Danni renali in corso di sindrome emolitico uremica

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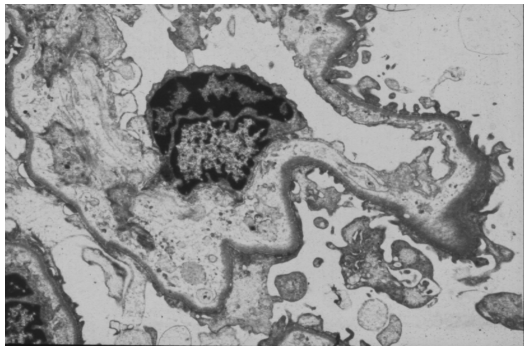
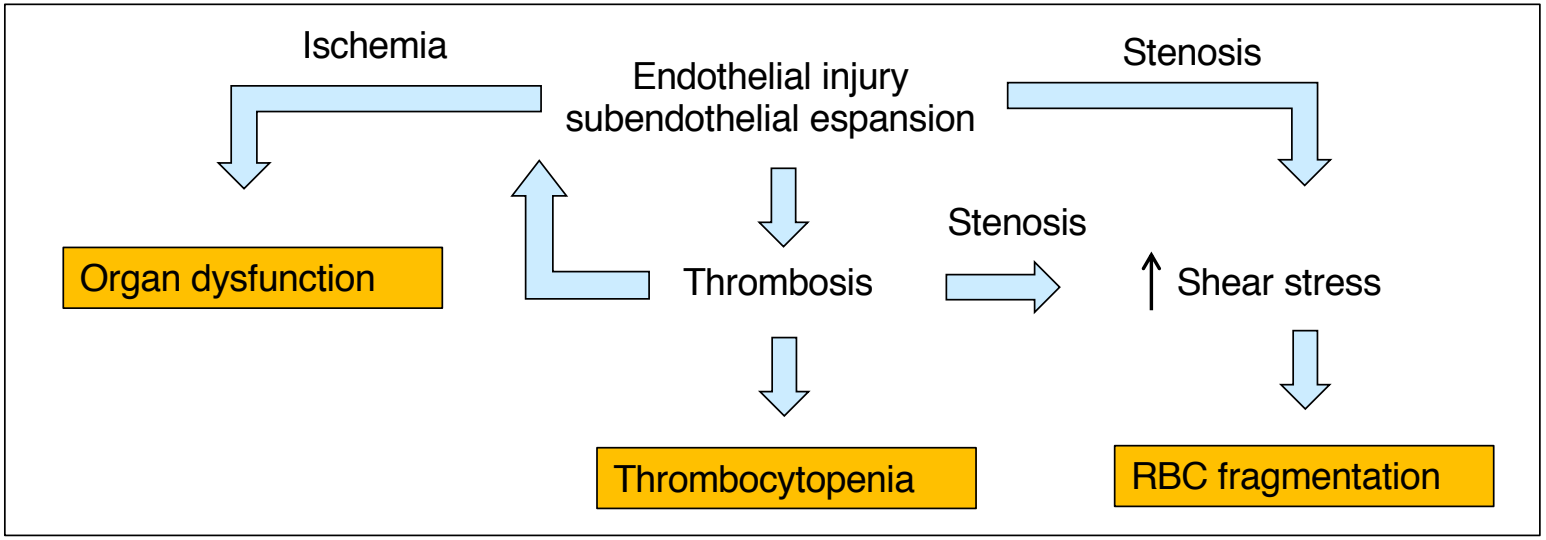
  - *Omeros (research grant)*

THROMBOTIC MICROANGIOPATHY

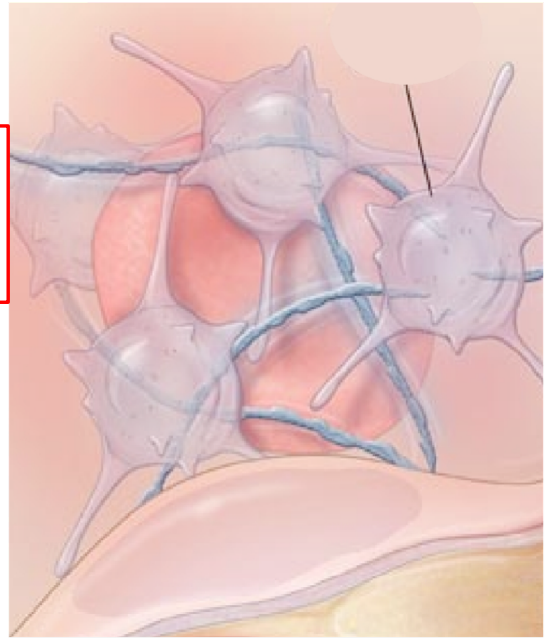
Histology lesions: Swelling and detachment of endothelial cells, accumulation of fluffy material in the subendothelium, thrombi and obstruction of the vessel lumina.





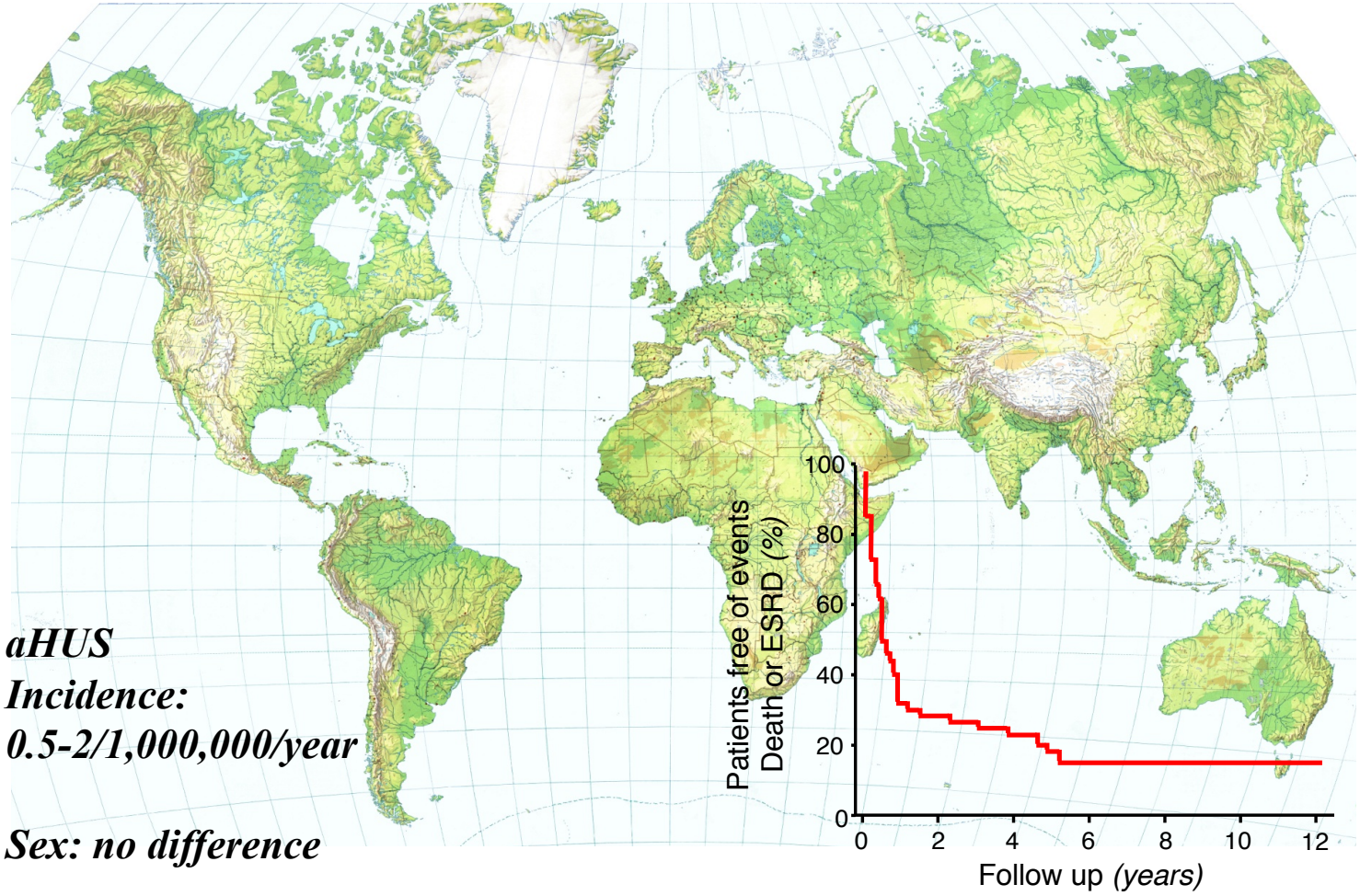
Ruggenenti and Remuzzi, Kidney Int 2001

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

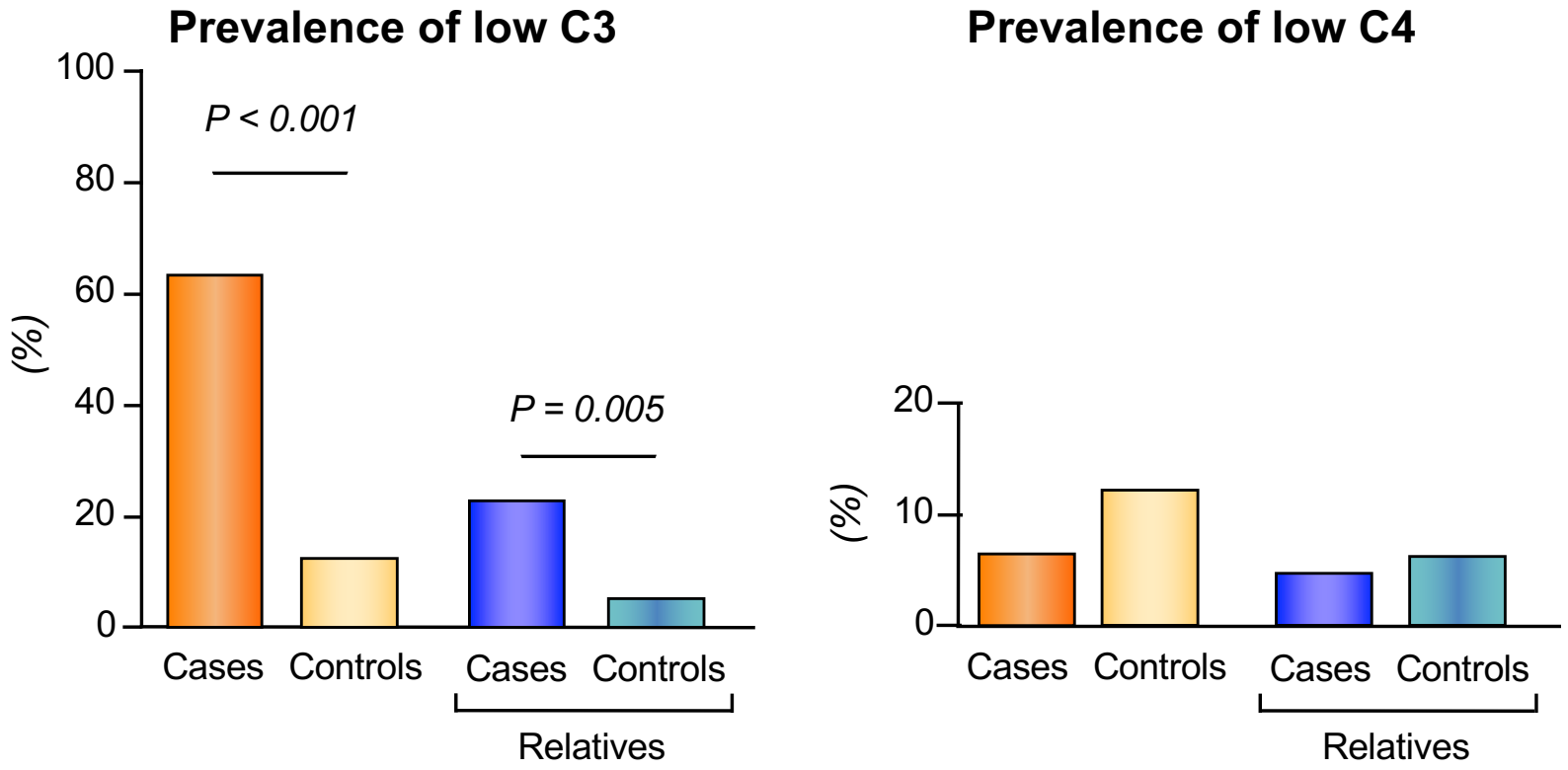


Microthrombi

ATYPICAL HEMOLYTIC UREMIC SYNDROME



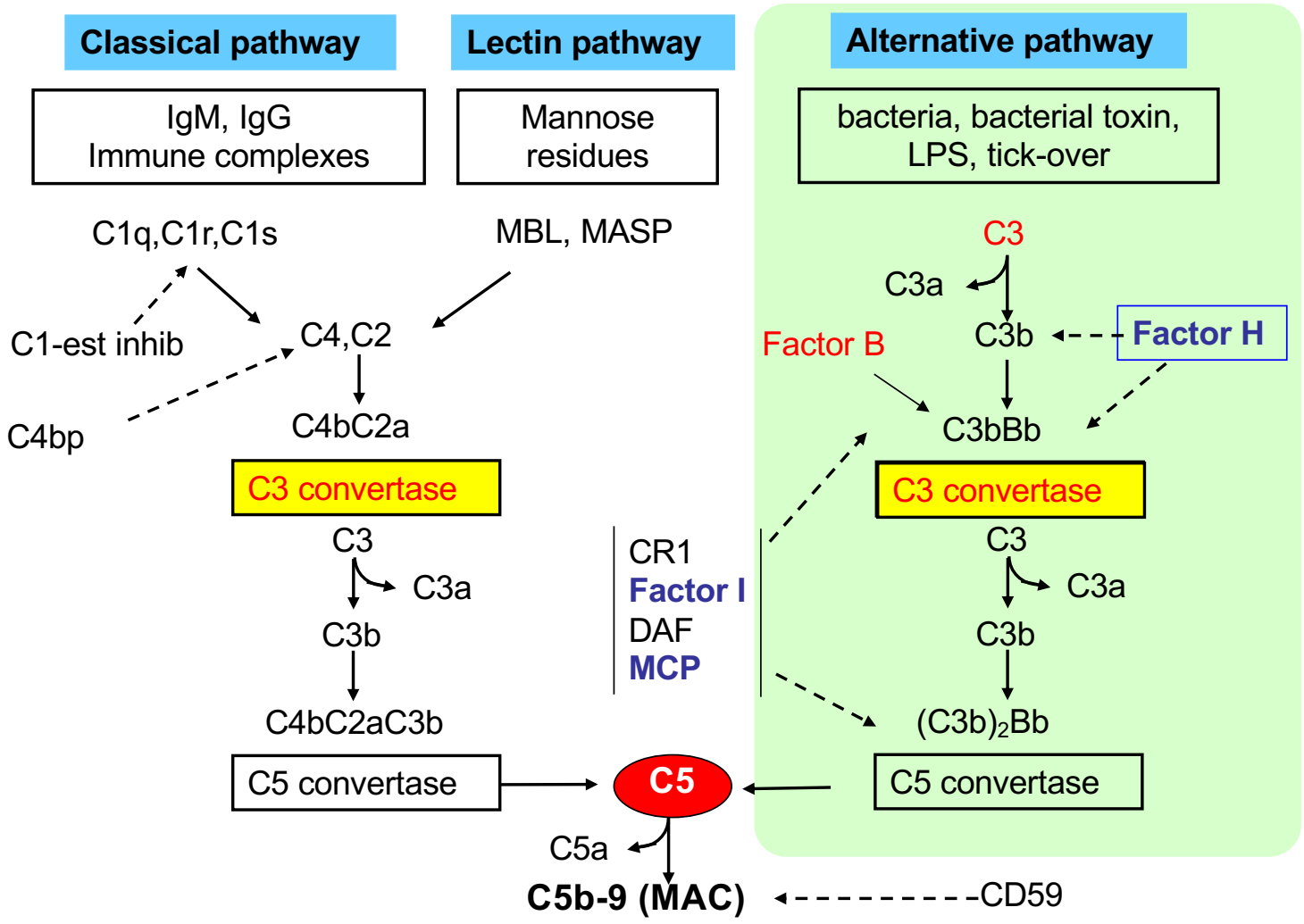
A life threatening multisystem disease of microangiopathic hemolytic anemia and thrombocytopenia with predominant but not exclusive renal involvement.



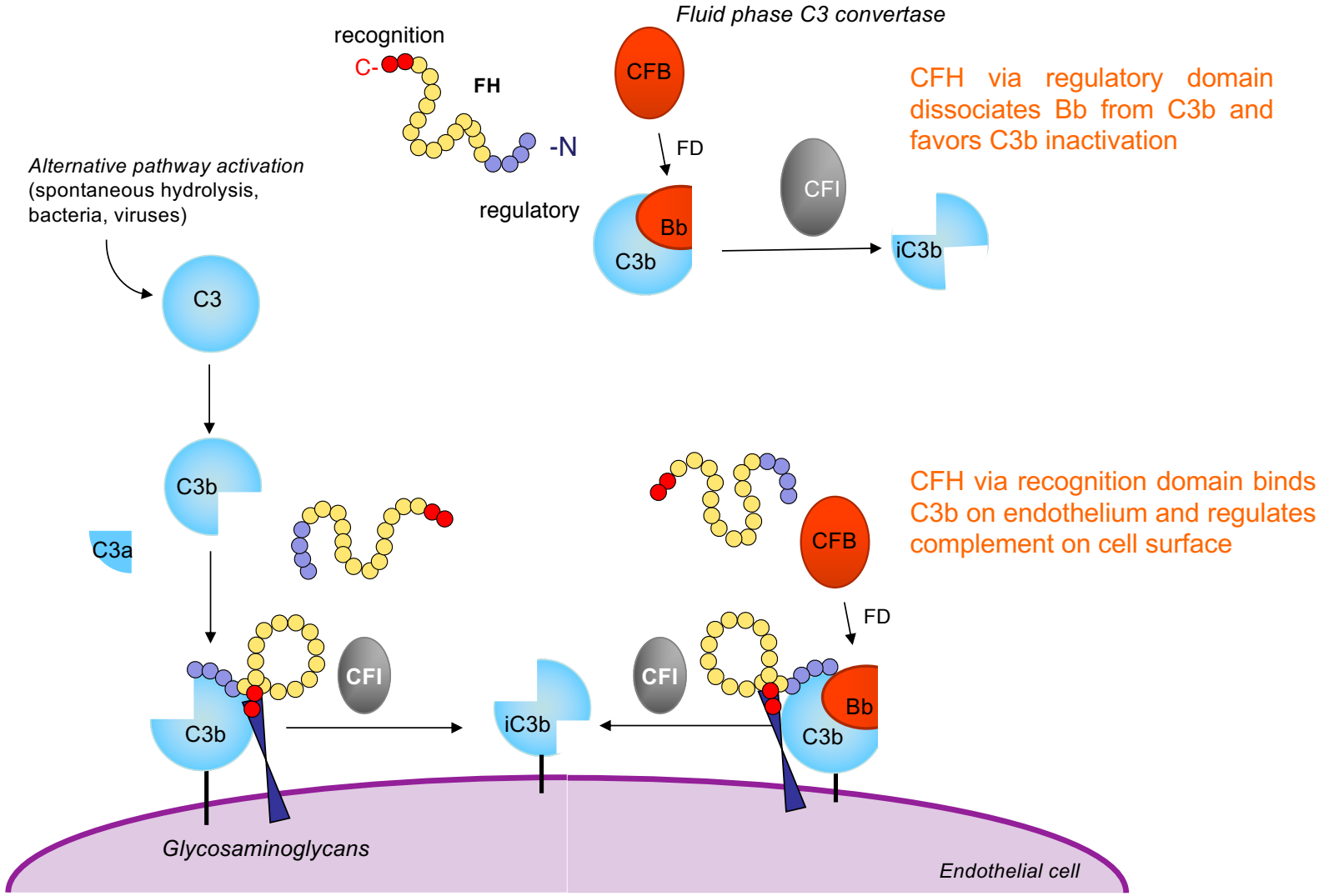
Within families, subjects with lower than normal C3 serum levels had a relative risk of aHUS of 16.5 as compared to subjects with normal C3 levels

Noris et al., *J Am Soc Nephrol,* 1999

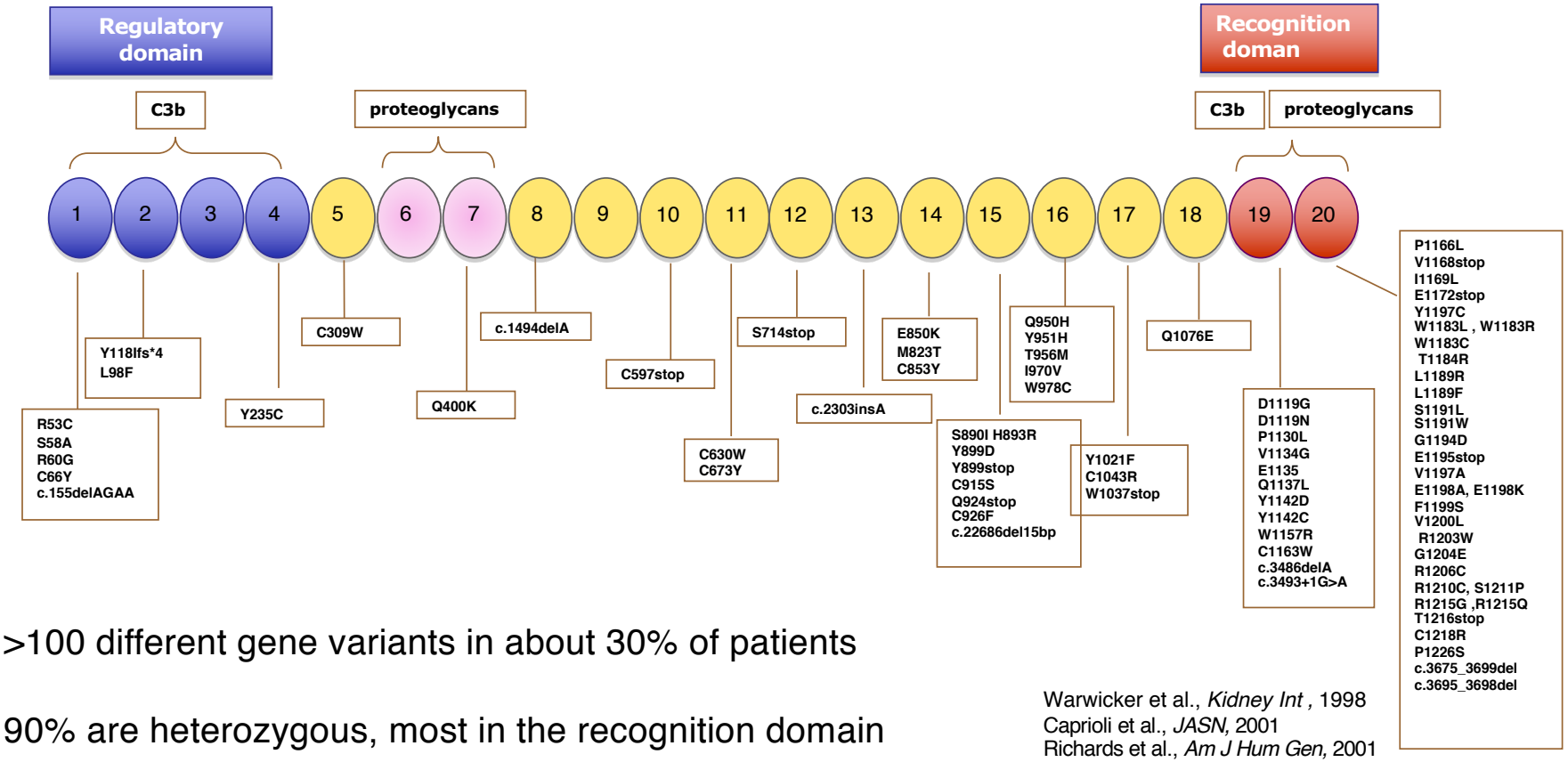
COMPLEMENT ACTIVATION PATHWAYS



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



CFH MUTATIONS IN ATYPICAL HEMOLYTIC UREMIC SYNDROME

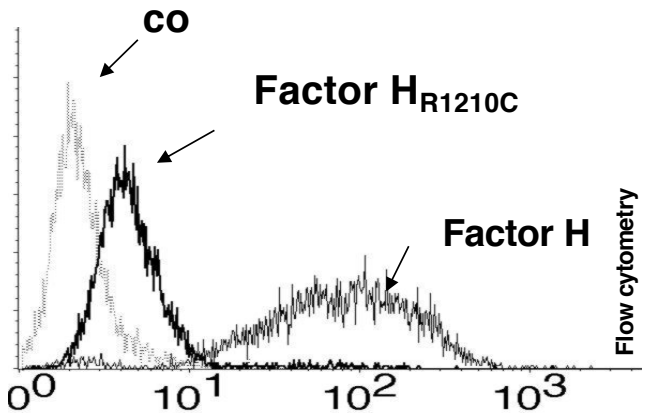


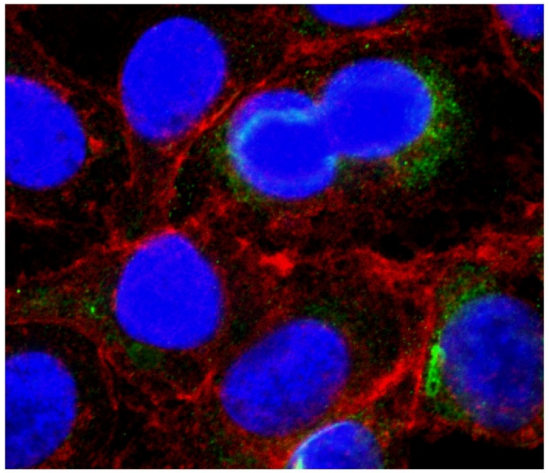
- >100 different gene variants in about 30% of patients
- 90% are heterozygous, most in the recognition domain

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

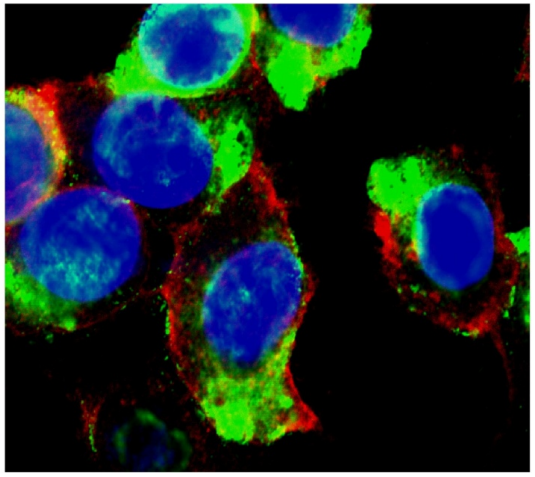
SINGLE AMINO ACID CHANGES IN SCR 20 OF FACTOR H AFFECT ENDOTHELIAL CELL BINDING

HUVEC incubated with recombinant wild type or mutated factor H stained with fluorescinated anti-factor H antibody and analyzed by FACS





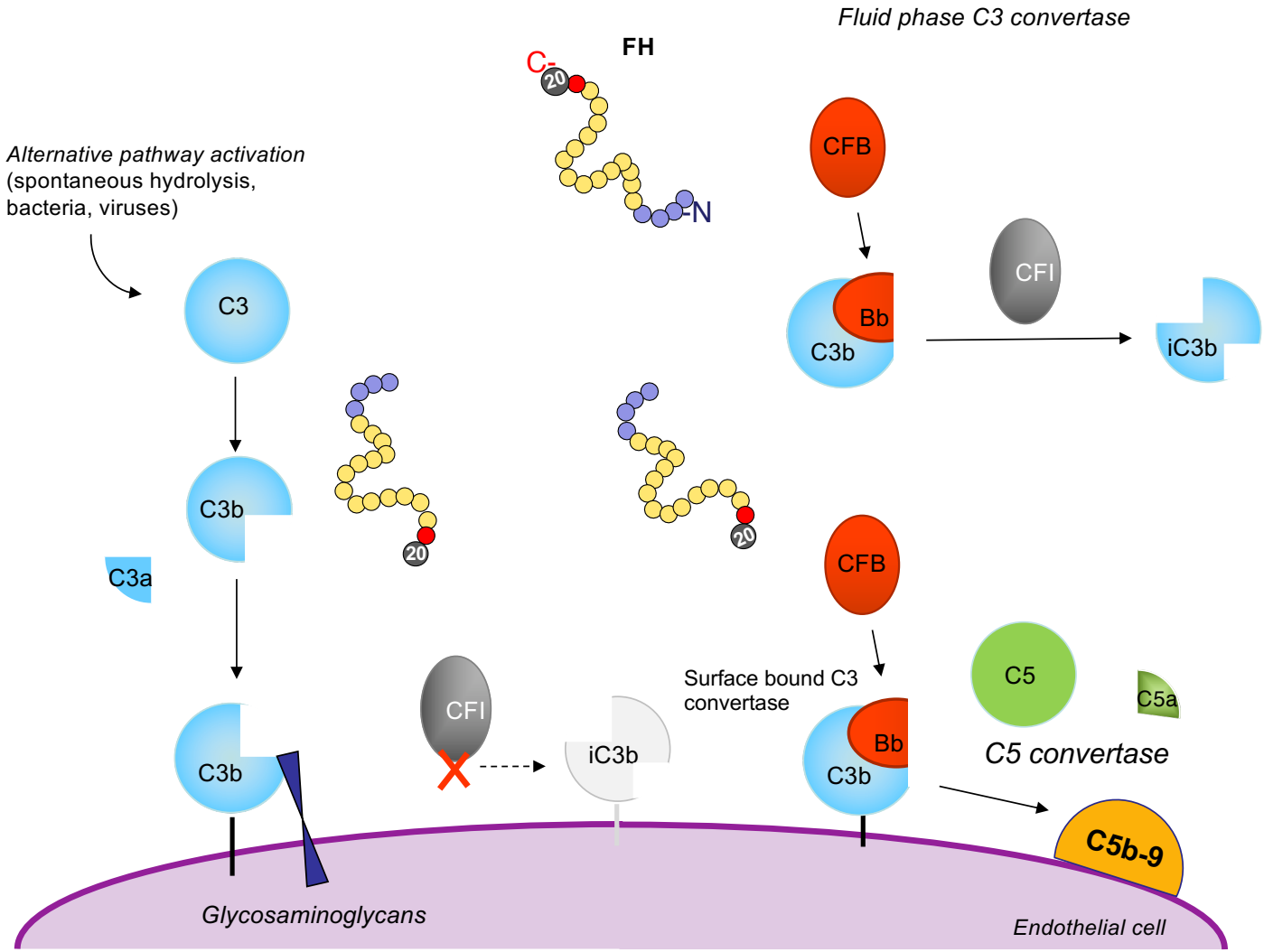
Factor H R1210C



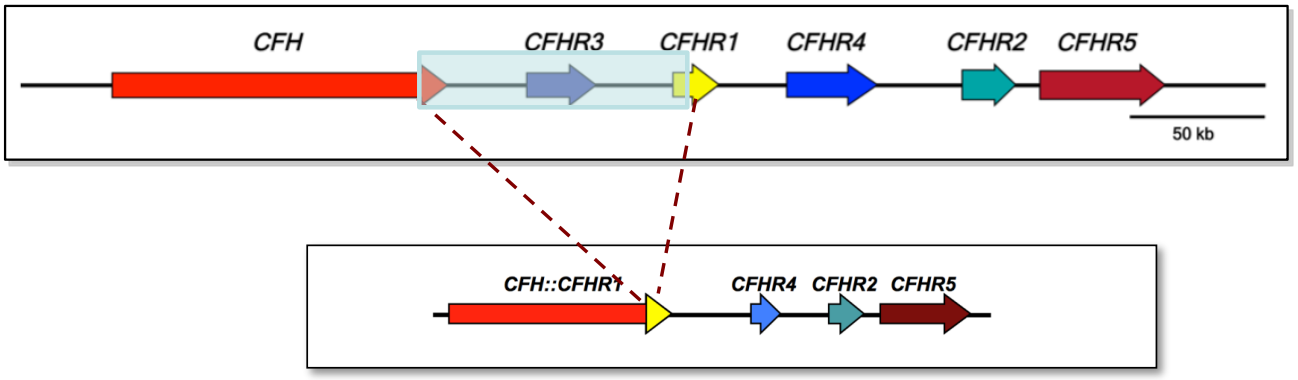
WT Factor H

Manuelian et al, *J Clin Invest* 2003

CONSEQUENCES OF CFH aHUS-ASSOCIATED GENETIC VARIANTS



HIGH HOMOLOGY IN THE REGULATORS OF COMPLEMENT ACTIVATION GENE CLUSTER



***CFH/CFHR1* hybrid gene**

- 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.

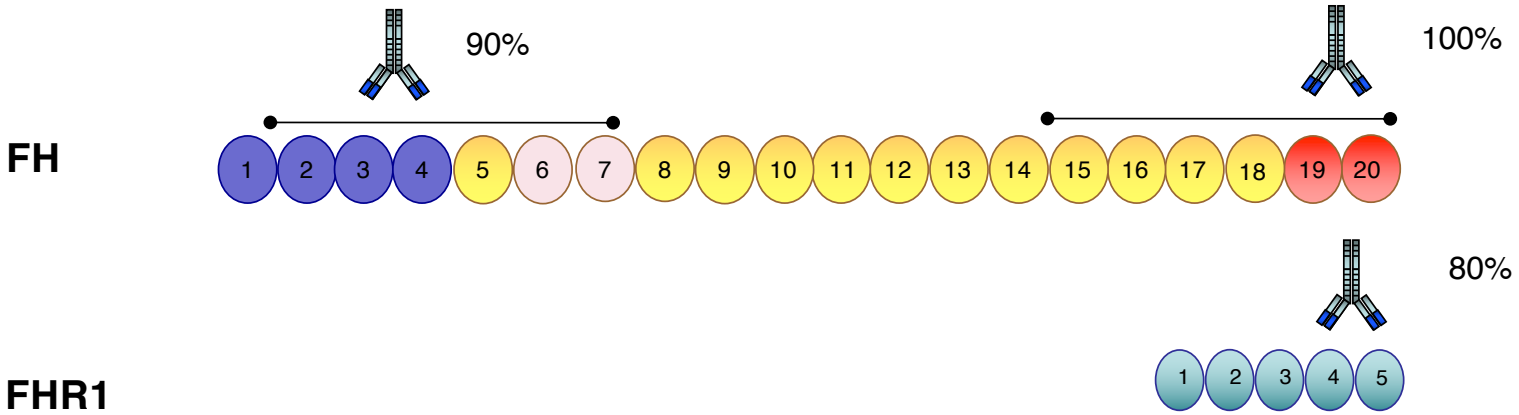
- Copy number variation assays (high resolution CGH arrays or MLPA) are required to detect hybrid genes

Venables et al., *Plos Medicine,* 2006

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



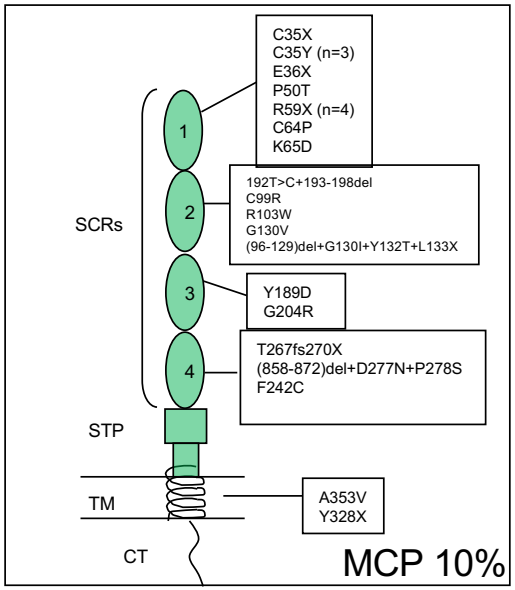
8-9% mostly children

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

- Anti-FH abs mainly target the FH C-terminus
- Three linear epitopes in SCR19 and 20 of FH (1157-1171; 1177-1191, 1207-1226) and one in SCR5 of FHR1 are recognized by anti-FH abs.
- Most patients with aHUS and FH autoantibodies are homozygous for a deletion of genes encoding FH related proteins 1 and 3.

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

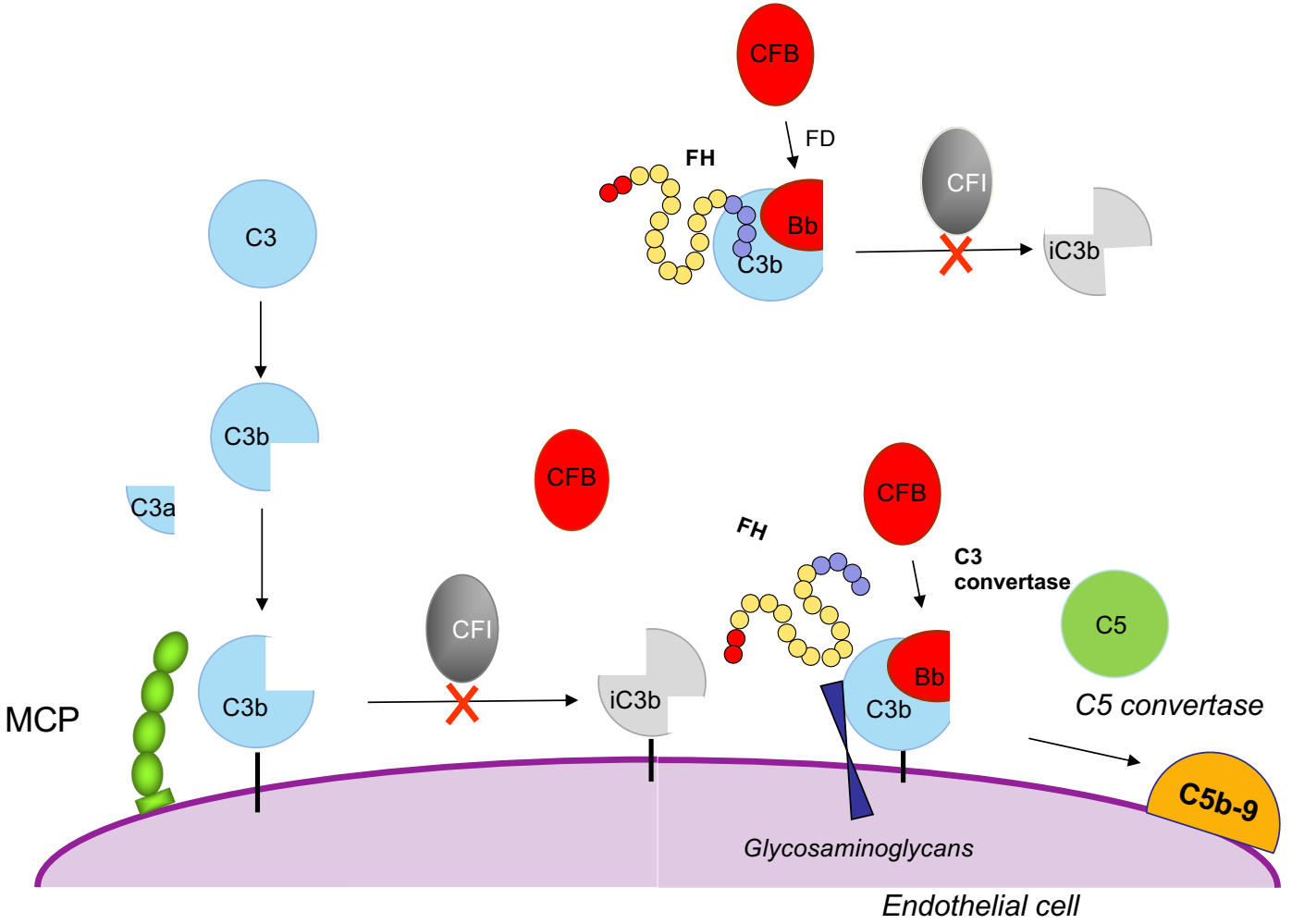
MCP VARIANTS IN aHUS



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

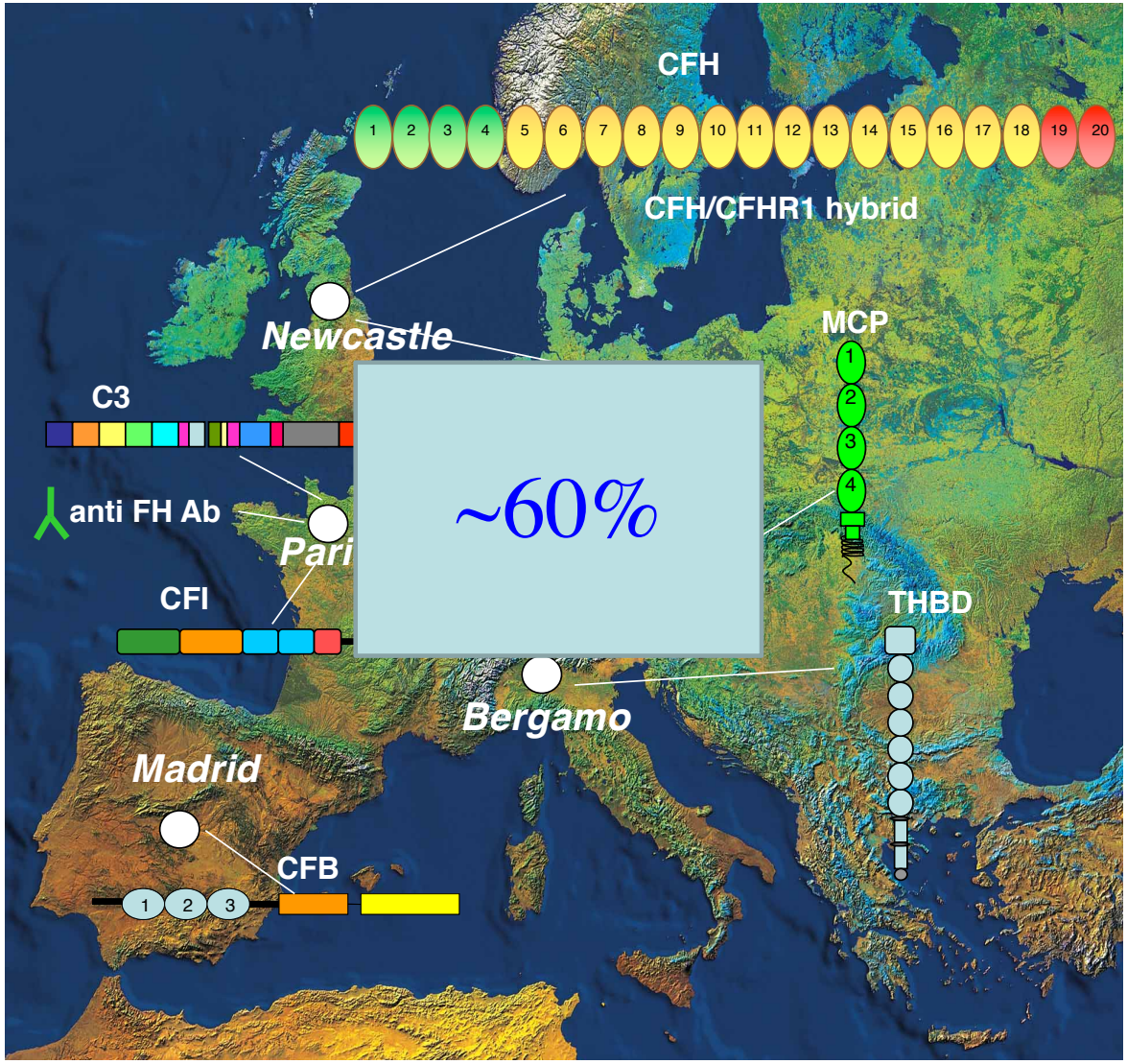
Noris et al, Lancet 2003
Richards et al, PNAS 2003
Caprioli et al, Blood 2006

C3 AND CFB GAIN OF FUNCTION VARIANTS IN aHUS

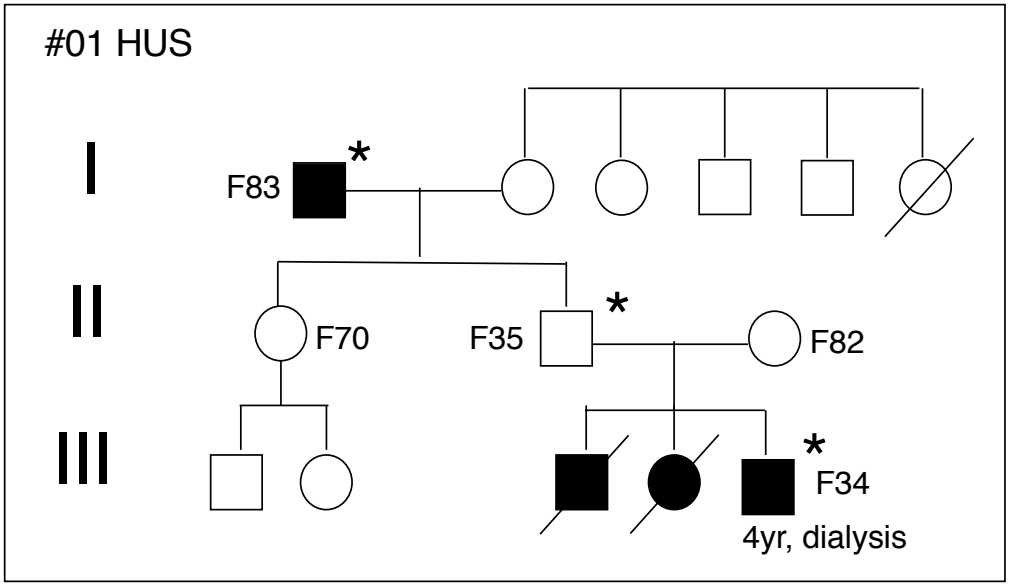


Goicoechea et al., *PNAS,* 2007
Roumenina et al., *Blood,* 2009
Fremeaux-Bacchi et al, Blood 2008

GENETICS OF AHUS: AN EUROPEAN DISCOVERY



INCOMPLETE PENETRANCE OF aHUS IN CARRIERS OF COMPLEMENT GENE VARIANTS

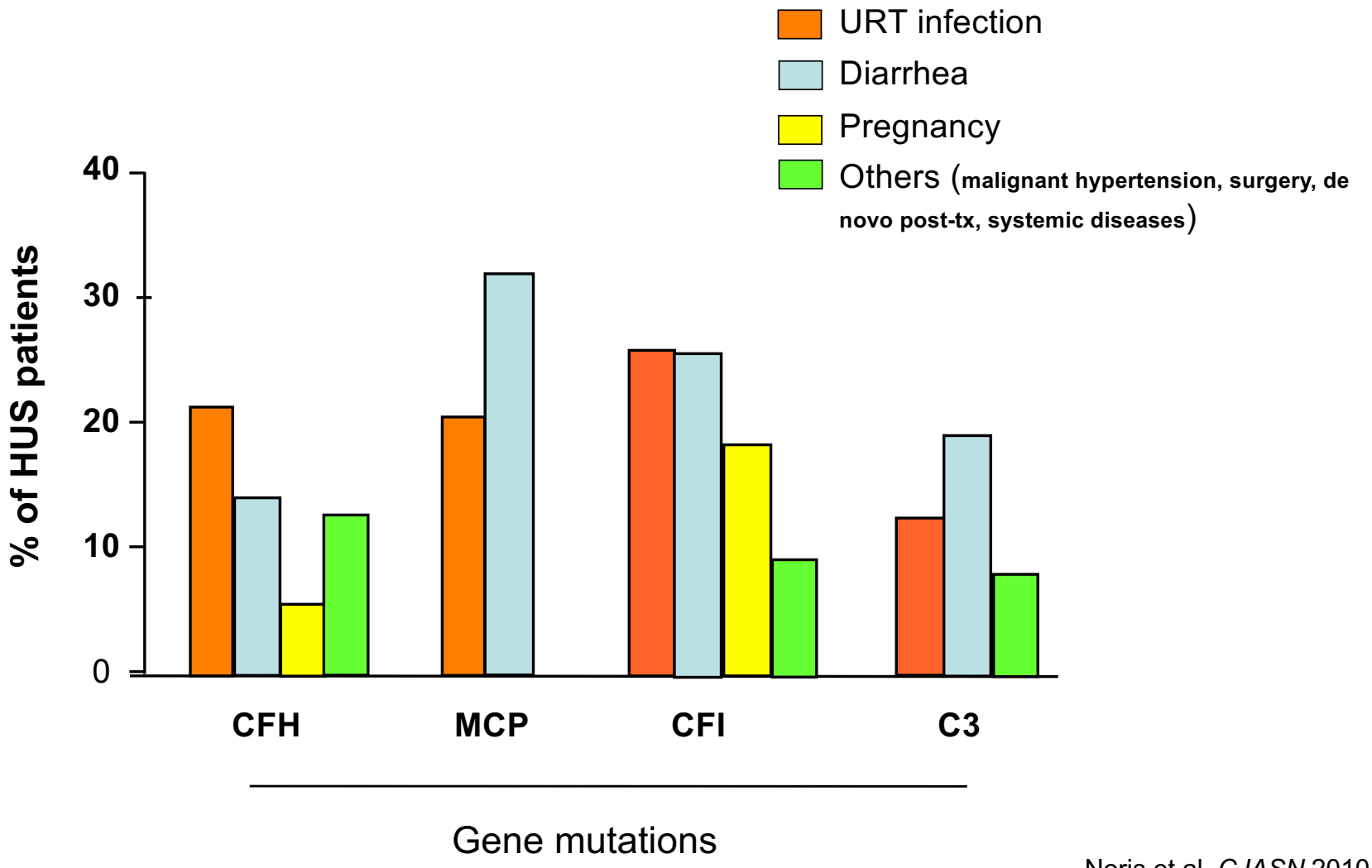


* R1215Q pathogenetic change in CFH

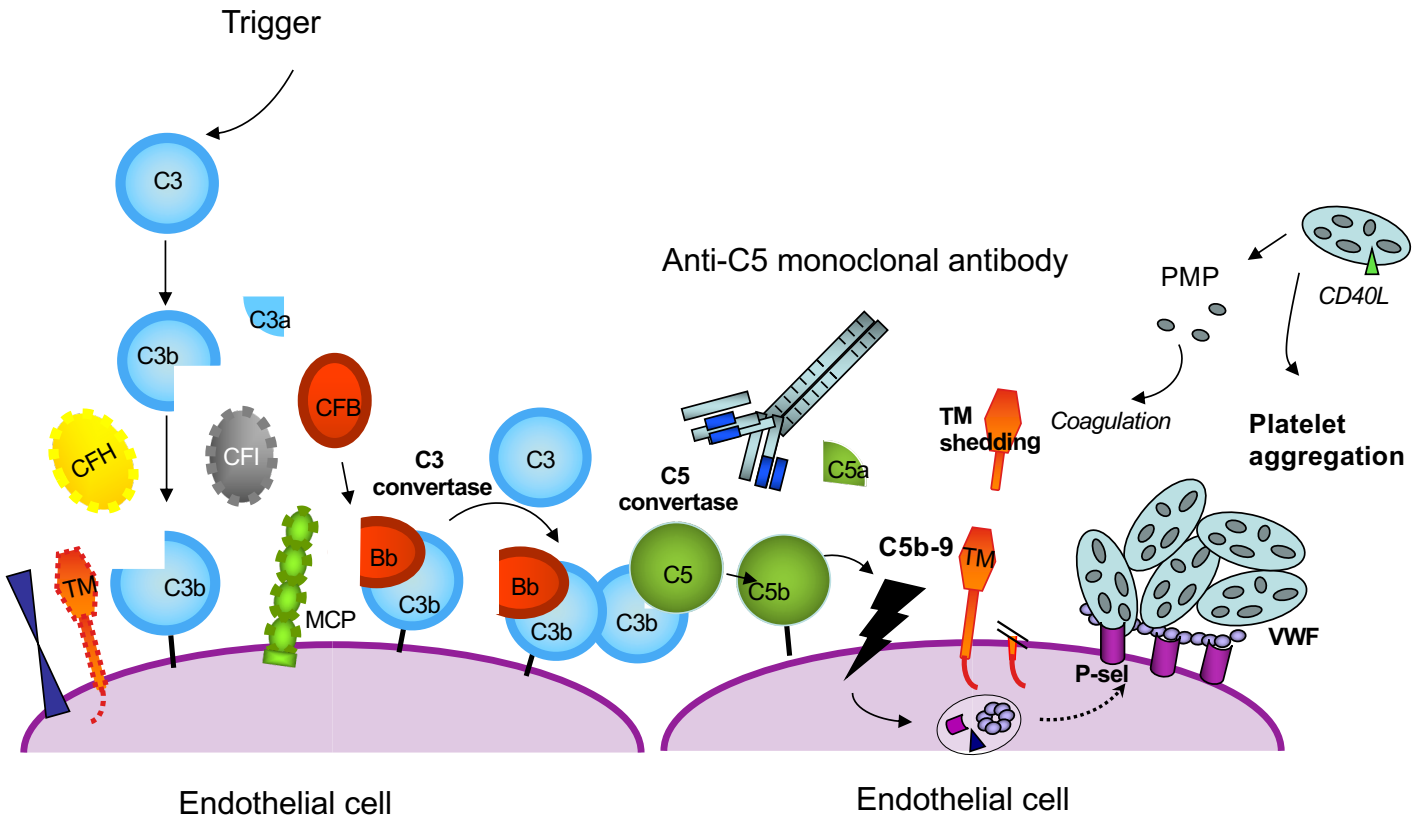
- 3 subjects in the III generation developed aHUS in infancy: 2 died, 1 reached ESRD
- F35 never developed aHUS
- Subject F83, carrier of the R1215Q mutation developed aHUS and died at 82 years of age

Noris et al., C*JASN 2010*

TRIGGERING /UNDERLYING CONDITIONS

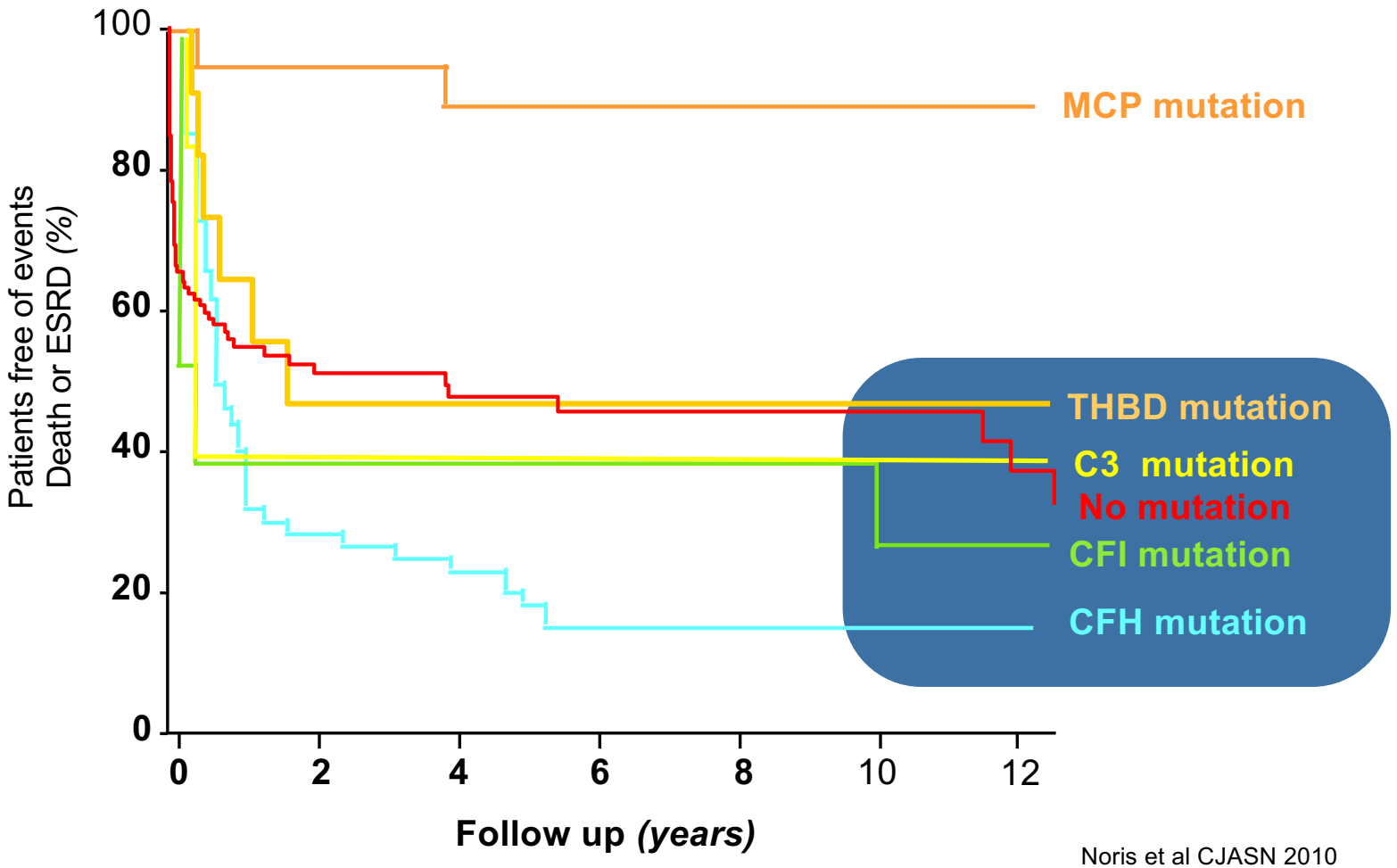


Noris et al, *CJASN* 2010



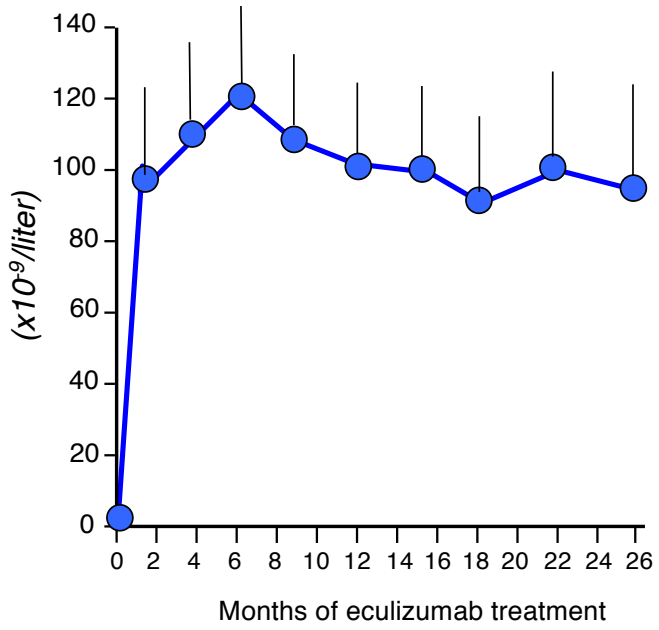
Modified from Noris and Remuzzi, NEJM 2009

LONG TERM OUTCOME OF aHUS PATIENTS

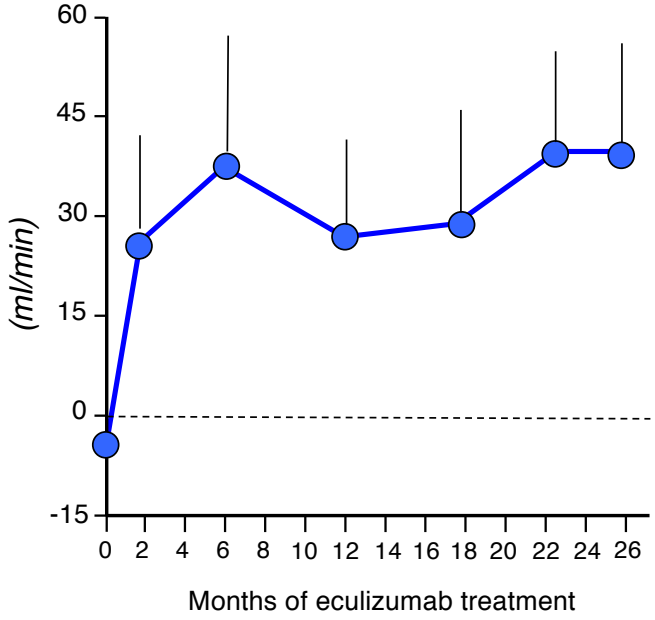


Noris et al CJASN 2010

Platelet count
mean change from baseline

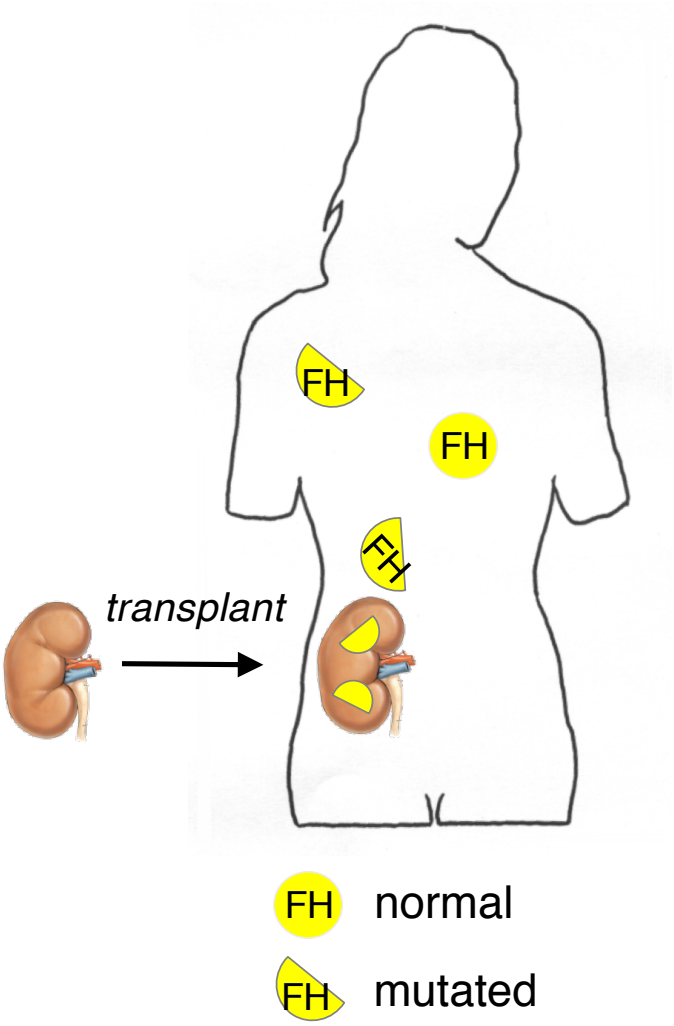


Estimated GFR
mean change from baseline



Treatment effect was sustained for up to 26 months

Licht et al., *Kidney Int,* 2015



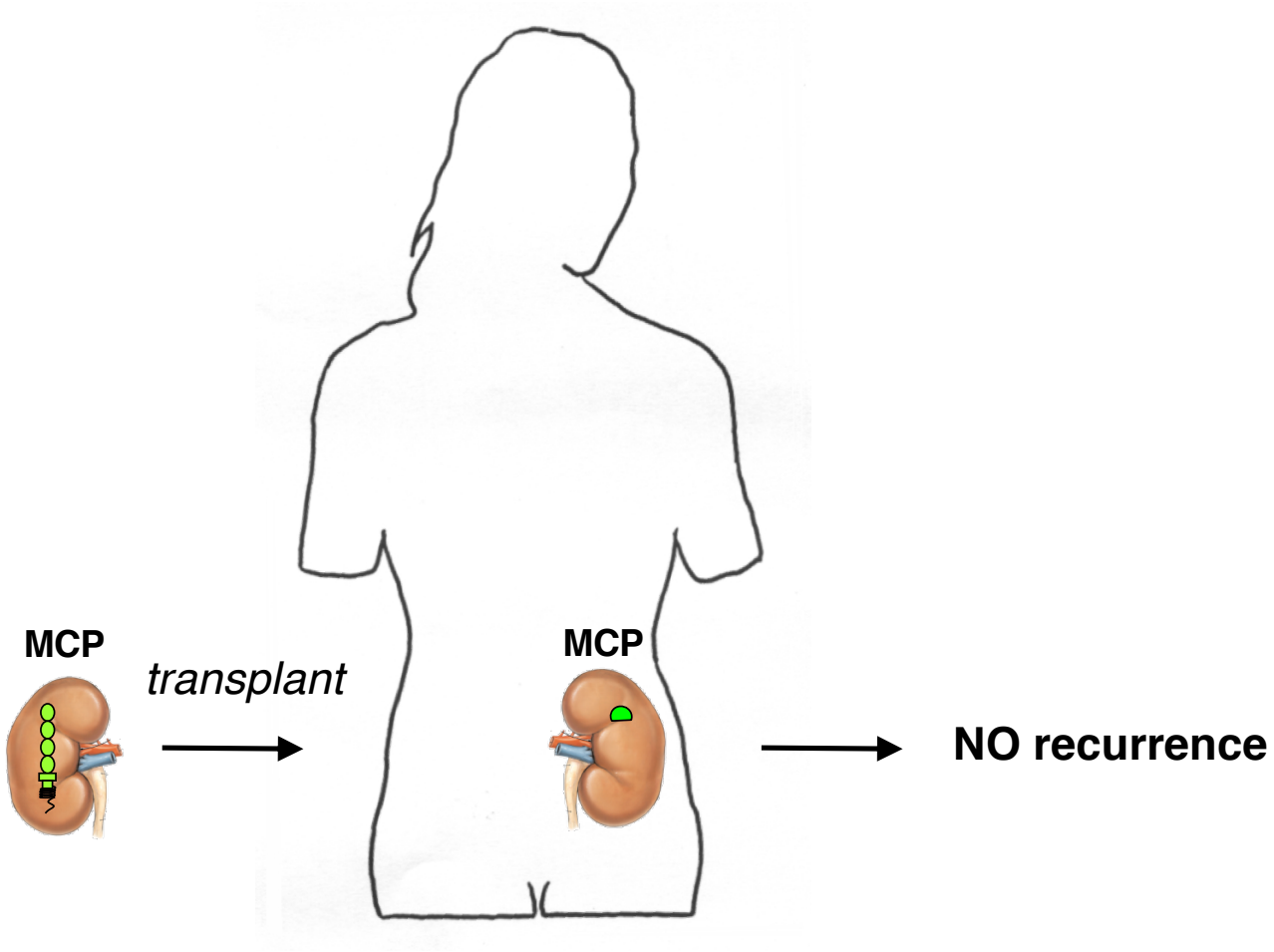
TRANSPLANTATION OUTCOMES

Kidney graft lost because aHUS recurrence

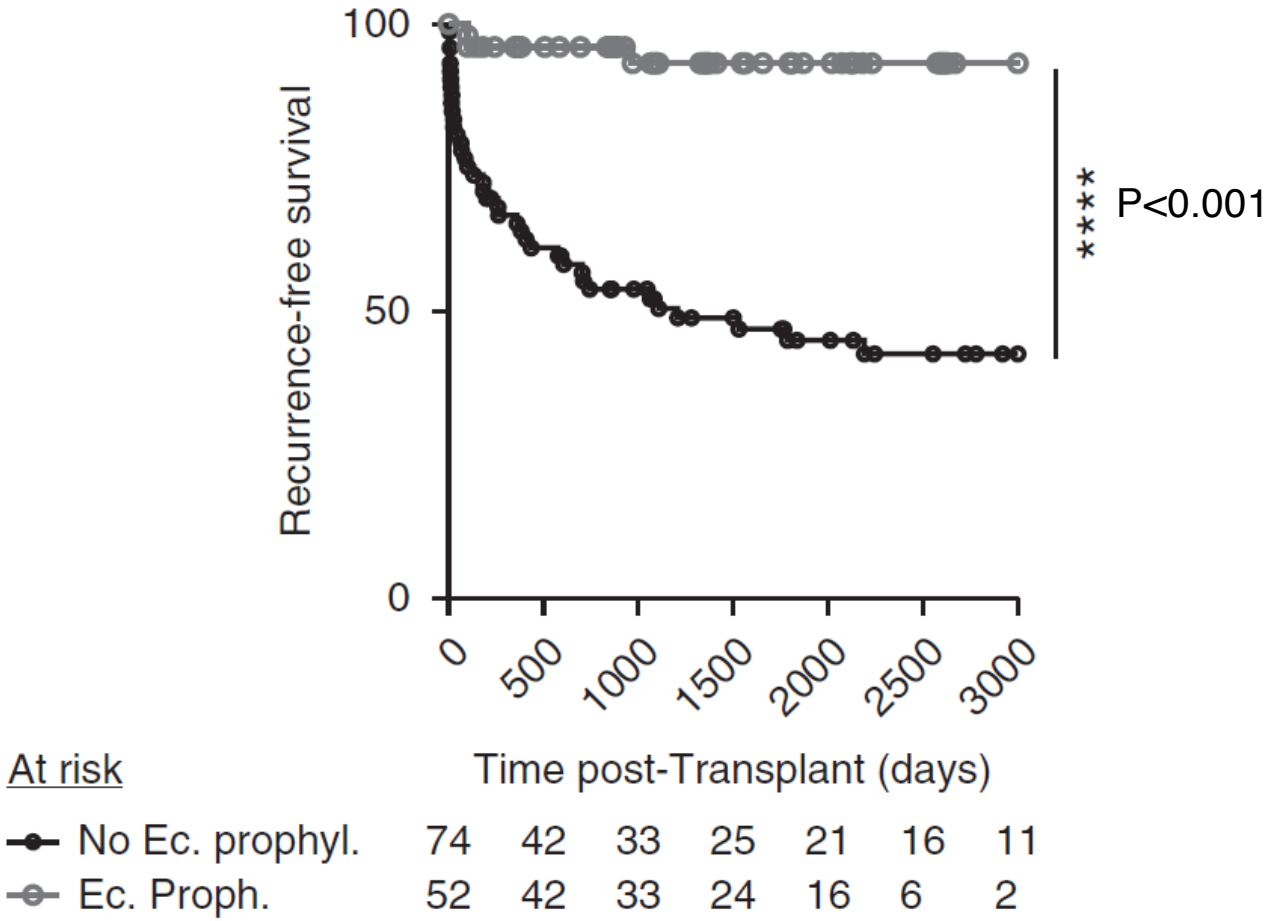
Patients with :

- CFH	mutations	12 out of 17
- CFI	mutations	13 out of 21
- CFB	mutations	2 out of 3
- C3	mutations	6 out of 21

Valoti et al., *J Nephrol,* 2012
Noris and Remuzzi, *Am J Transplant,* 2011


MCP
transplant
MCP
NO recurrence

SURVIVAL OF KIDNEY TRANSPLANT IN aHUS

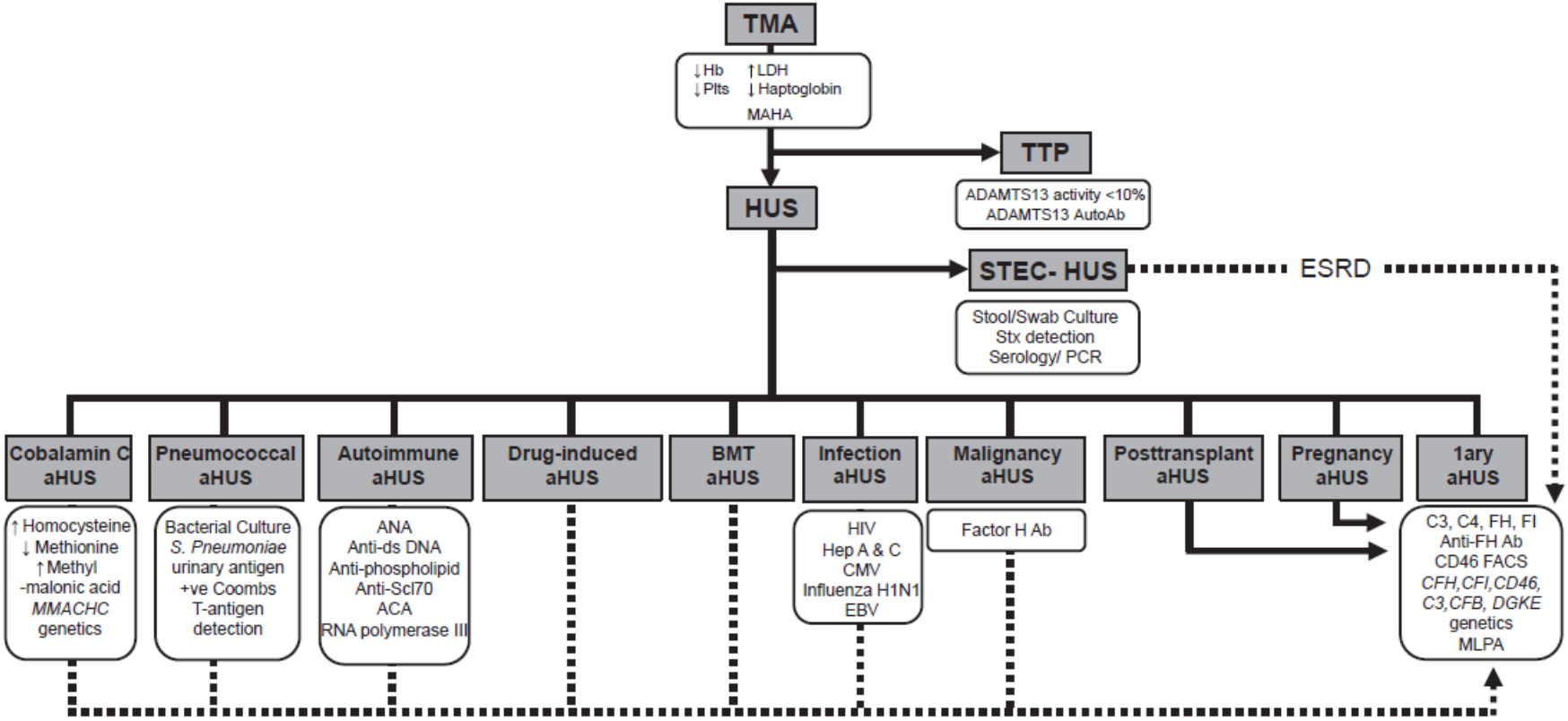


Zuber et al , JASN 2019

Eculizumab in aHUS

- Life-long treatment is recommended in the EMA-approved label for all patients
- Requires one day-hospitalizations for intravenous infusions every two weeks
- Carries the risk of severe infections, mainly meningococcal meningitis and gonococcal infections
- The drug is extremely expensive (460.000 euro/year in adults and 150.000-330.000 euro/year in children)

COMPLEMENT-MEDIATED HUS: HOW TO DIAGNOSE AND MONITOR?



Genetic analysis requires time and is not always informative.
Specific and sensitive markers of complement activation in aHUS are lacking.

Goodship et al , Kidney Int 2017

Case

- 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 complement mediated aHUS?***

  - No, because circulating complement parameters are normal

  - Yes, could be, but further tests are needed

BLOOD, 11 SEPTEMBER 2014

CLINICAL TRIALS AND OBSERVATIONS

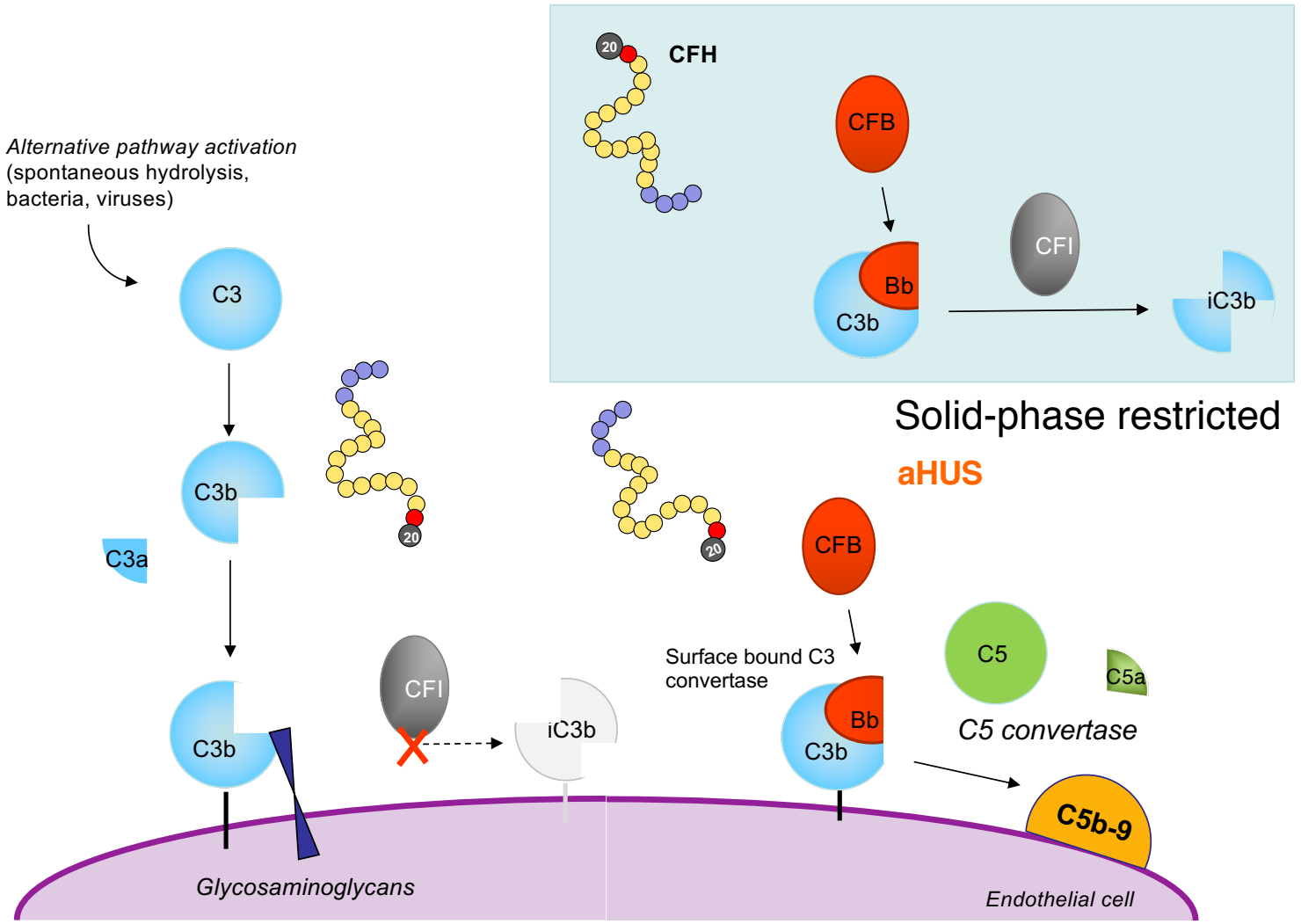
Dynamics of complement activation in aHUS and how to monitor eculizumab therapy

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]

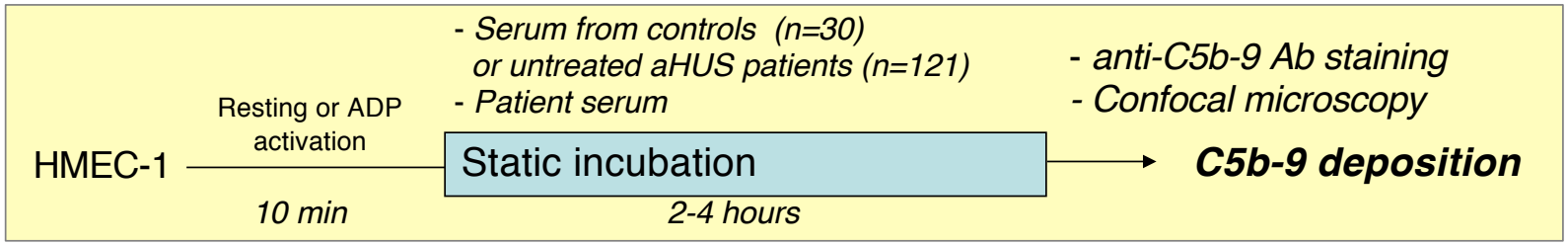
Both during the acute phase of the disease and at remission about half of aHUS patients had normal serum C3 and plasma sC5b-9 levels

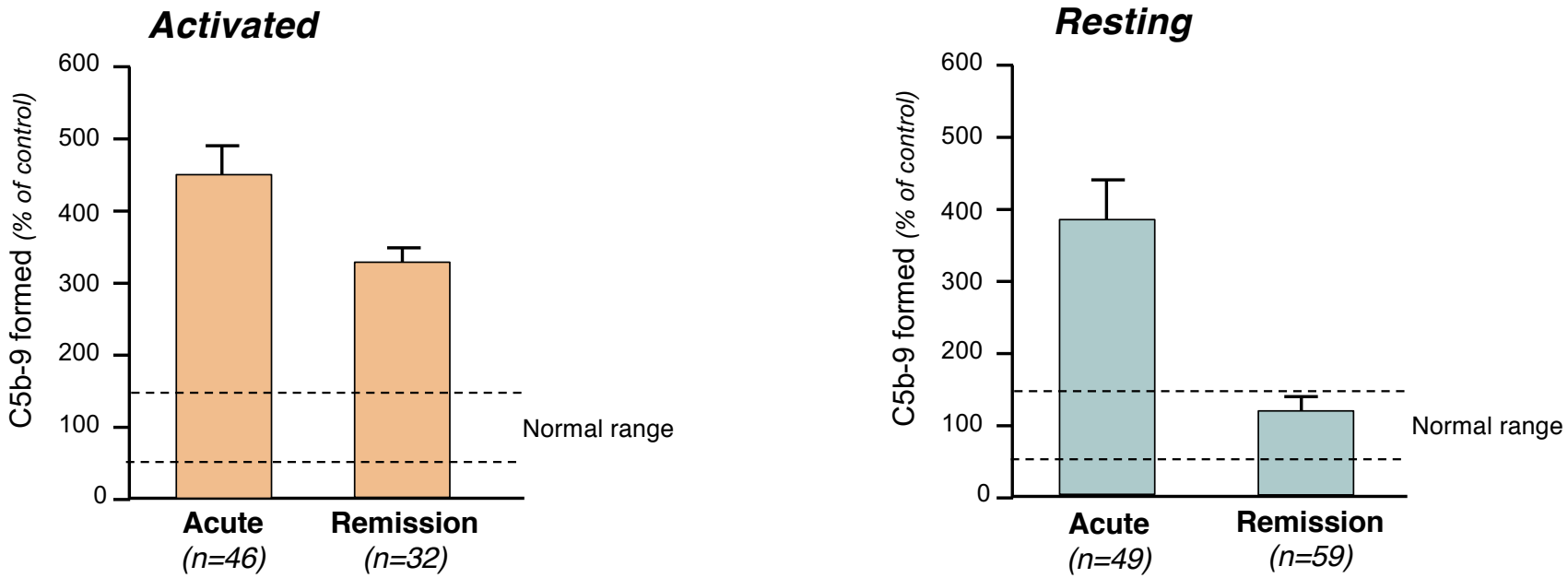
Noris et al. Blood, 2014, volume 124, Number 11

SOLID-PHASE RESTRICTED COMPLEMENT ACTIVATION IN aHUS



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



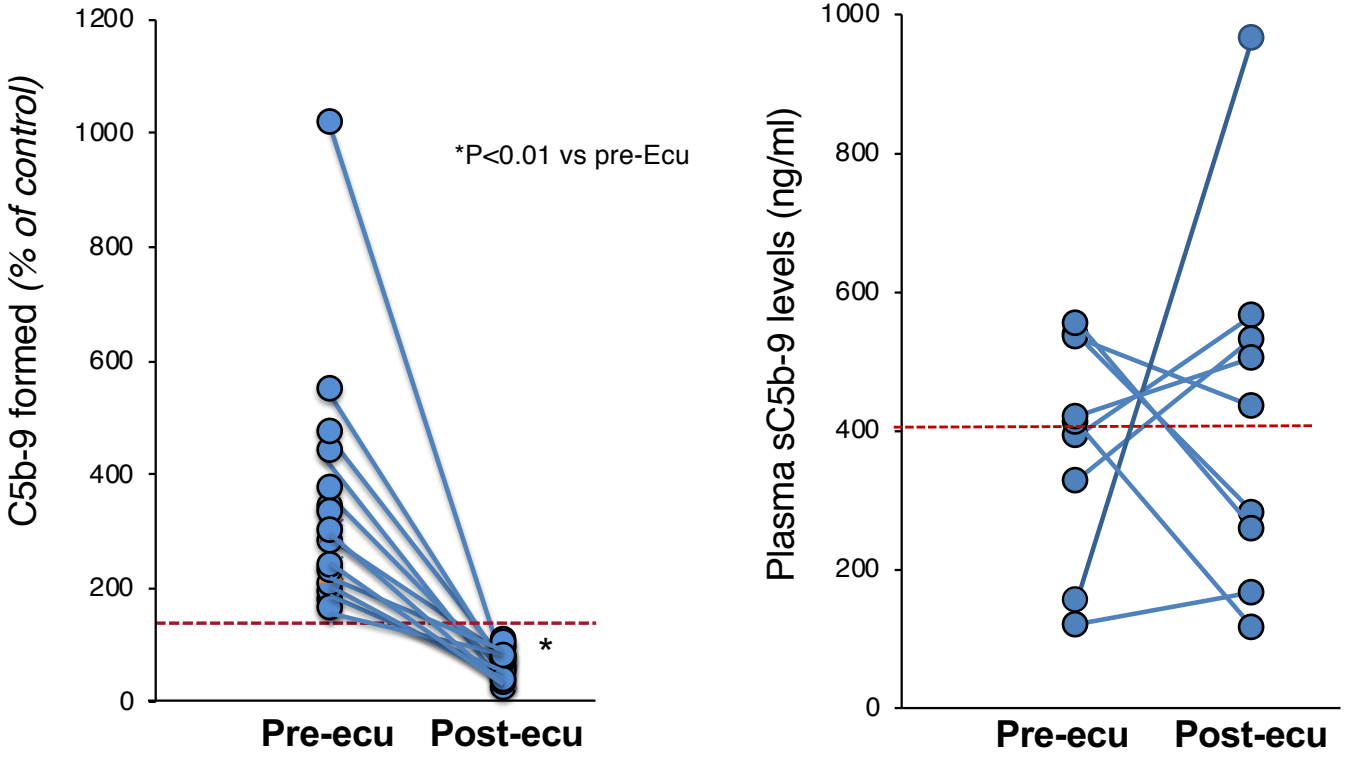


Activated: complement dysregulation (sensitivity 100 %)

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

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

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

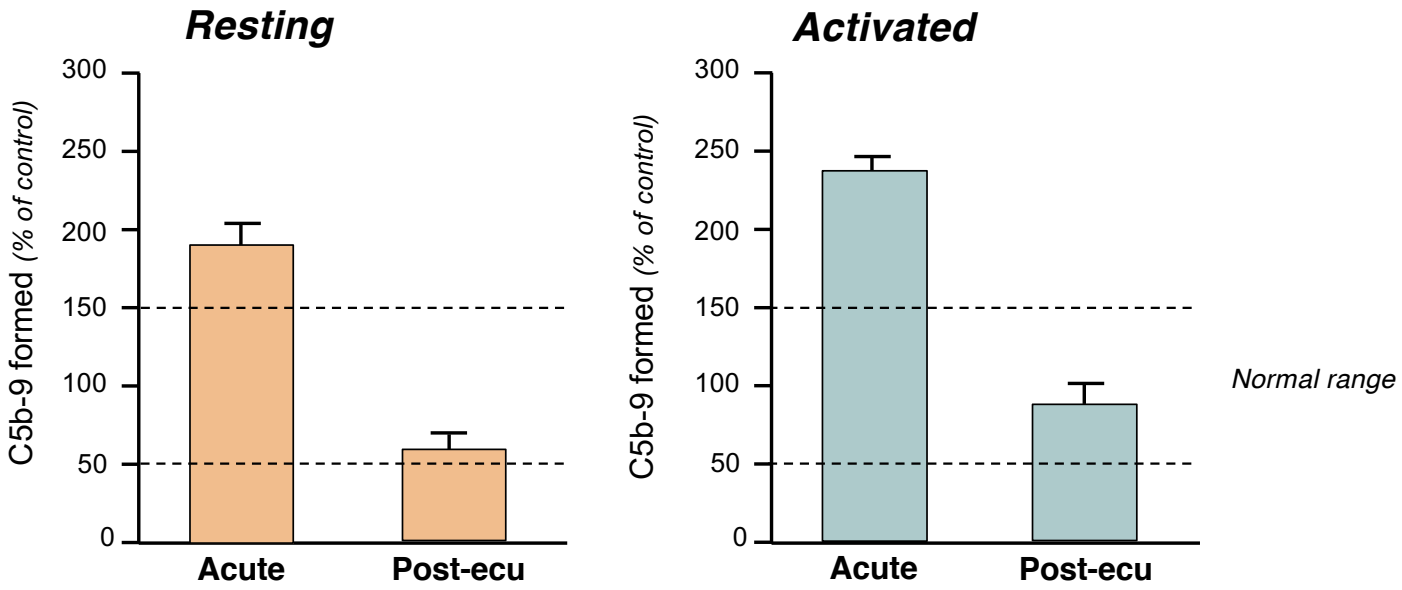


- *In 20 aHUS cases treated with Eculizumab, serum-induced C5b-9 deposits on ADP-activated HMEC-1 ex-vivo normalized after treatment*
- *No significant change was observed in pre- and post-Eculizumab plasma sC5b-9 levels*

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

Case

- Serum-induced C5b-9 deposits were higher than normal on resting and on activated HMEC-1
- Eculizumab treatment was started 10 days after onset and normalized hematological and renal parameters
- Serum-induced C5b-9 deposits normalized after eculizumab



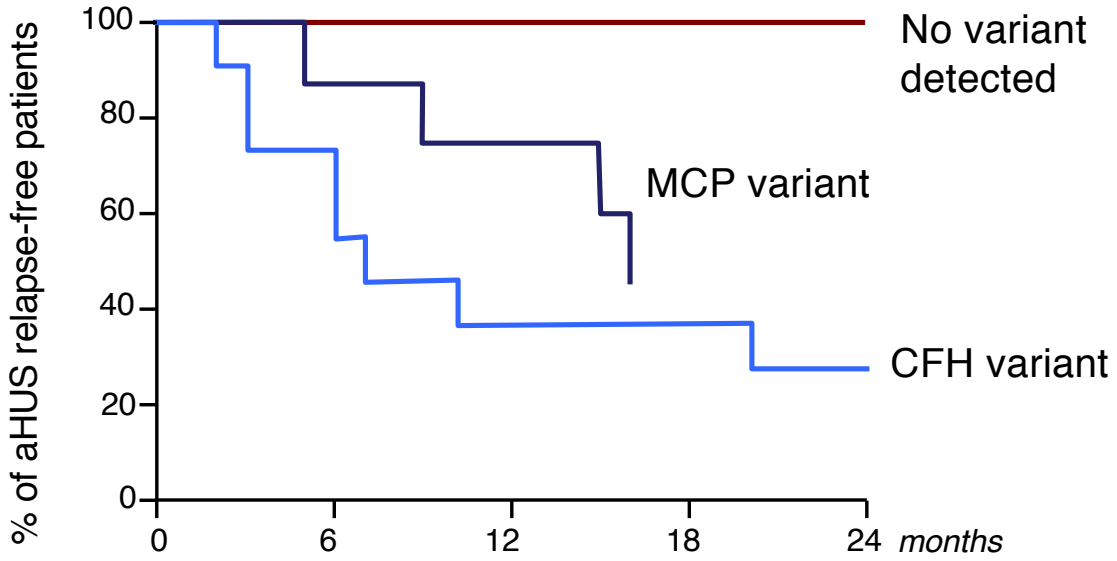
Content based on unpublished data and/or speaker experience

Every 15 days forever?

How to monitor and possibly tapering?

RISK OF ATYPICAL HEMOLYTIC UREMIC SYNDROME RELAPSE AFTER ECULIZUMAB DISCONTINUATION

38 patients (24 women - 9 children and 29 adults)

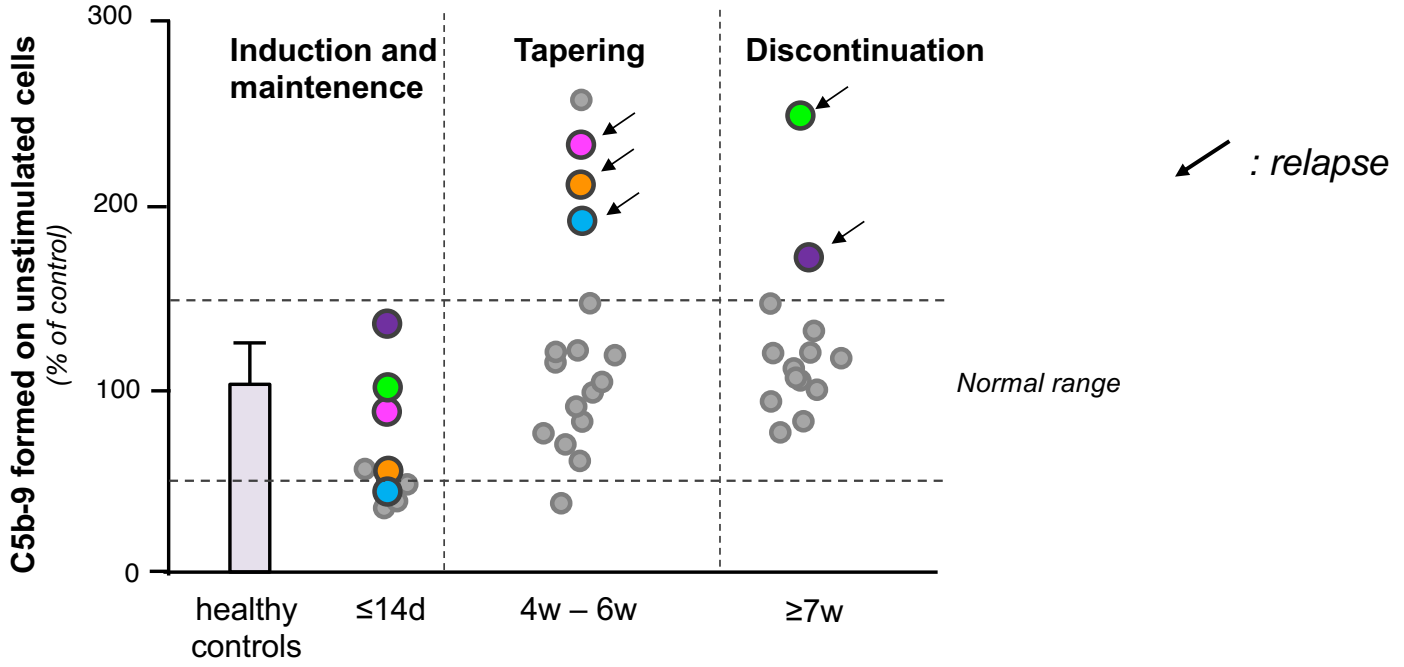


The decision to stop eculizumab was made by the clinician in charge of the patient after at least 3 months of stabilization of renal function and proteinuria

"Our series does not provide sufficient data for patients with C3 (n=1), CFI (n=2), or anti-FH antibodies (n=1)"

Fakhouri et al, *CJASN*, 2017

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

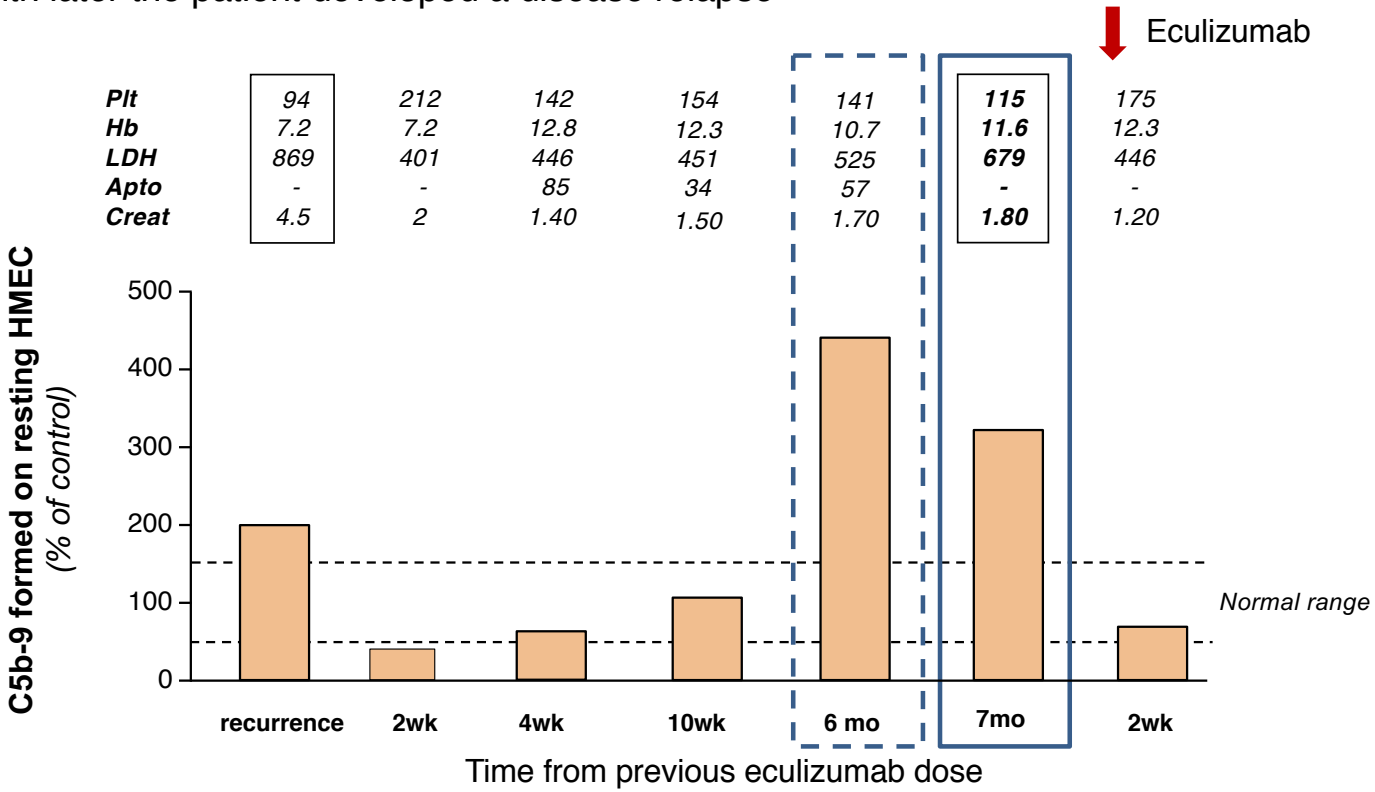
Case #2

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



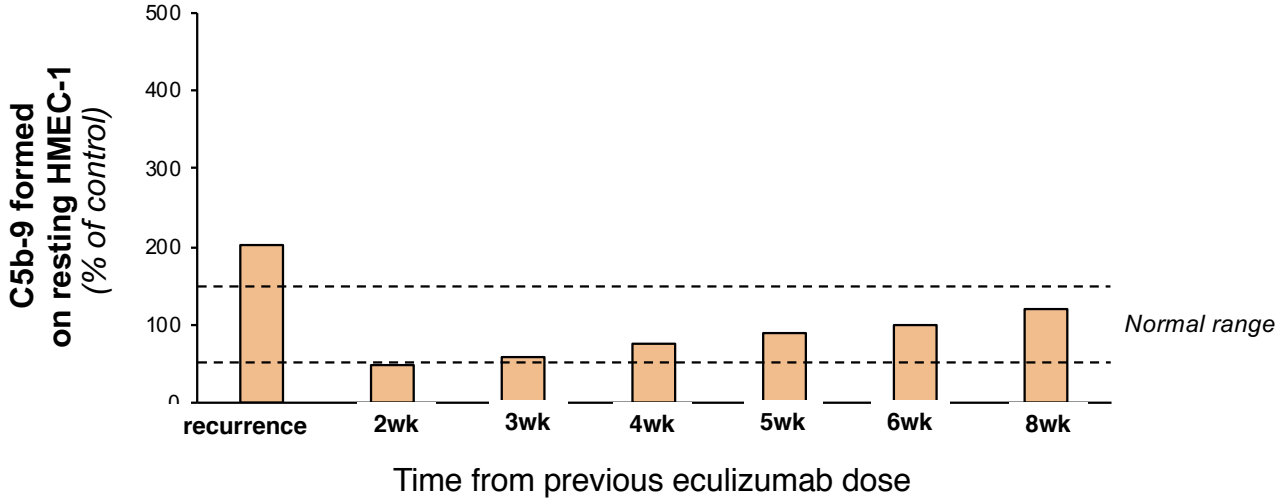
Galbusera et al., *Am J Kidney Dis,* 2019

Case #3

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

Summary

- About 90% of childhood cases of HUS are caused by infections with bacteria producing shiga-like toxins that induce endothelial P-selectin expression, C3 activation and microvascular thrombosis
- 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.
- A subgroup of aHUS patients carry anti-FH autoantibodies
- In aHUS local complement activation on endothelial surface (rather than fluid phase) plays a pathogenic role.
- The ex-vivo serum-induced C5b-9 deposition on endothelial cells is a sensitive and specific test of complement activation in aHUS and may represent a tool toward individualized anticomplement therapy

.

Thanks!

Caterina Mele

Roberta Donadelli

Elisabetta Valoti

Rossella Piras

Marta Alberti

Paola Cuccarolo

Matteo Breno

Miriam Galbusera

Sara Gastoldi

Erica Daina

Elena Bresin

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Sara Gamba

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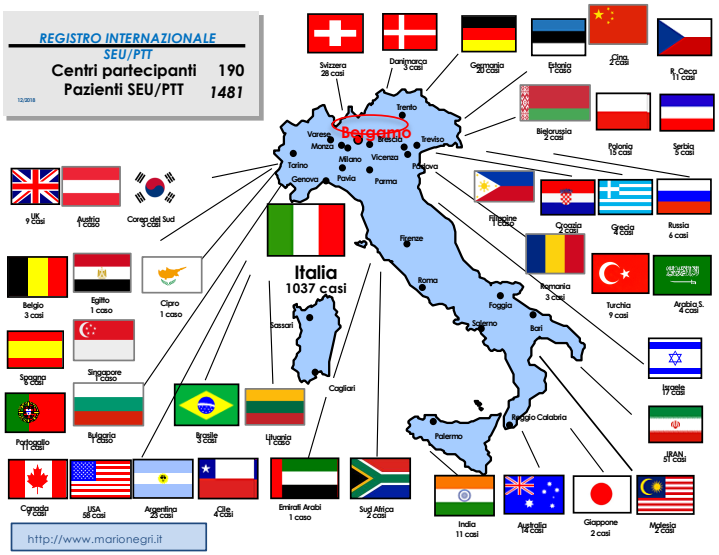

Santiago Rodriguez de Cordoba

Tim Goodship

Veronique-Fremeaux Bacchi

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