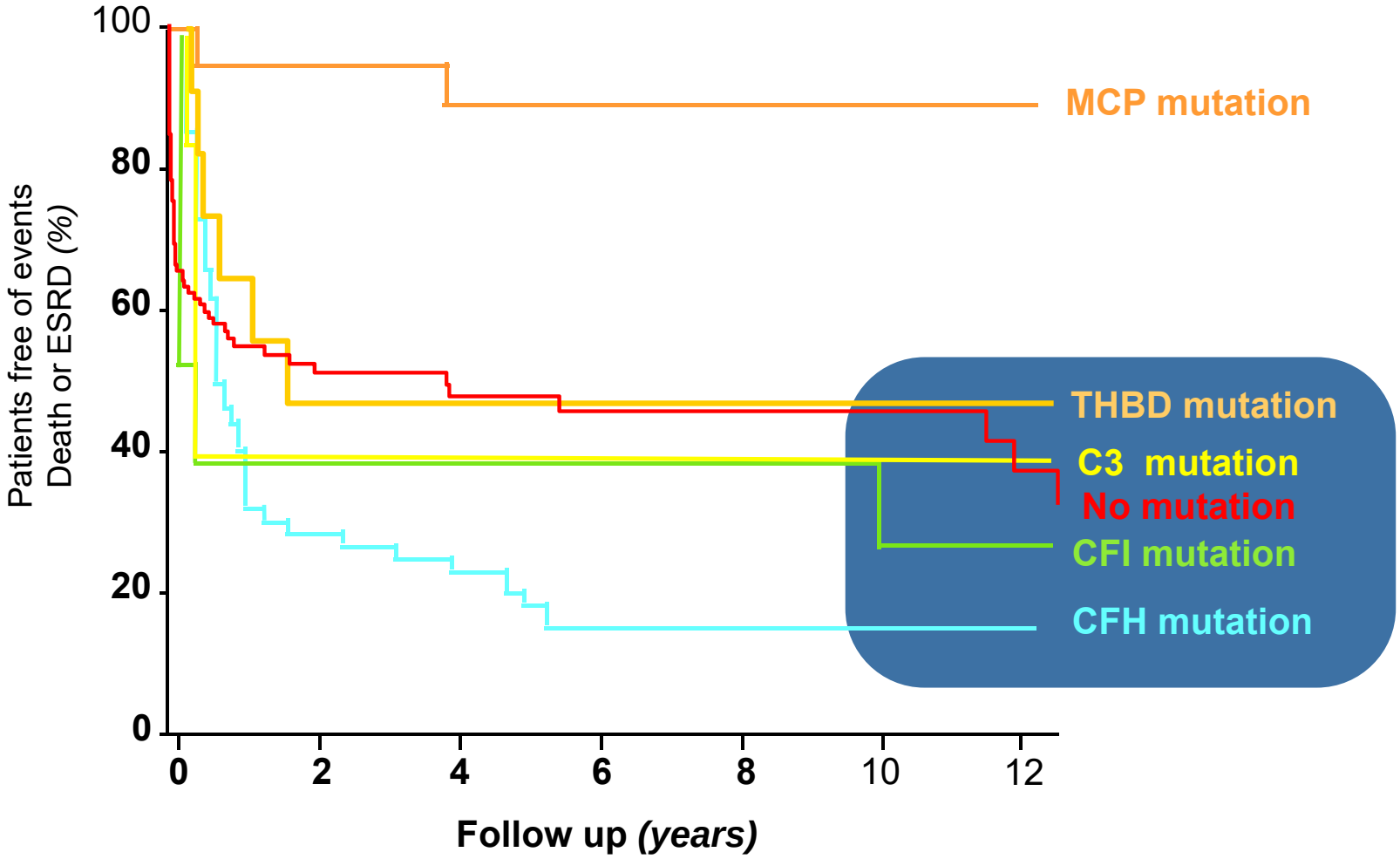
P-sel

Endothelial cell

Endothelial cell

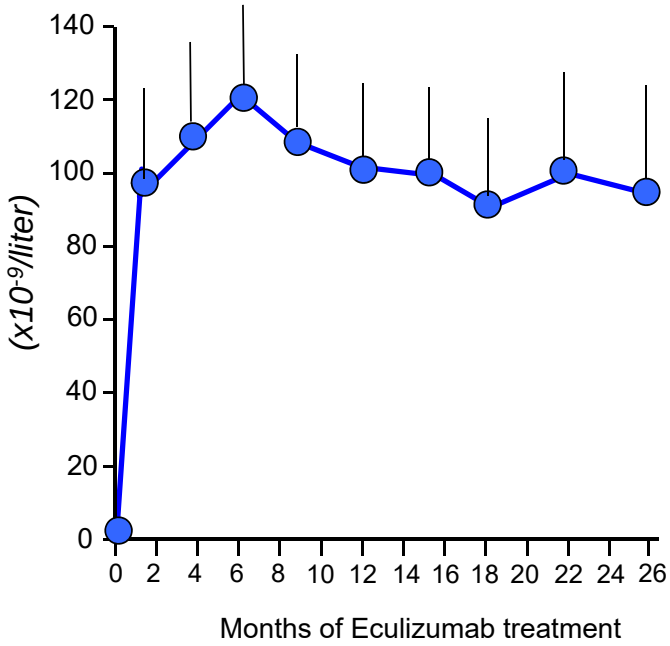
Modified from Noris and Remuzzi, *NEJM,* 2009

LONG TERM OUTCOME OF aHUS PATIENTS

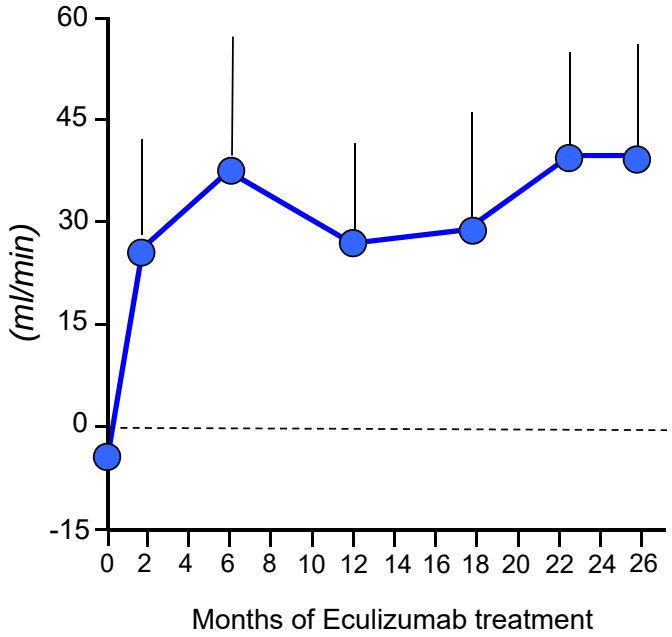


Noris et al CJASN 2010

Platelet count
mean change from baseline



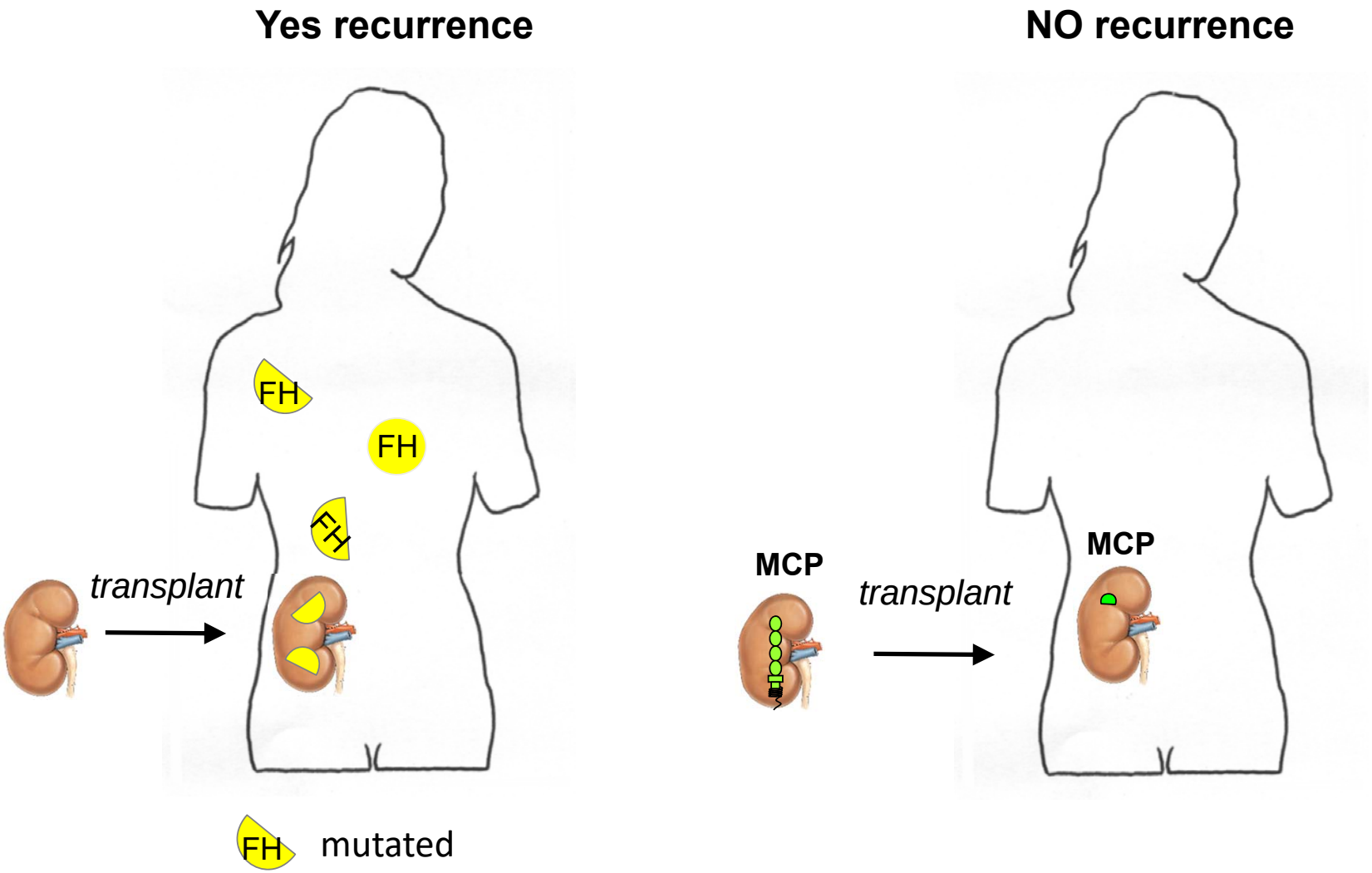
Estimated GFR
mean change from baseline



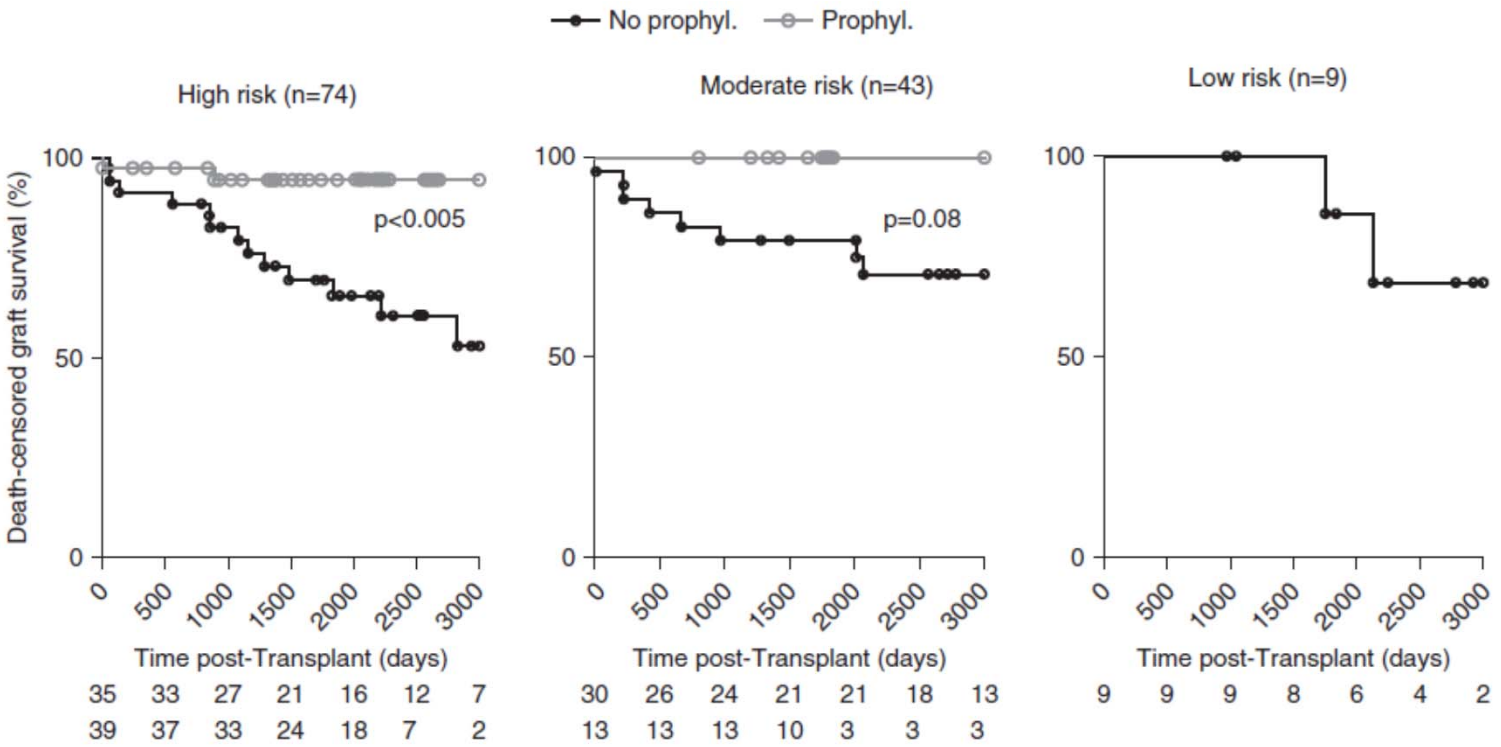
Treatment effect was sustained for up to 26 months

Licht et al., *Kidney Int,* 2015

THE RISK OF POST-TRANSPLANT RECURRENCE DEPENDS ON THE GENETIC ABNORMALITY



ECULIZUMAB TREATMENT TAILORING BASED ON THE RISK STRATIFICATION IMPROVED GRAFT SURVIVAL IN THE HIGH RISK GROUP



- High risk: recurrence in a previous graft, and/or pathogenic variants of CFH/C3/CFB
- Moderate risk: negative complement screening results or a pathogenic variant in CFI
- Low risk: isolated variants in MCP or anti-CFH autoantibody no longer detected at the time of transplantation

Zuber et al., *JASN*, 2019

•A 26-year old male presents with suspicion of TMA after 1 week of vomiting and progressive asthenia:

- •Platelets: 39 x10^3/µL
- •LDH: 1900 IU/L
- •Hemoglobin: 8.2 g/dL
- •Creatinine: 2.7 mg/dL
- •ADAMTS13: >10%
- •STEC tests: negative

•Serum C3, and C4 levels: normal

•Plasma C5b-9 levels: normal

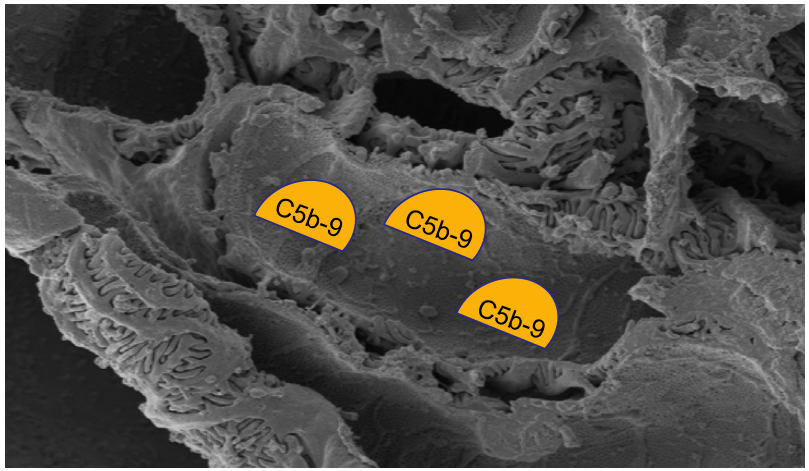
•Can this be aHUS?

- •No, because circulating complement parameters are normal
- •Yes, could be, but further tests are needed ⬅

Dynamics of complement activation in aHUS and how to monitor eculizumab therapy

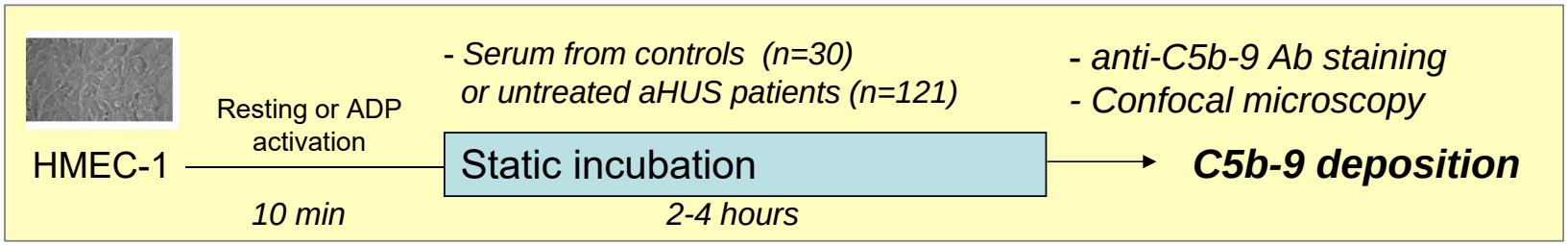
Marina Noris,[1] Miriam Galbusera,[1] Sara Gastoldi,[1] Paolo Macor,[2] Federica Banterla,[1] Elena Bresin,[1] Claudio Tripodo,[3] Serena Bettoni,[1] Roberta Donadelli,[1] Elisabetta Valoti,[1] Francesco Tedesco,[4] Alessandro Amore,[5] Rosanna Coppo,[5] Piero Ruggenenti,[6] Eliana Gotti,[6] and Giuseppe Remuzzi[1,6]

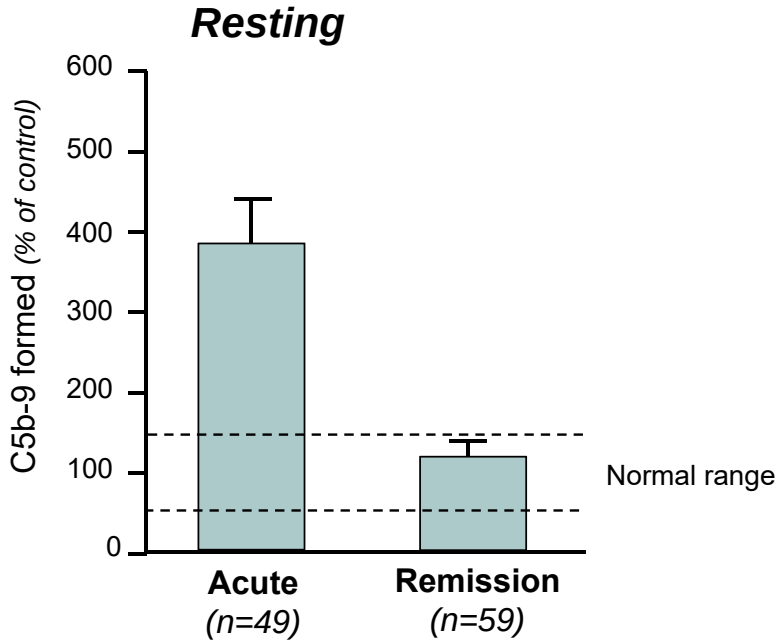
BLOOD, 11 SEPTEMBER 2014

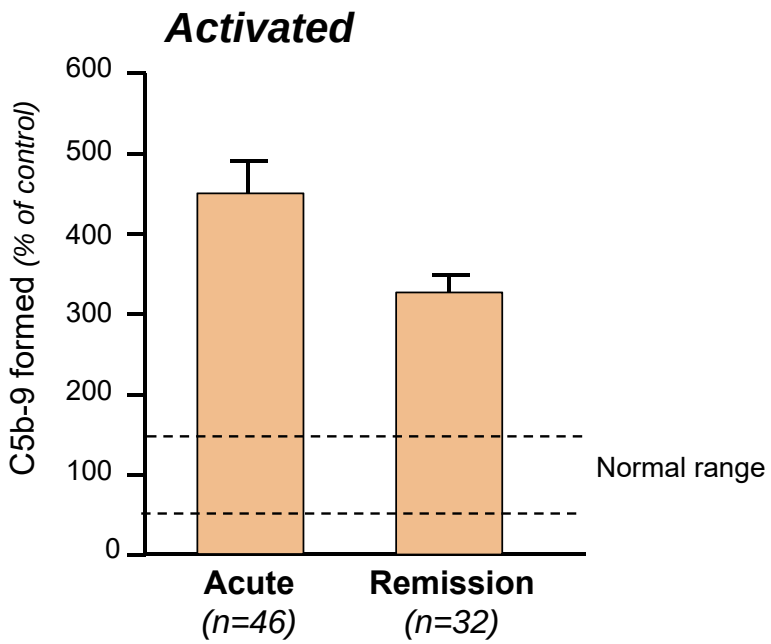


Both during the acute phase of the disease and at remission about half of aHUS patients had normal serum C3 and plasma sC5b-9 levels

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





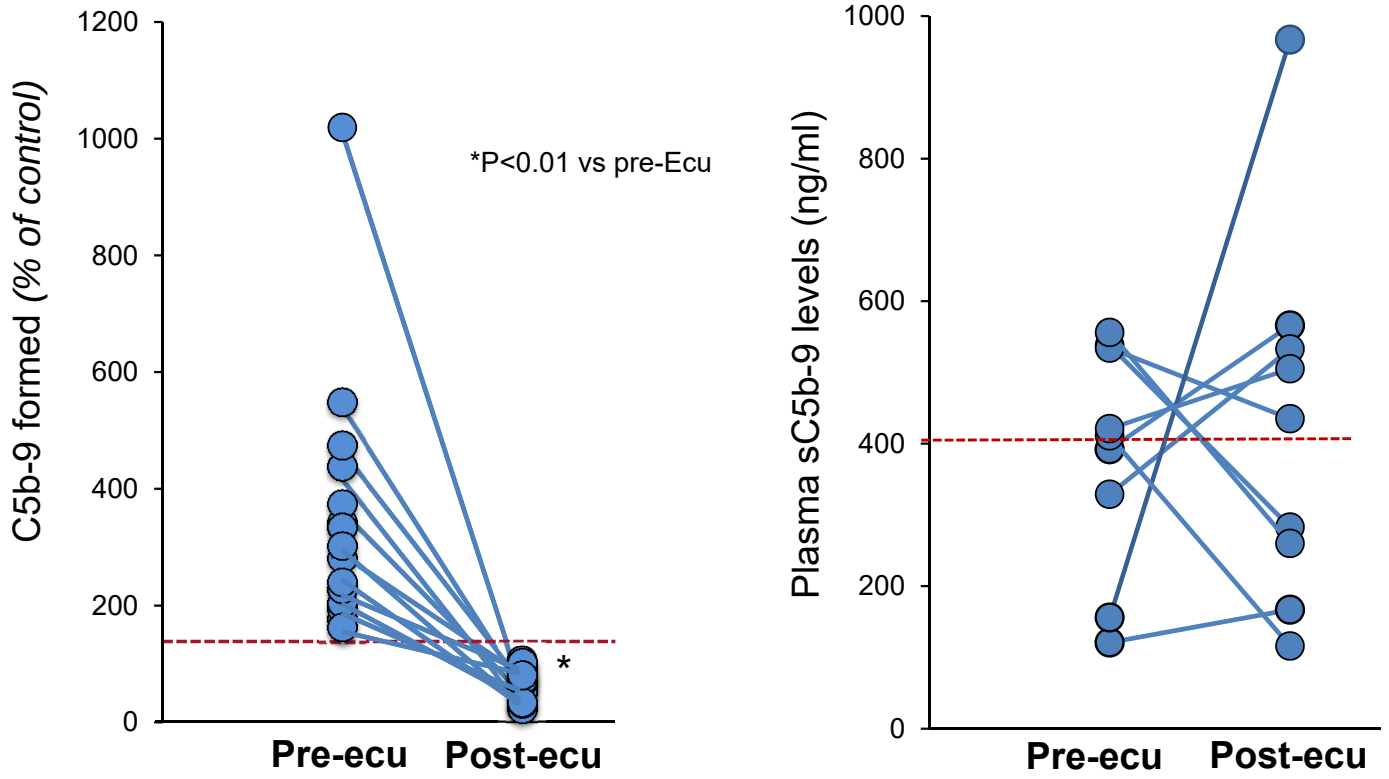


Resting : disease activity (sensitivity 100%, specificity 97%)

Activated: complement dysregulation (sensitivity 100 %)

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

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

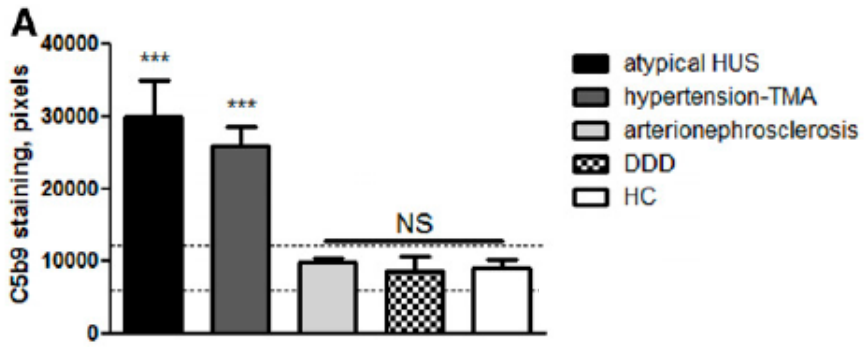


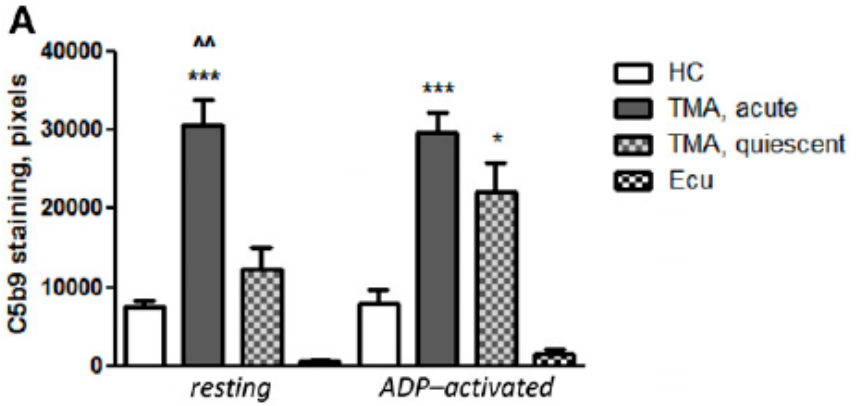
- *In 20 aHUS cases treated with Eculizumab, serum-induced C5b-9 deposits on ADP-activated HMEC-1 ex-vivo normalized after treatment*
- *No significant change was observed in pre- and post-Eculizumab plasma sC5b-9 levels*

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

The assay must be reproduced by other specialized laboratories so that clinicians could consider using it

SERUM FROM PATIENTS WITH HYPERTENSION-ASSOCIATED HUS INDUCED C5b-9 DEPOSITS ON HMEC-1





- Compared with serum from healthy controls (HC), serum from patients with aHUS or hypertension-associated HUS induced abnormal C5b-9 formation on HMEC-1. C5b-9 deposits normalized after eculizumab treatment.

Timmermans S et al *JASN,* 2018

- Either serum or activated plasma from patients with acute aHUS induced more C5b-9 deposition on HMEC-1 than control sera.

- C5b-9 deposits were at control levels or lower with activated-plasma from aHUS patients treated with eculizumab

Palomo M et al, C*JASN,* 2019

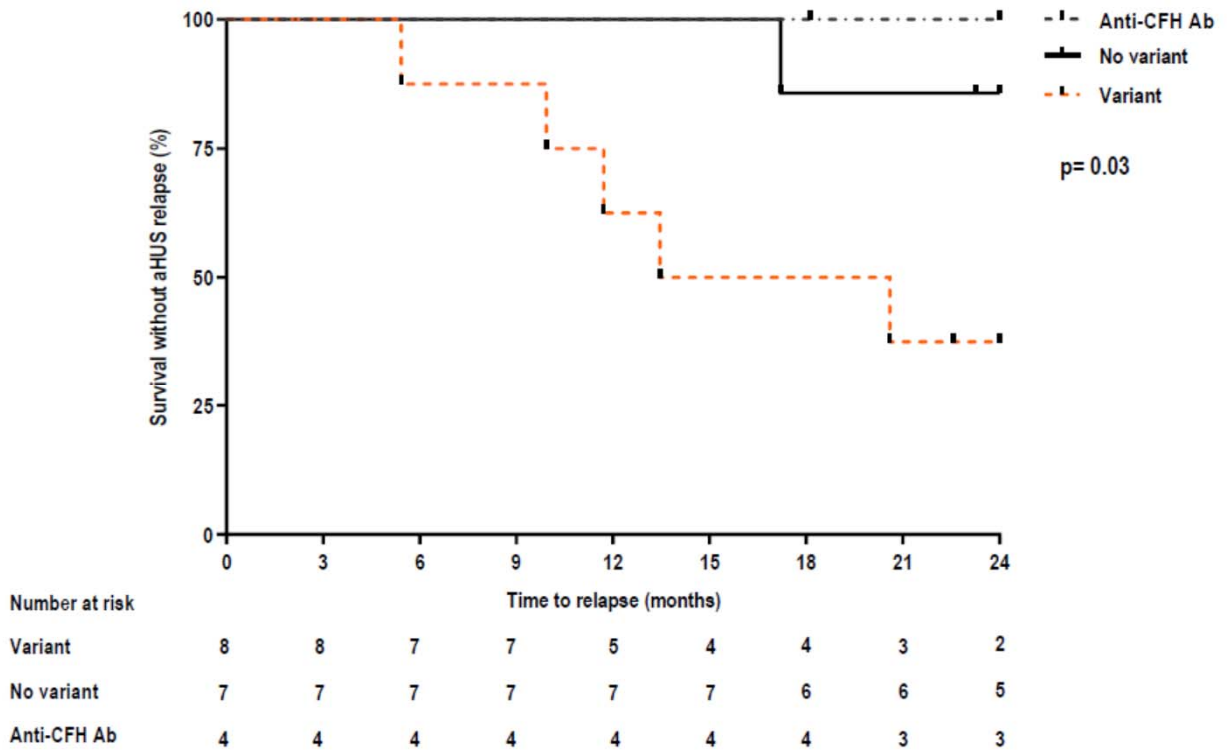
Every 15 days forever?

How to monitor and possibly tapering?

RISK OF AHUS RELAPSE AFTER ECULIZUMAB DISCONTINUATION: A PROSPECTIVE NATIONAL MULTICENTRIC OPEN-LABEL STUDY

55 patients (19 children and 36 adults) discontinued eculizumab (mean duration of treatment, 16,5 months)

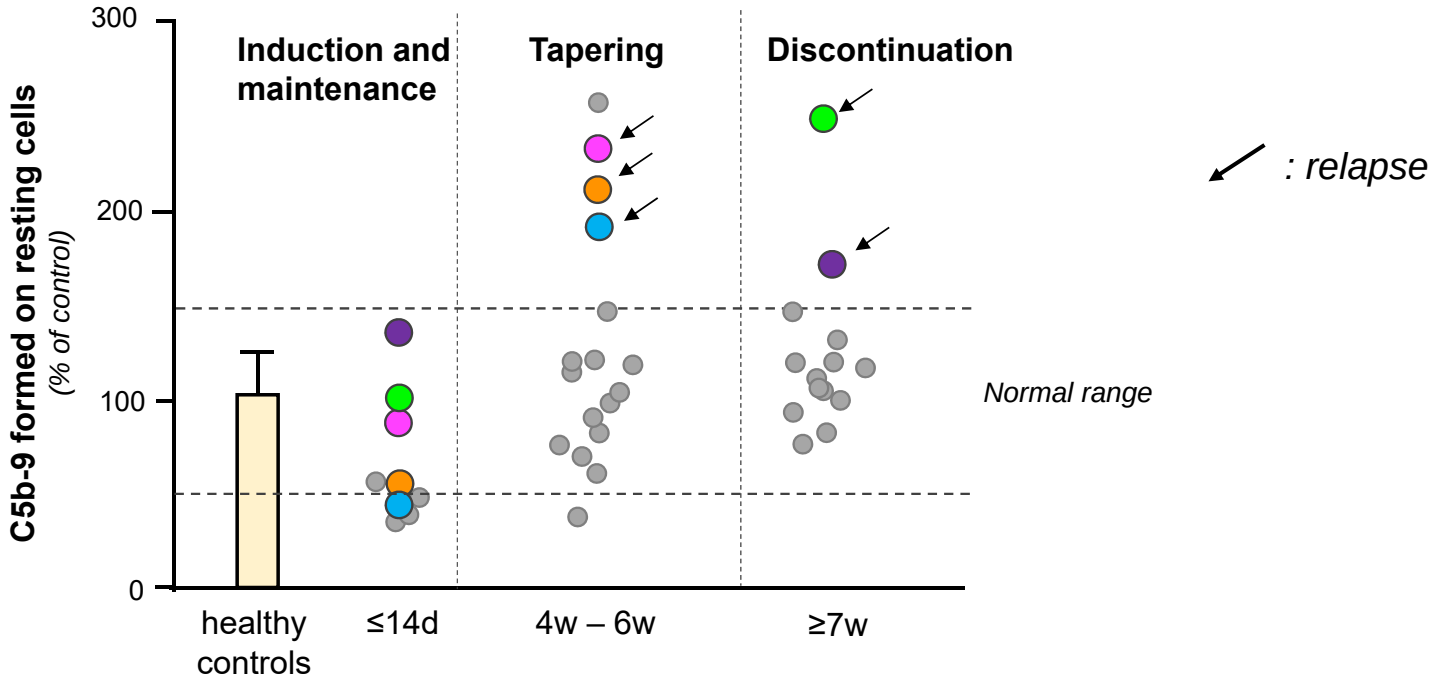
Children



During follow-up 13 (23%) patients experienced aHUS relapse. In multivariable analysis, the presence of a rare complement gene variant was associated with an increased risk of relapse (OR 16.20 [1.78-147.72]).

Fakhouri et al, *Blood*, 2020

EX VIVO AHUS SERUM-INDUCED C5b-9 FORMATION ON RESTING HMEC-1 DURING ECULIZUMAB TAPERING/DISCONTINUATION

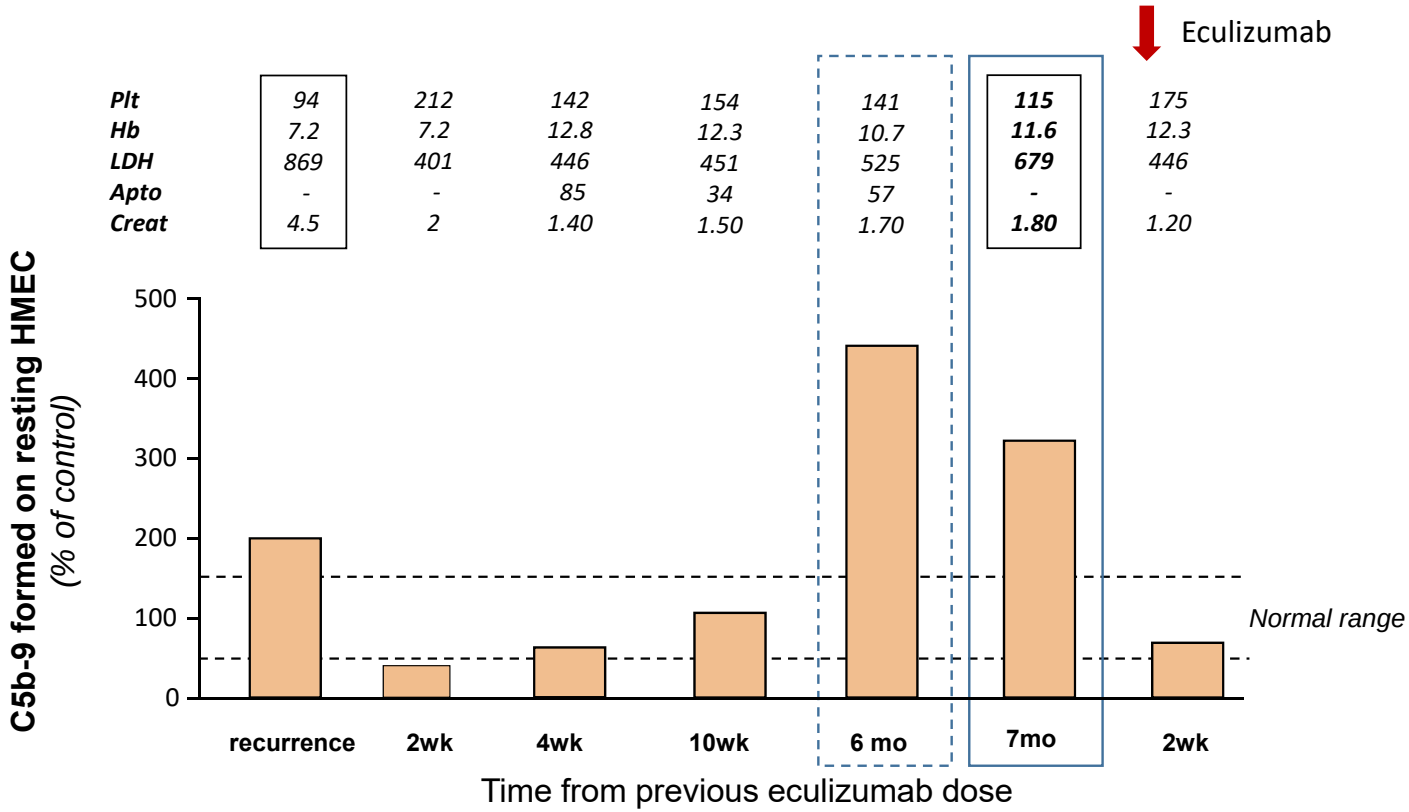


- The large majority of patients taking eculizumab at extended interdose intervals retained normal serum-induced C5b-9 deposits on unstimulated endothelium
- All five patients (colored dots) manifesting relapses showed elevated C5b-9 deposition on resting endothelial cells, in concomitance with or before worsening of clinical parameters (sensitivity for disease relapse 100%)

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

42 year old woman
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 months after eculizumab cessation, C5b-9 deposits on resting HMEC-1 rose above normal levels
- One month later the patient developed a disease relapse

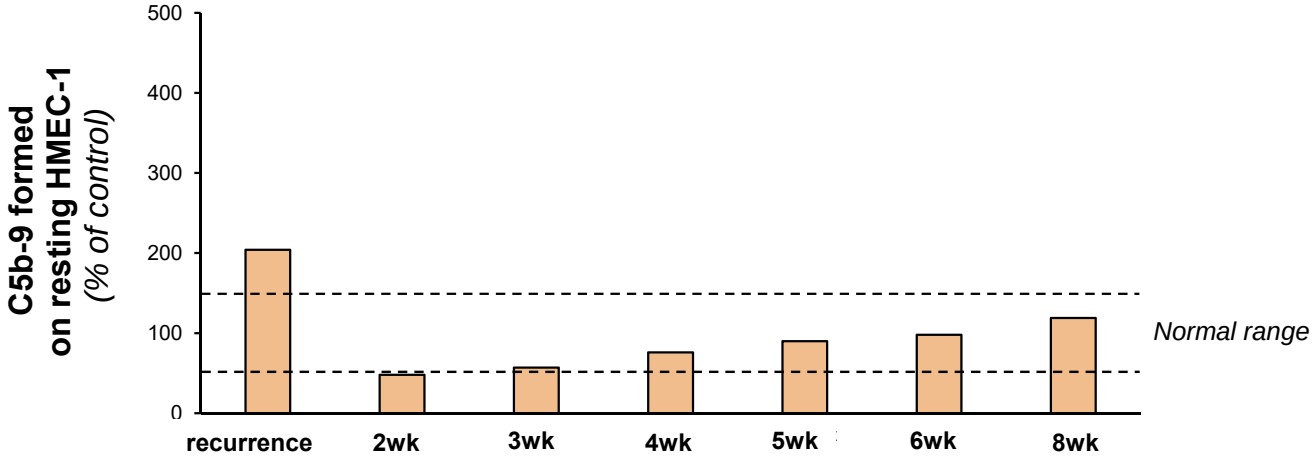


Galbusera et al., *Am J Kidney Dis,* 2019

16 year old girl
- recurrent aHUS (onset at 14 years of age)
- no identified complement gene abnormalities

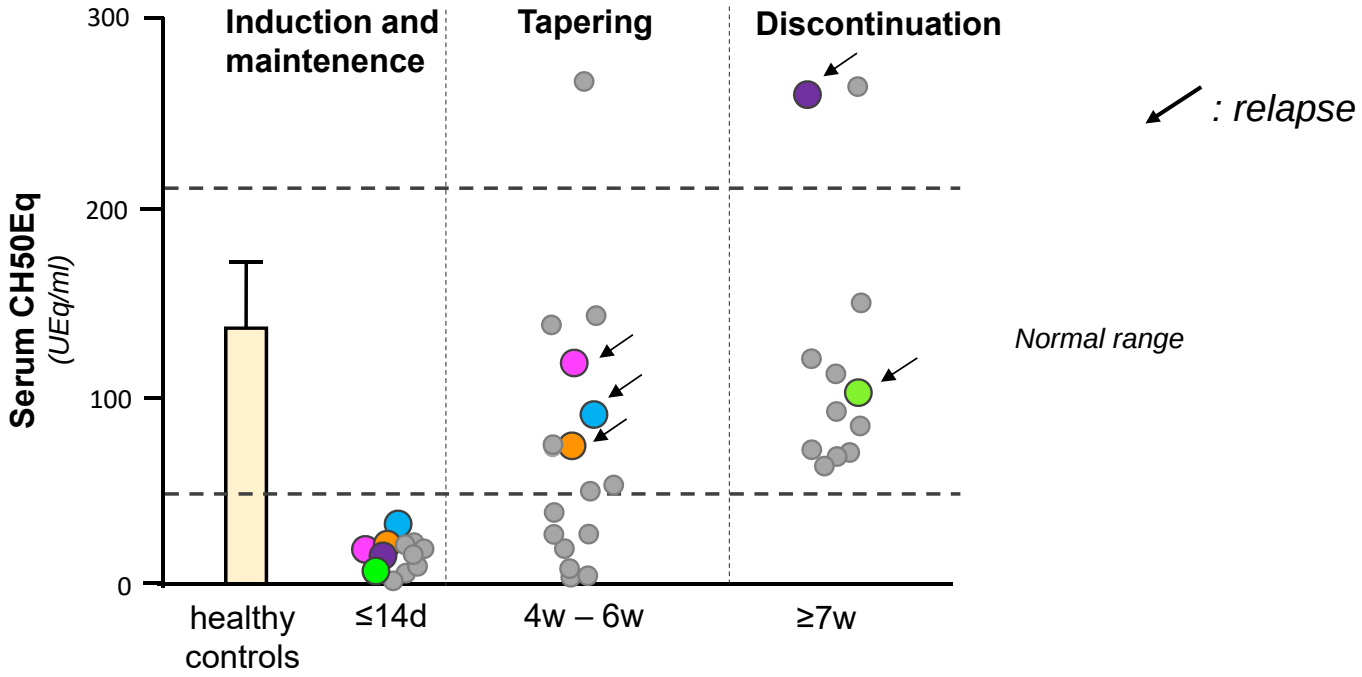
- During a recurrence the patient was treated with eculizumab resulting in disease remission
- After 2 year eculizumab every 2 weeks, the interval between doses was progressively increased
- Hematological and renal parameters and C5b-9 deposits on resting HMEC-1 remained stably normal

Plt	*180*	*147*	*190*	*181*	*203*	*175*	*172*
Hb	*9*	*11*	*11.7*	*11.4*	*11.6*	*11.3*	*10.6*
LDH	*-*	*301*	*349*	*341*	*378*	*313*	*408*
Apto	*-*	*50*	*82*	*49*	*74*	*83*	*55*
Creat	*1.3*	*0.74*	*0.81*	*0.8*	*0.79*	*0.66*	*0.67*



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

CH50 DID NOT EFFICIENTLY DETECT RELAPSE DURING ECULIZUMAB TAPERING/DISCONTINUATION



- At 5-week or longer intervals between eculizumab doses, total complement activity (CH50) levels did not differ between patients who had relapse (coloured dots,132±92 UEq/ml) and those who did not (grey dots,113±72 UEq/ml).

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

EX VIVO C5b-9 DEPOSITION HIGHLIGHTS COMPLEMENT DYSREGULATION AND DISEASE RELAPSES WHILE THE OTHER BIOMARKERS DO NOT

	Acute aHUS	Remission aHUS		Eculizumab discontinuation	
		No therapy	Eculizumab	No relapse	Relapse
Plasma sC5b-9	N/↑	N/↑	N/↑	N/↑	N/↑
CH50	N	N	↓↓	N	N
C5b-9 deposition on:					
- resting HMEC	↑↑	N	N	N	↑↑

- Plasma sC5b-9 levels did not differ between acute aHUS and remission and did not consistently change during eculizumab treatment or after discontinuation
- CH50 was normal in aHUS, was suppressed by eculizumab and returned to normal after discontinuation, independently from disease relapses
- C5b-9 deposits on resting HMEC were elevated only in patients with acute aHUS and in those with signs of relapses during eculizumab discontinuation

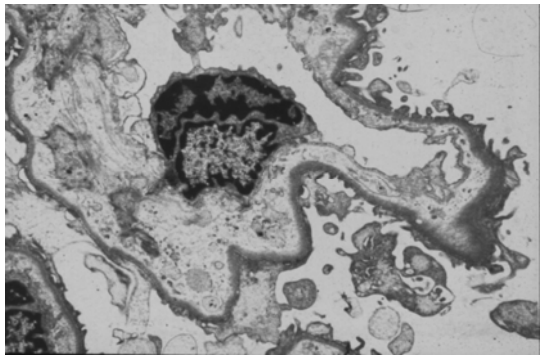
Galbusera et al., *Am J Kidney Dis,* 2019

Take Home Messages

- In Atypical HUS local complement activation on endothelial surface (rather than fluid phase) plays a pathogenic role.
- Genetic abnormalities (mainly heterozygous) causing loss of function of AP complement regulators or gain of function of the components of the AP C3 convertase predispose to aHUS.
- Both gene mutations and risk polymorphism concur to determine disease penetrance.
- C5-inhibition efficiently induces disease remission in aHUS and prevents disease recurrences
- The presence of a pathogenetic complement gene variant increases the risk of relapse upon anti-C5 therapy discontinuation, however relapses are still difficult to predict and early and reliable markers are required

ATYPICAL HEMOLYTIC UREMIC SYNDROME: A DISEASE IN A DISH

Diagnosis



Monitoring

Thanks!

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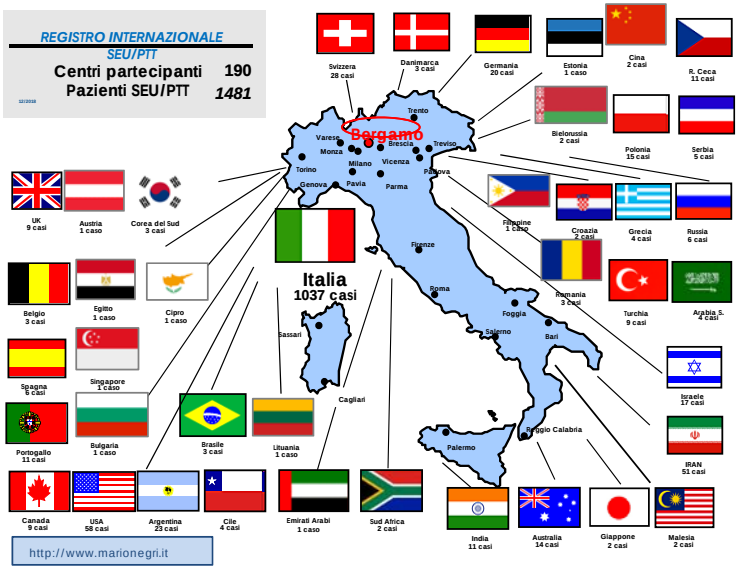

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- Speaker: **Rosa Vargas Poussou**
- Topic: **Dent Disease**

- **ERKNet/ERA-EDTA Advanced Webinars on Rare Kidney Disorders**
- Date: **29 June 2021**
- Speaker: **Jürgen Floege**
- Topic**: Update on KDIGO on Immune Glomerulopathies**

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