

Complement and its role in eHUS



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DISCLOSURES

**R.W. has received
unrestricted educational grants from,
and attended expert meetings organized by,
Alexion Pharmaceuticals (Cheshire, CT),
the maker of eculizumab.**

CONTENTS

HUS Forms

History of eculizumab therapy

Summary

HUS / aHUS / eHUS

**HUS: Hemolytic uremic syndrome,
a thrombotic microangiopathy defined by**

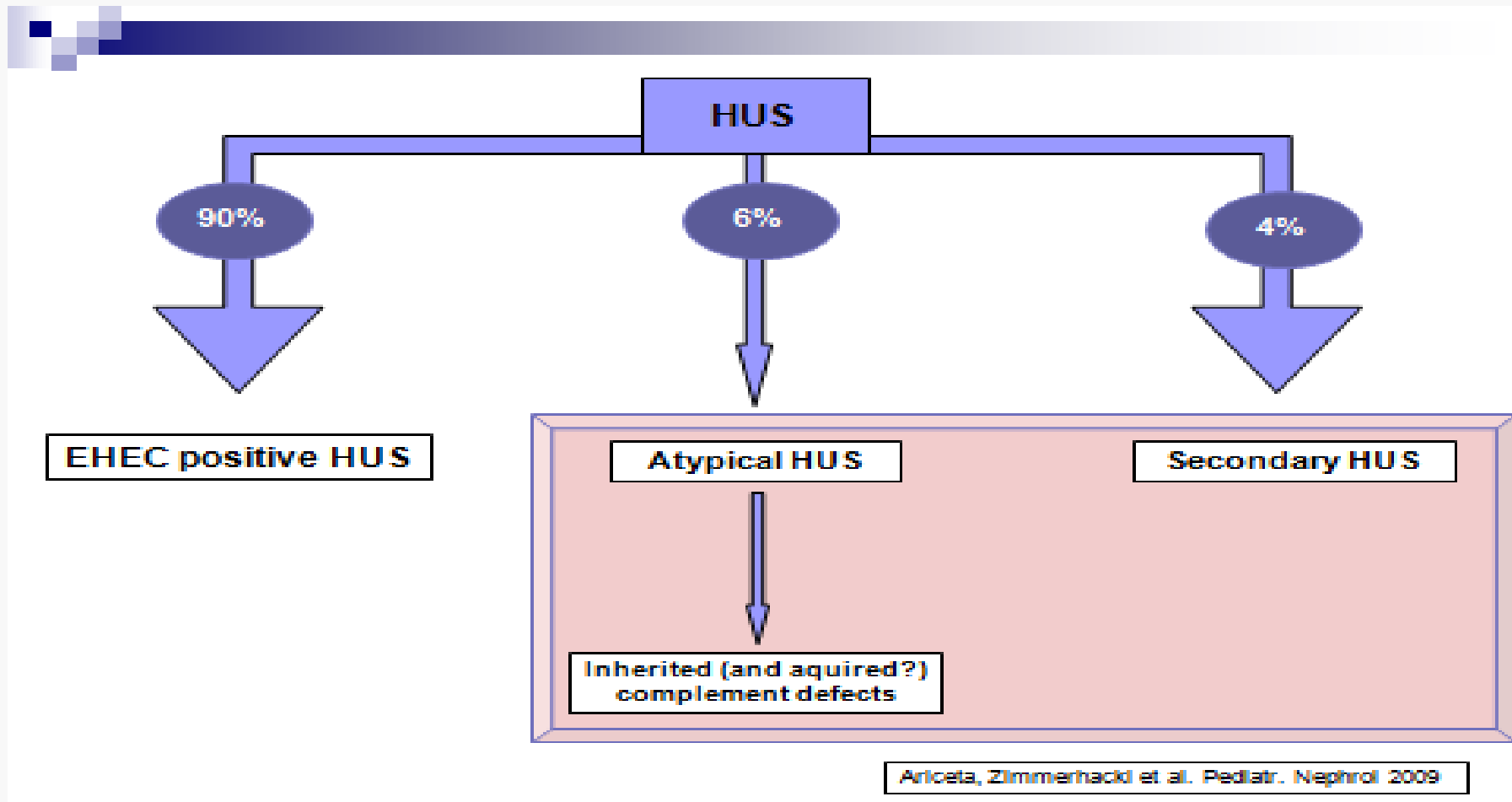
- Hemolytic anemia: Hb < 10g/dL with fragmented erythrocytes
- Thrombocytopenia: < 150×10^9 platelets/L
- Acute renal failure: Creatinine > upper normal limit for age

aHUS: Atypical HUS

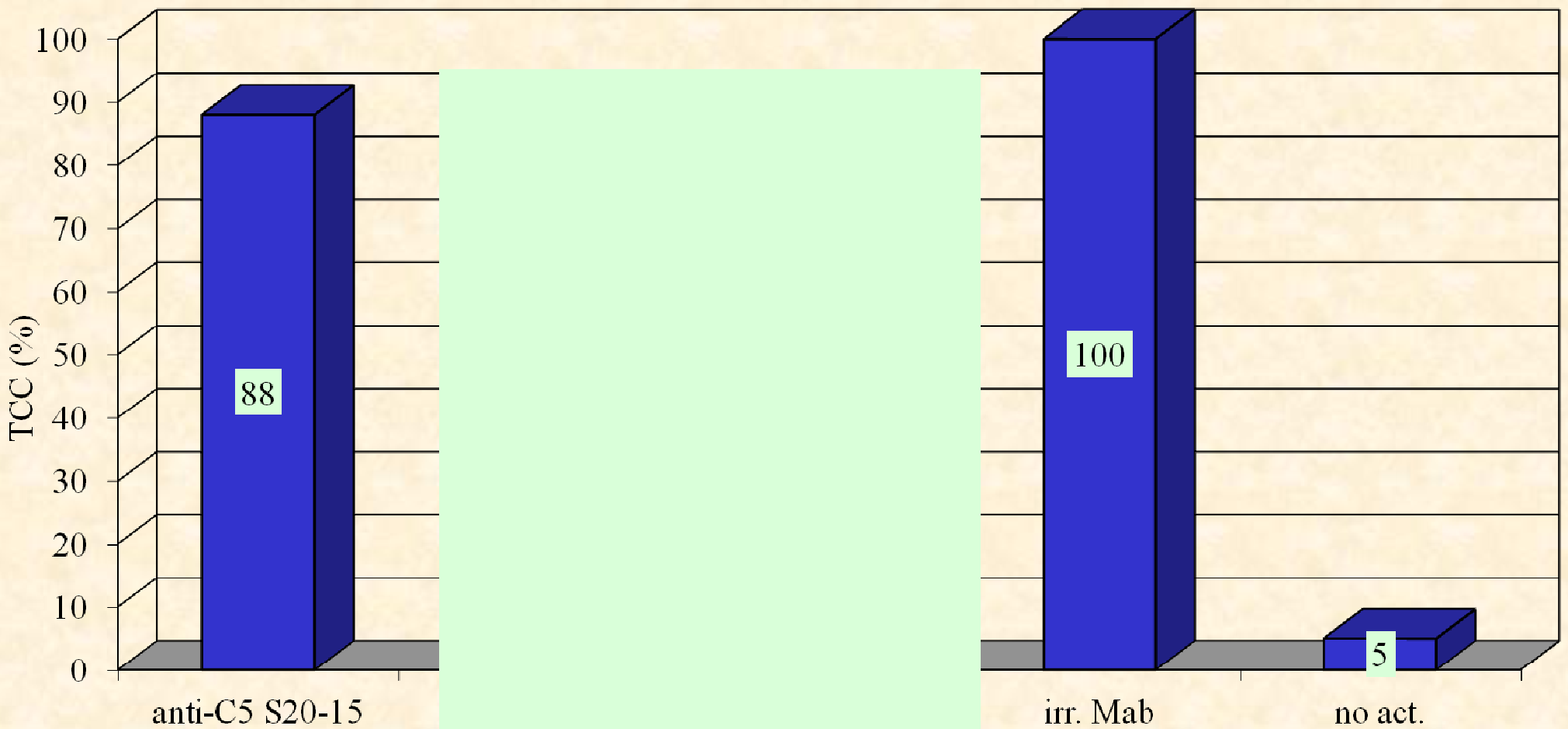
eHUS: Escherichia coli-associated HUS

Secondary HUS – no abbreviation!

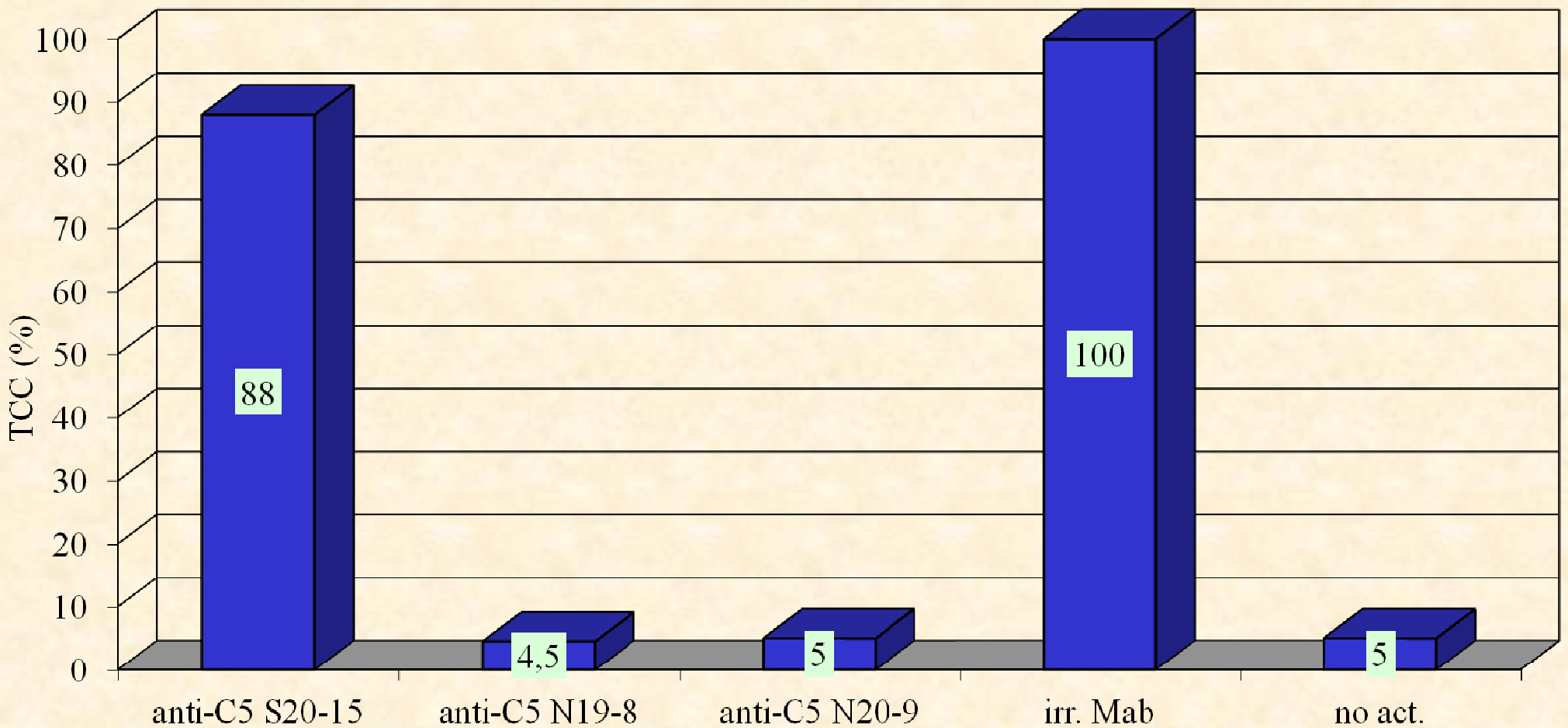
HUS FORMS - FREQUENCIES



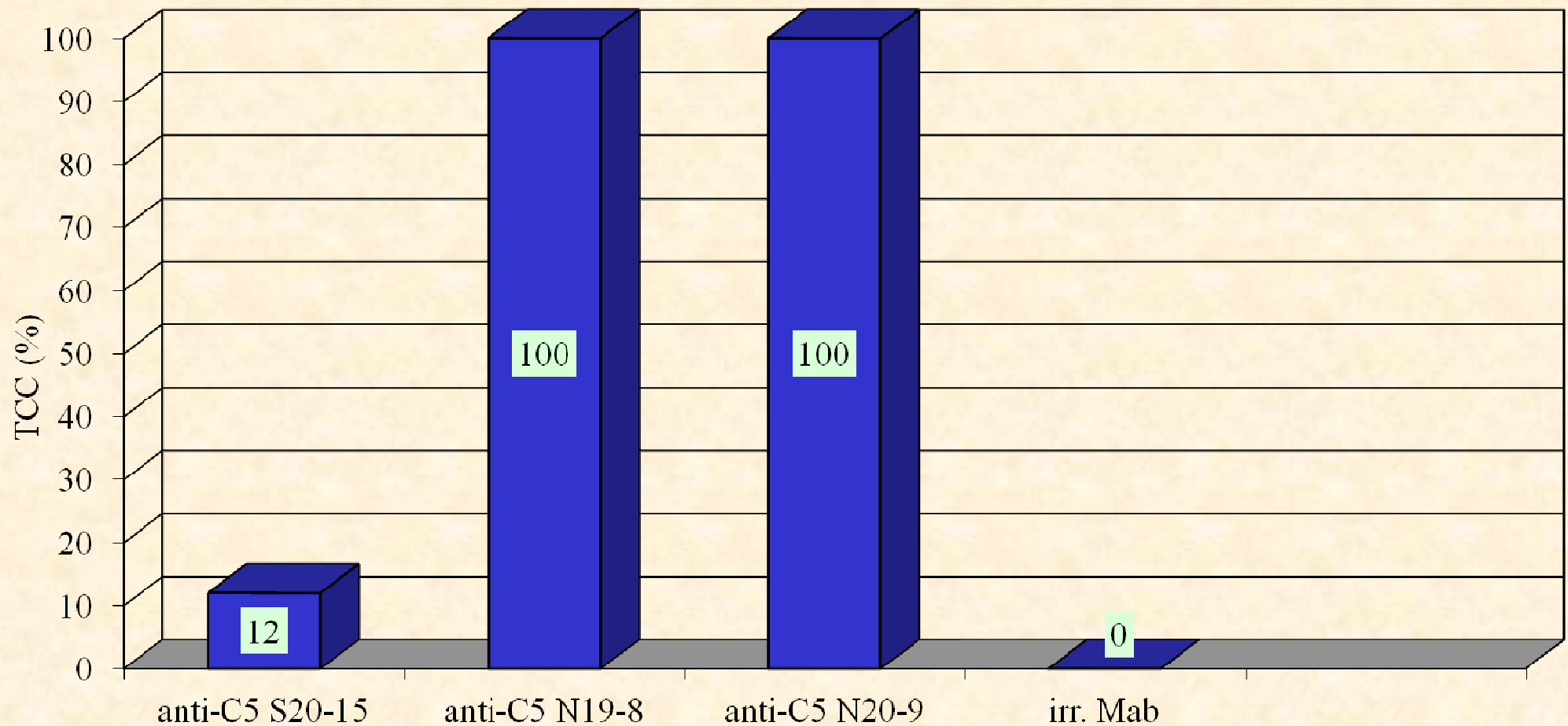
INHIBITION OF TCC FORMATION



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INHIBITION OF TCC FORMATION



Complement Inflamm 1991;8:328

EHUS & COMPLEMENT & ECULIZUMAB – TIMELINE I

**1991: First anti-human C5 monoclonal antibody
inhibiting TCC formation: N19-8**

Complement Inflamm 1991;8:328

**1995: Alexion shows preservation of inhibitory
function in N19-8 recombin. single chain Fv**

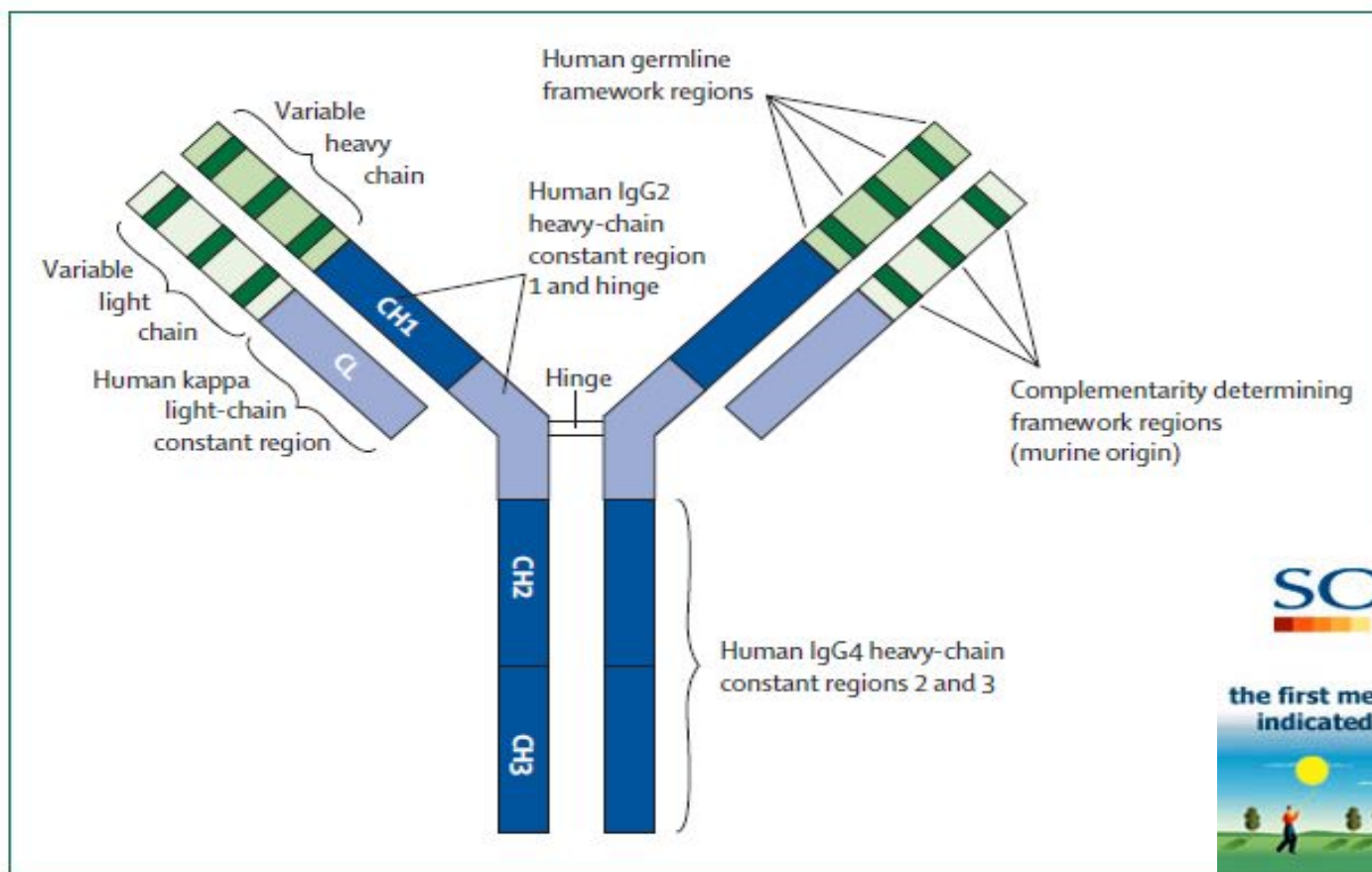
Mol Immunol 1995;32:1183

1996: Alexion introduces 5G1.1

Mol Immunol 1996;33:1389

2002: Description of eculizumab

Curr Op Invest Drugs 2002;3:1017



SOLIRIS™
(eculizumab)

the first medication specifically
indicated to treat all patients
with PNH



EHUS & COMPLEMENT & ECULIZUMAB – TIMELINE I

**1991: First anti-human C5 monoclonal antibody
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Complement Inflamm 1991;8:328

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1996: Alexion introduces 5G1.1

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2004: Use of eculizumab in PNH

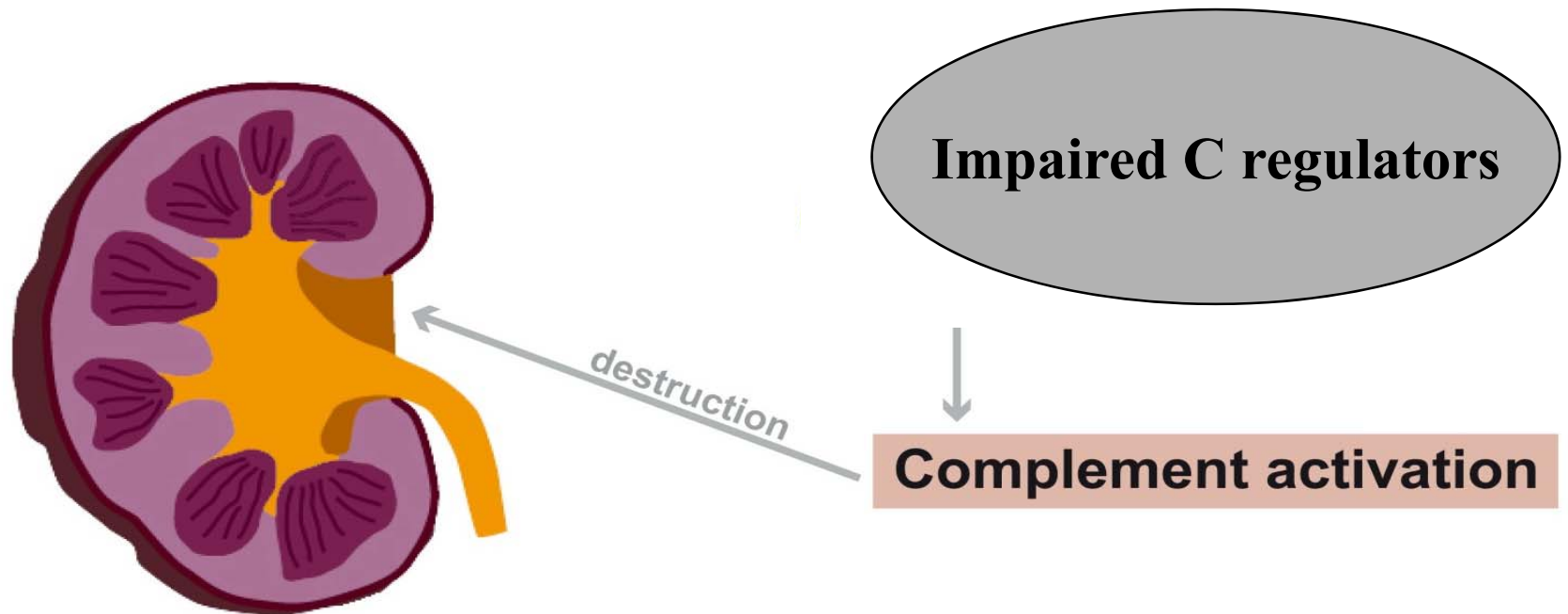
NEJM 2004;350:552

2008: Use of eculizumab in aHUS

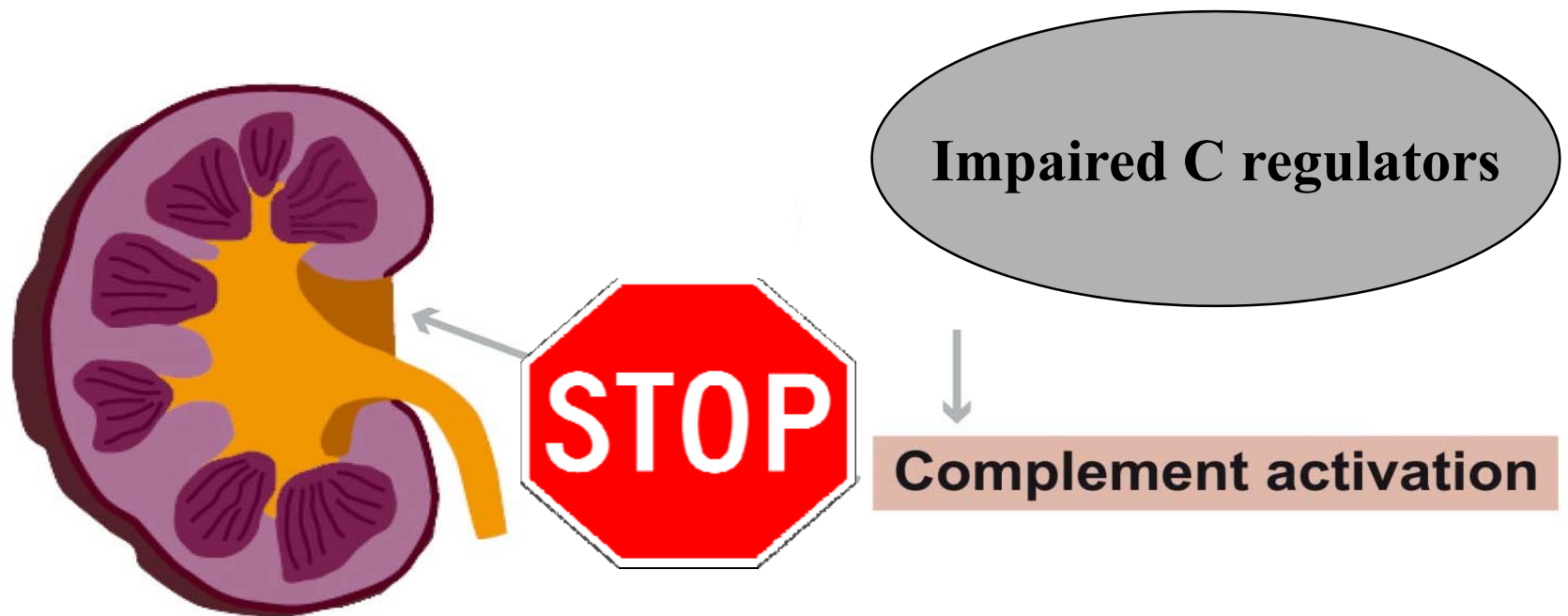
NEJM 2009;360:542

NEJM 2010;362:1746

PATHOPHYSIOLOGY OF ATYP. HUS



PATHOPHYSIOLOGY OF ATYP. HUS

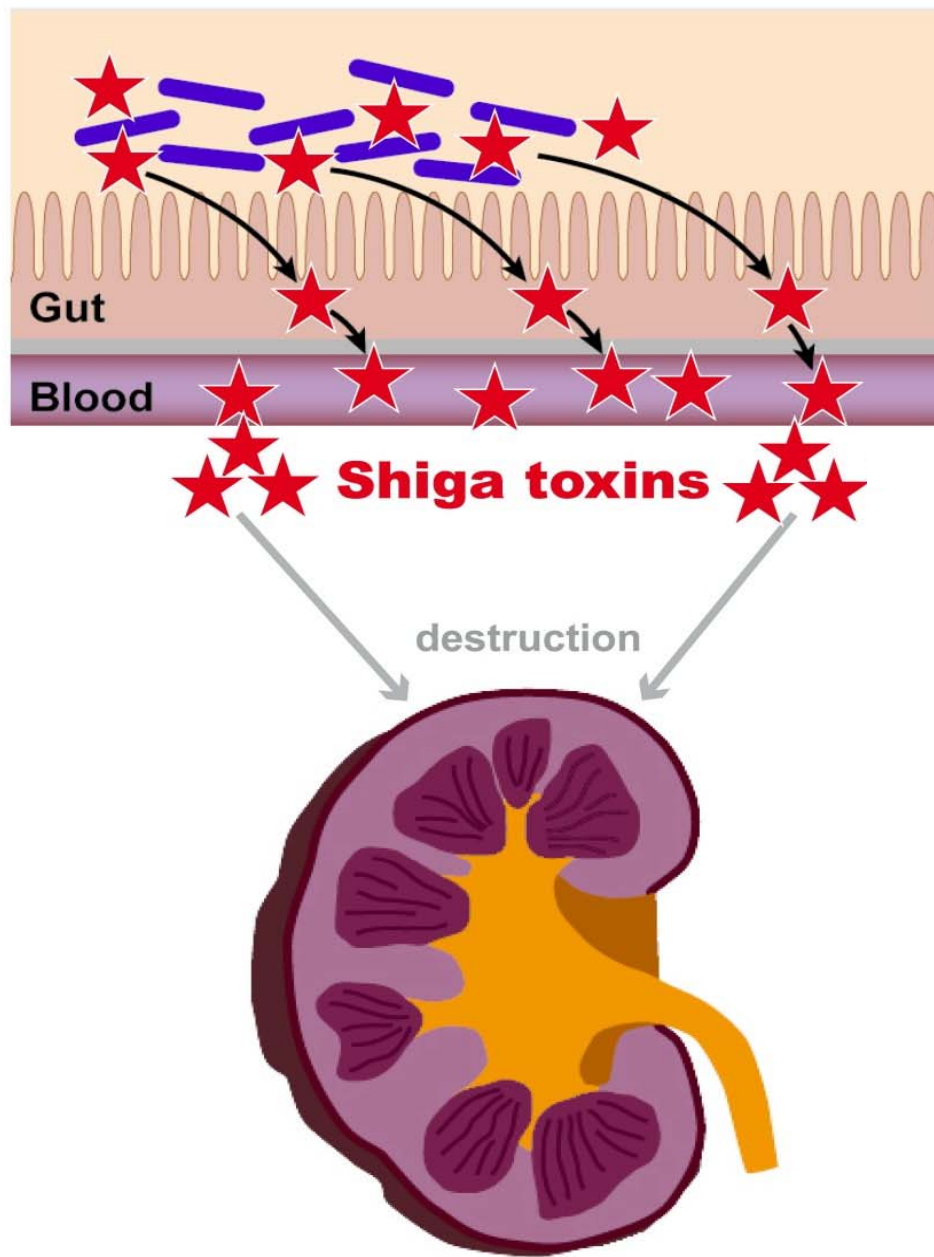


eHUS / SHIGATOXIN 2

Enterohemorrhagic Escherichia coli (EHEC) cause hemolytic uremic syndrome (eHUS) in 5-20% of the cases, which is lethal in 5%

Shiga toxin 2 (Stx 2) is considered to represent the most important virulence factor of EHEC

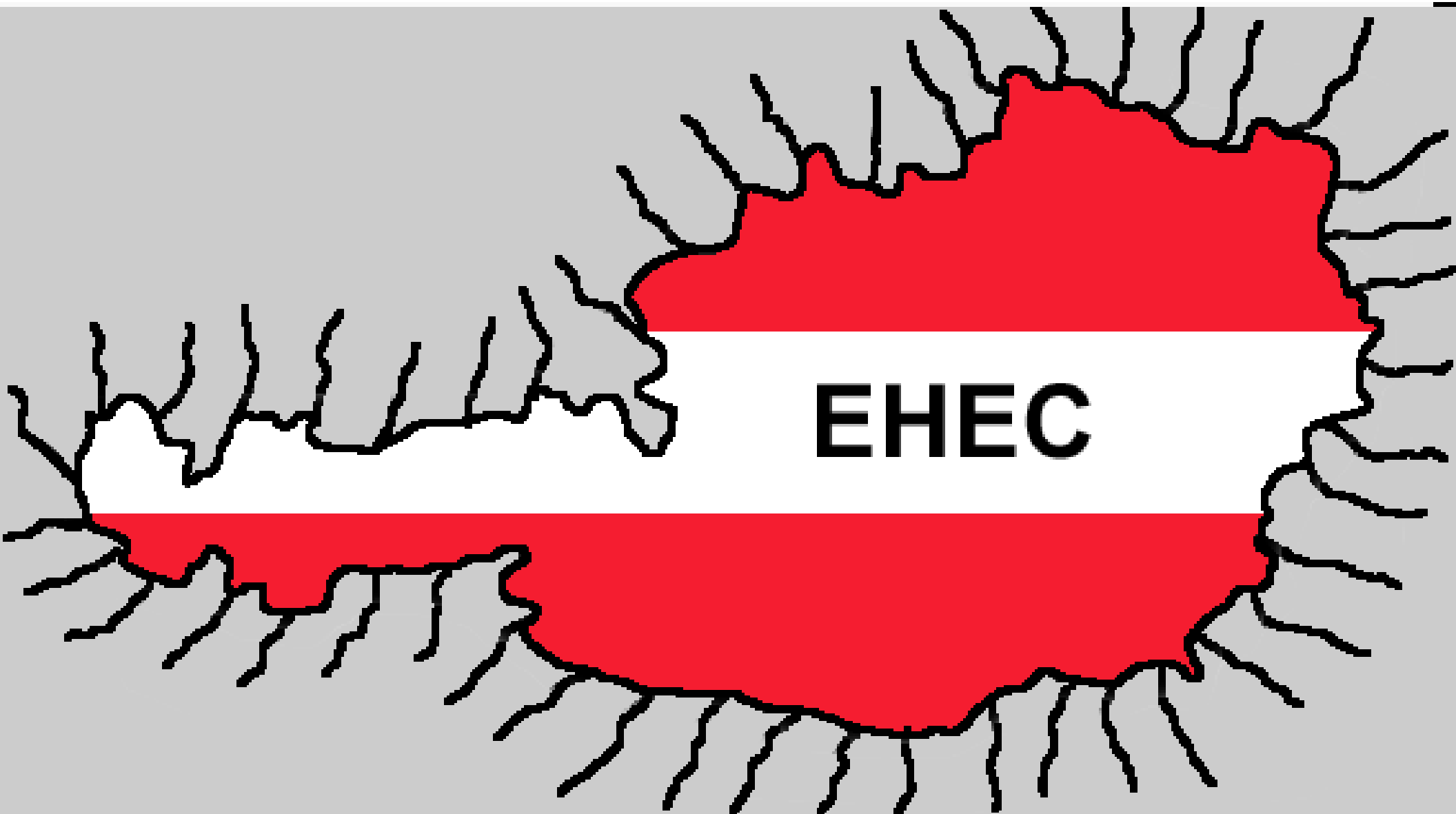
Why was a complement inhibitor, eculizumab, used in the German 2011 EHEC outbreak?



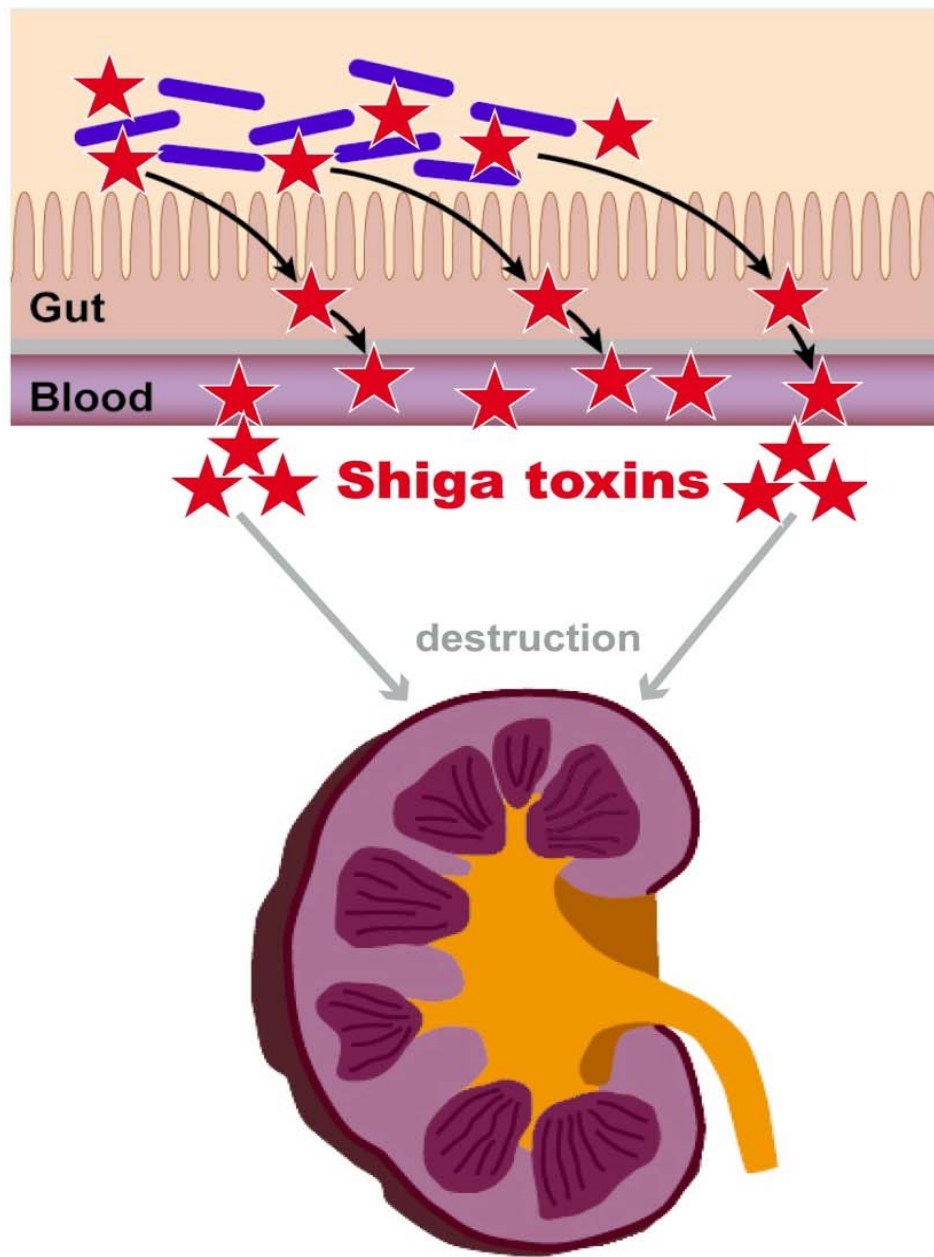
E. COLI-ASSOCIATED HUS (eHUS)

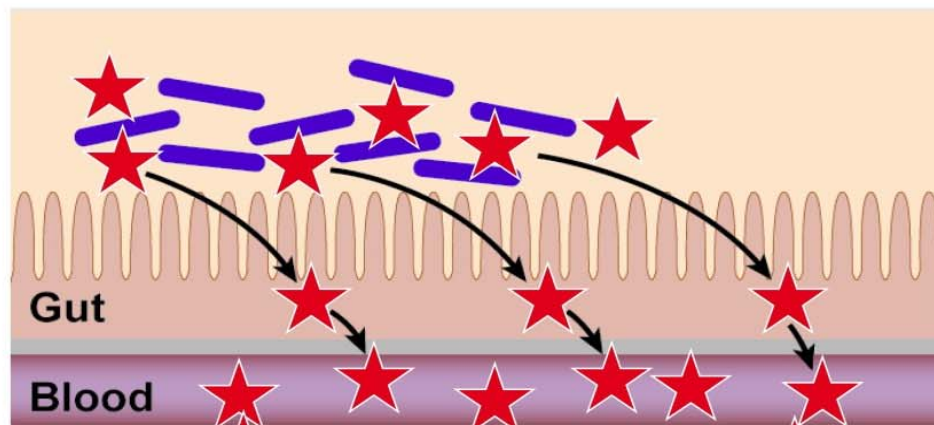
**Only a minority (5-20%) develops eHUS
→ host factors may be involved as well!**

Is complement also involved in eHUS?

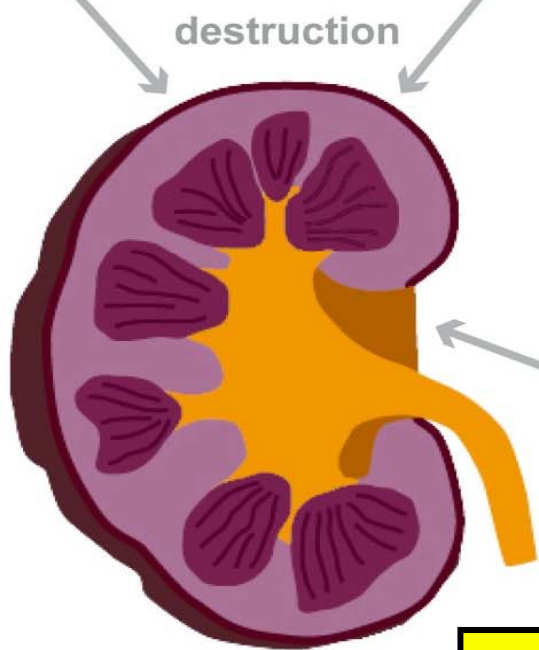


Austrian Reference Centre for EHEC till 2009
Director: Reinhard Würzner





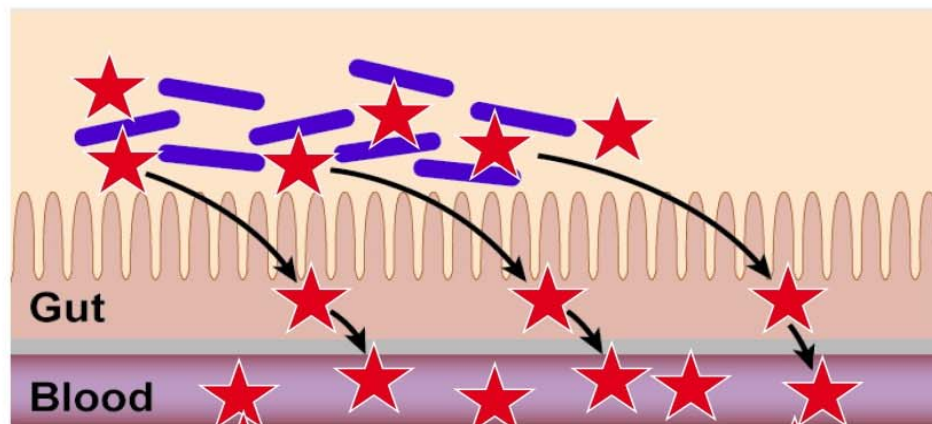
Shiga toxins



destruction

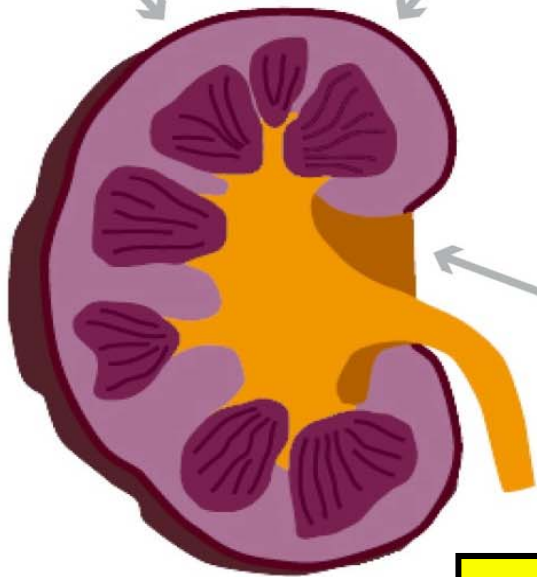
Complement activation

J Immunol 2009;154:4734

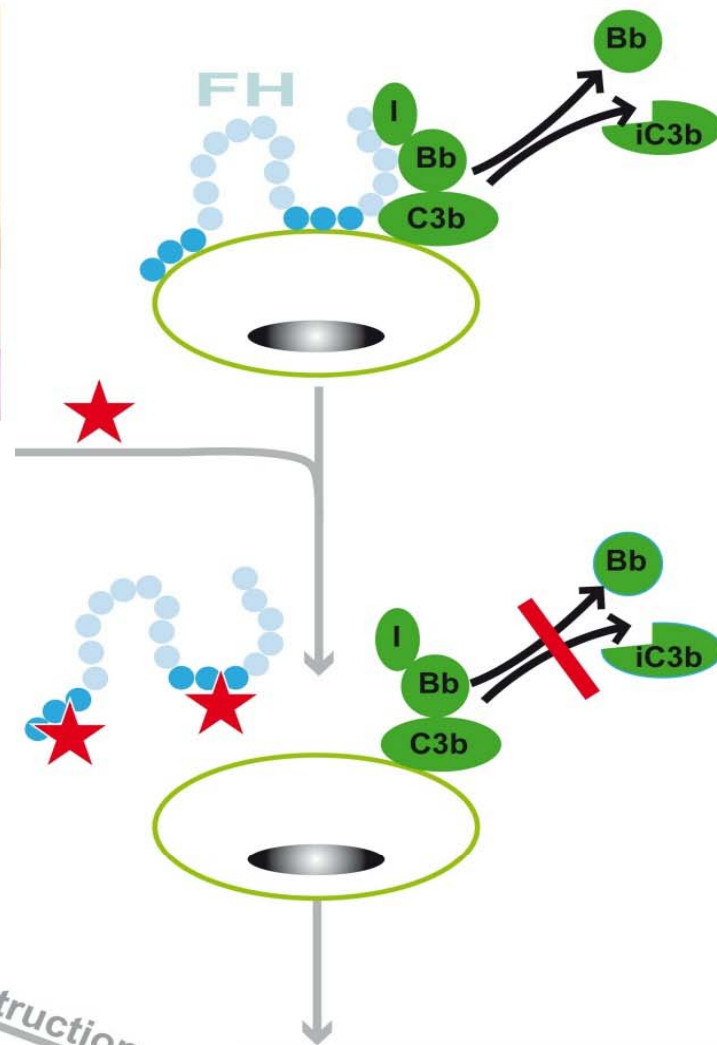


Shiga toxins

destruction



destruction



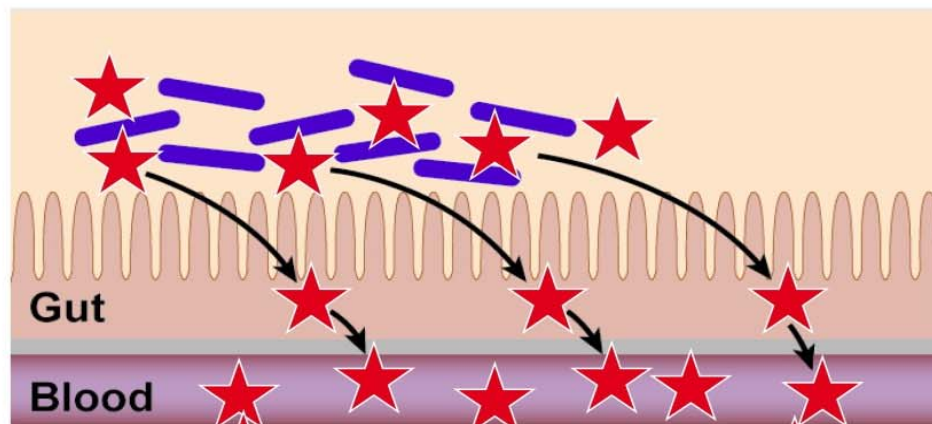
Complement activation

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SHIGA TOXIN ATTACKS COMPLEMENT

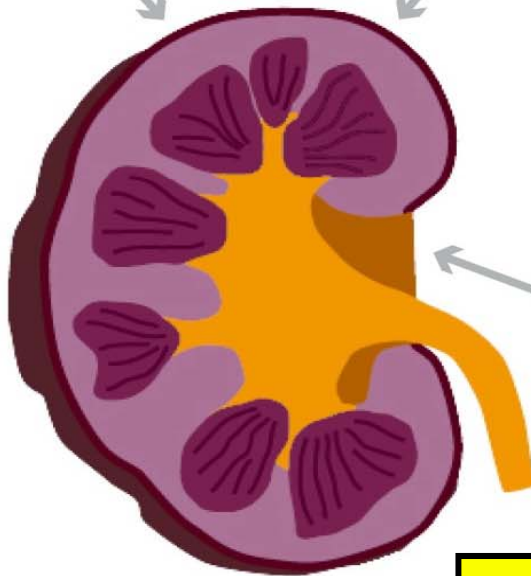
**Stx2 attacks complement in a dual way:
it directly activates complement enzymatically &
decelerates FH cofactor activity on target cells.**

**Complement inhibition in eHUS
may be clinically relevant!**

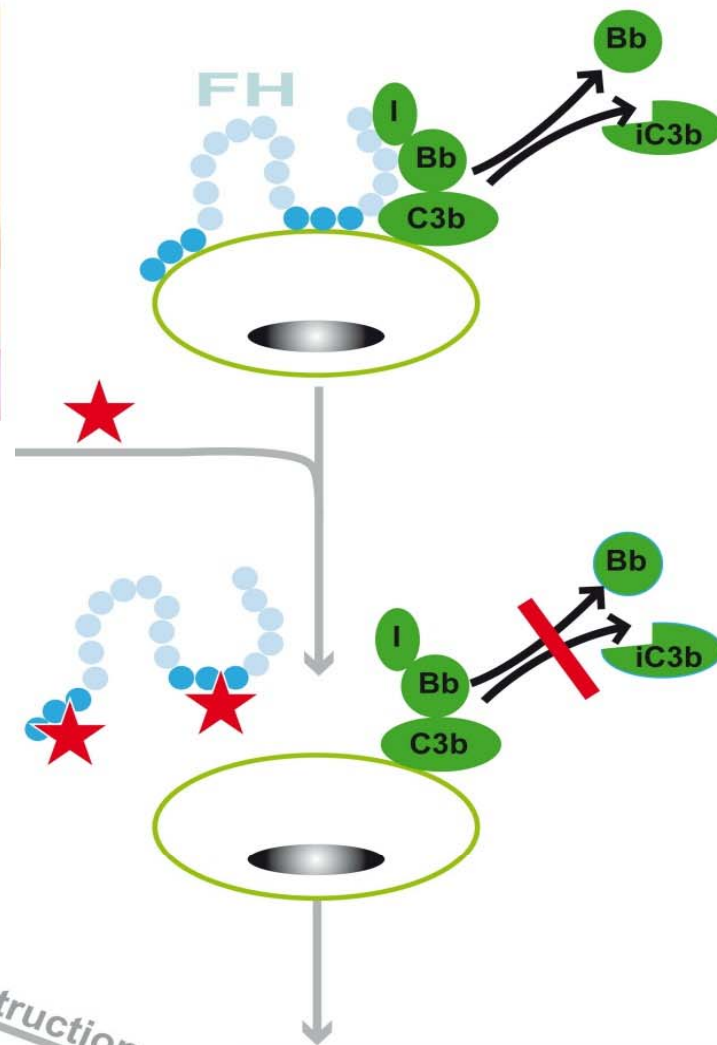


Shiga toxins

destruction

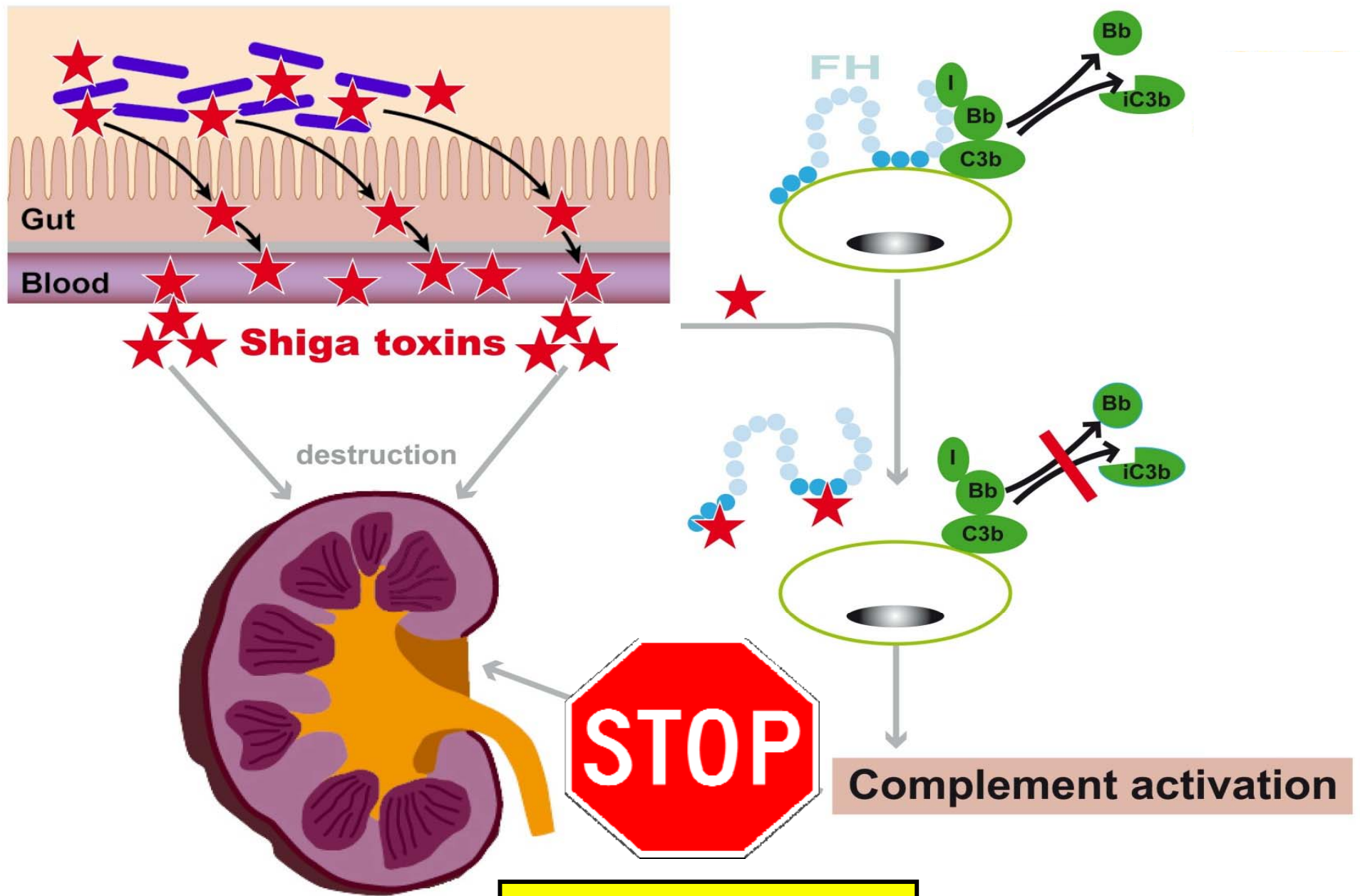


destruction



Complement activation

J Immunol 2009;154:4734



J Immunol 2009;154:4734

eHUS & COMPLEMENT & ECULIZUMAB – TIMELINE II

C activation products in HUS serum

CJASN 2009;4:1920

C activation products on HUS blood cells

Blood 2011;117:5503

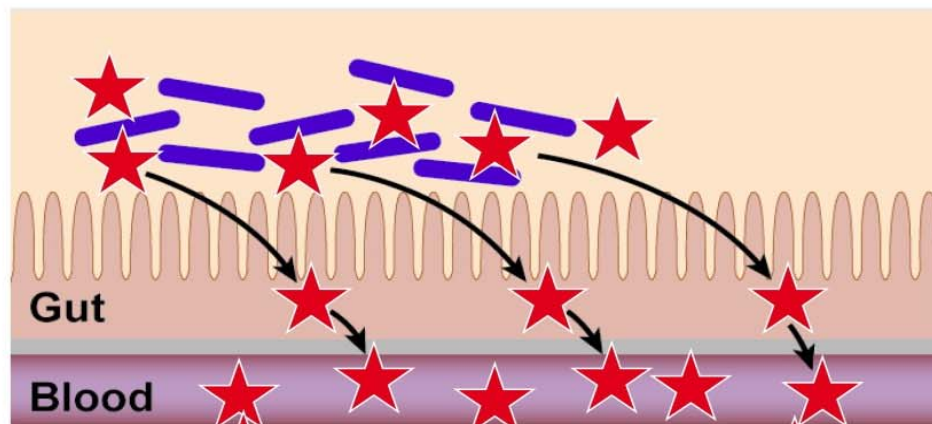
J Immunol 2011;187

**Marked clinical improvement by eculizumab in three
3 yr. old eHUS patients with devastating prognoses**

NEJM 2011;364:2561

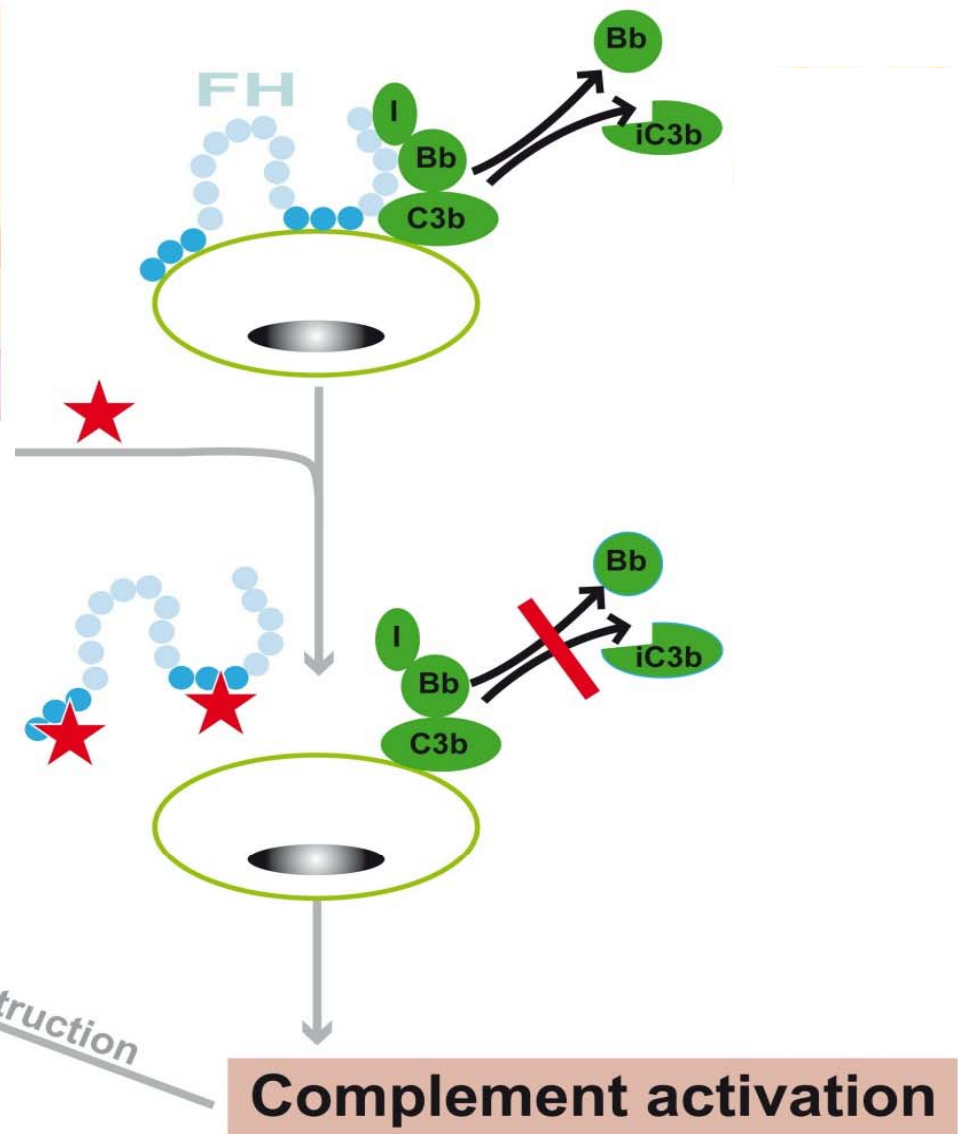
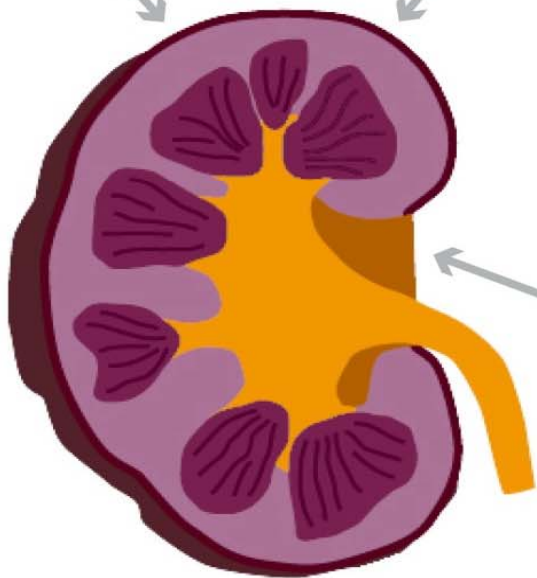
**EHEC O104:H4 outbreak 2011:
eculizumab improved the outcome of
many of the ~400 treated HUS patients**

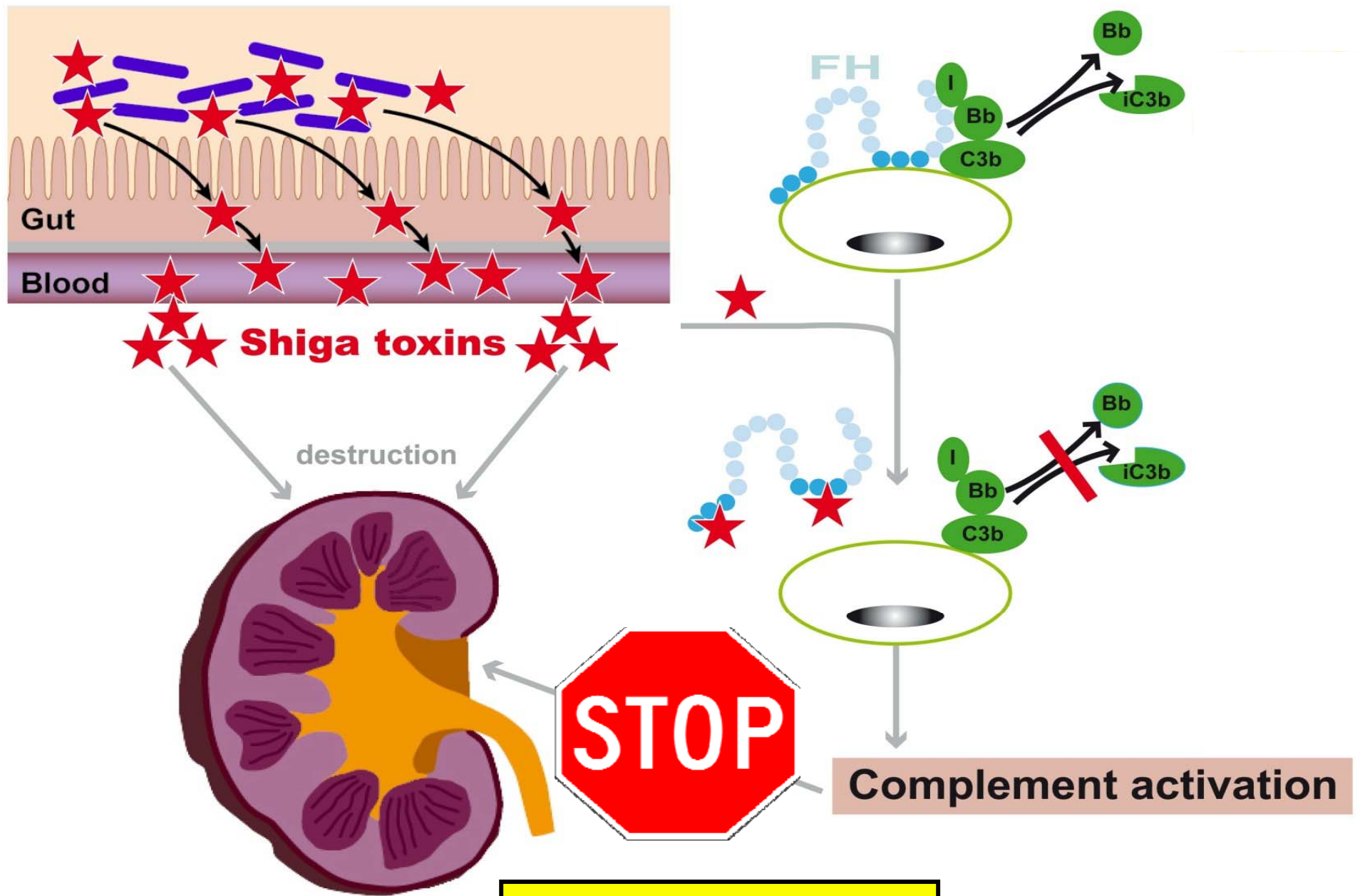
**EMBO Mol Med
2011;3:617**



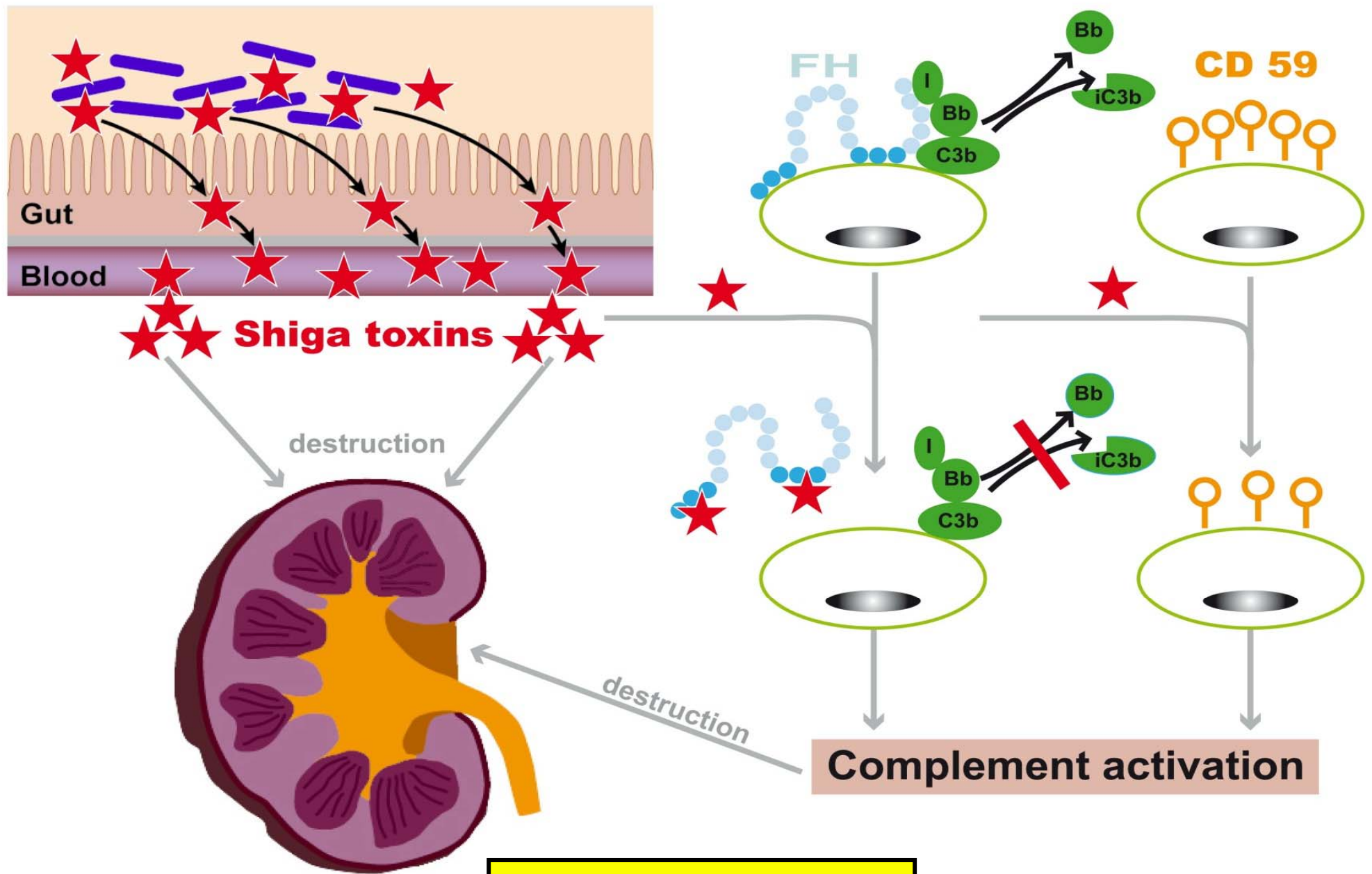
Shiga toxins

destruction

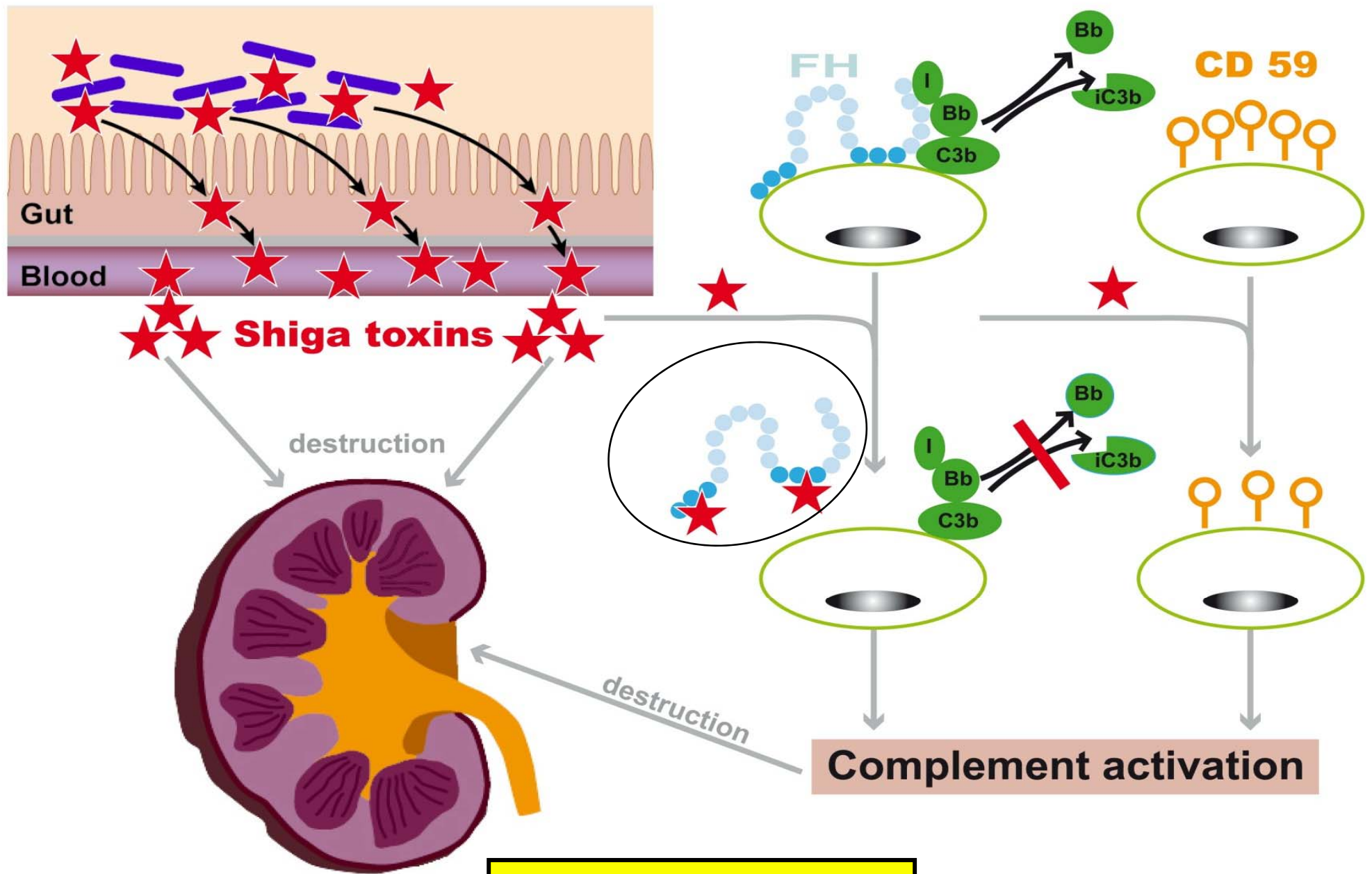




EMBO Mol Med 2011;3:617

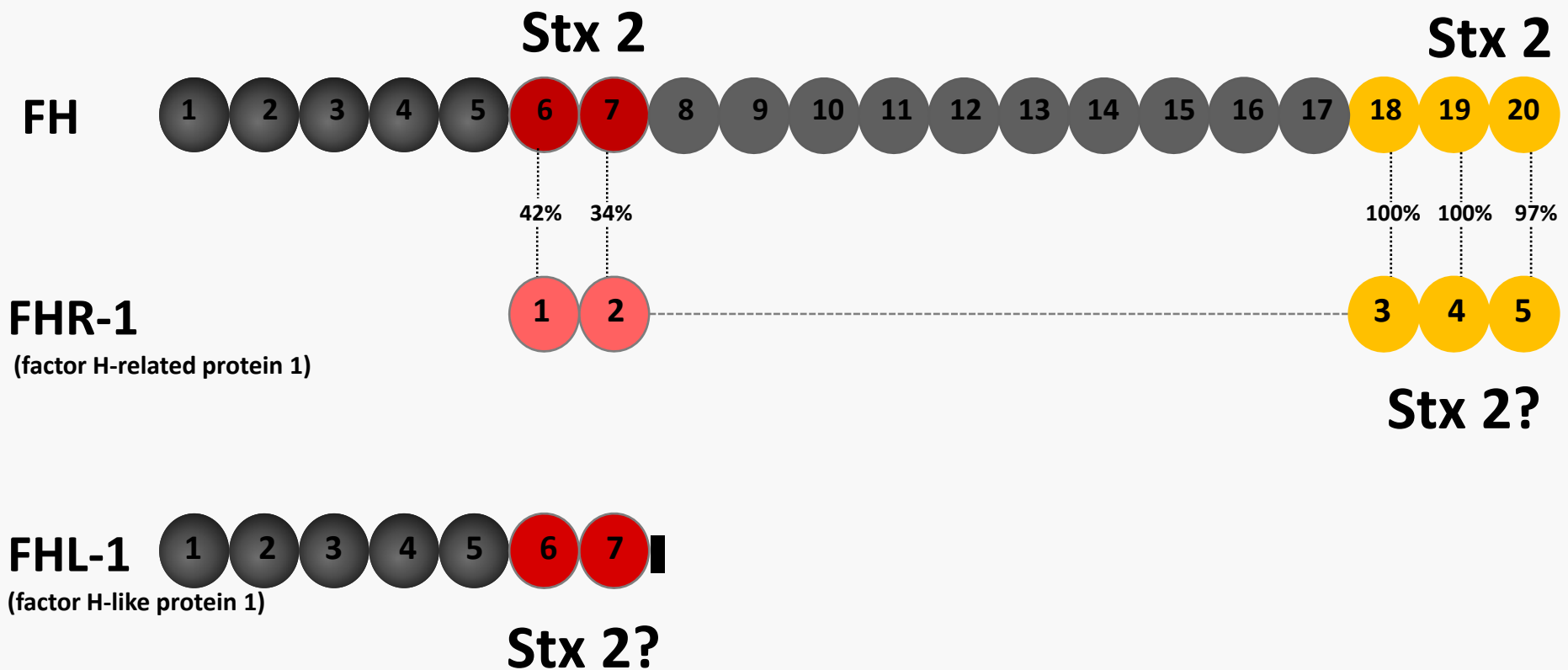


Infect Immun 2013;81:2678

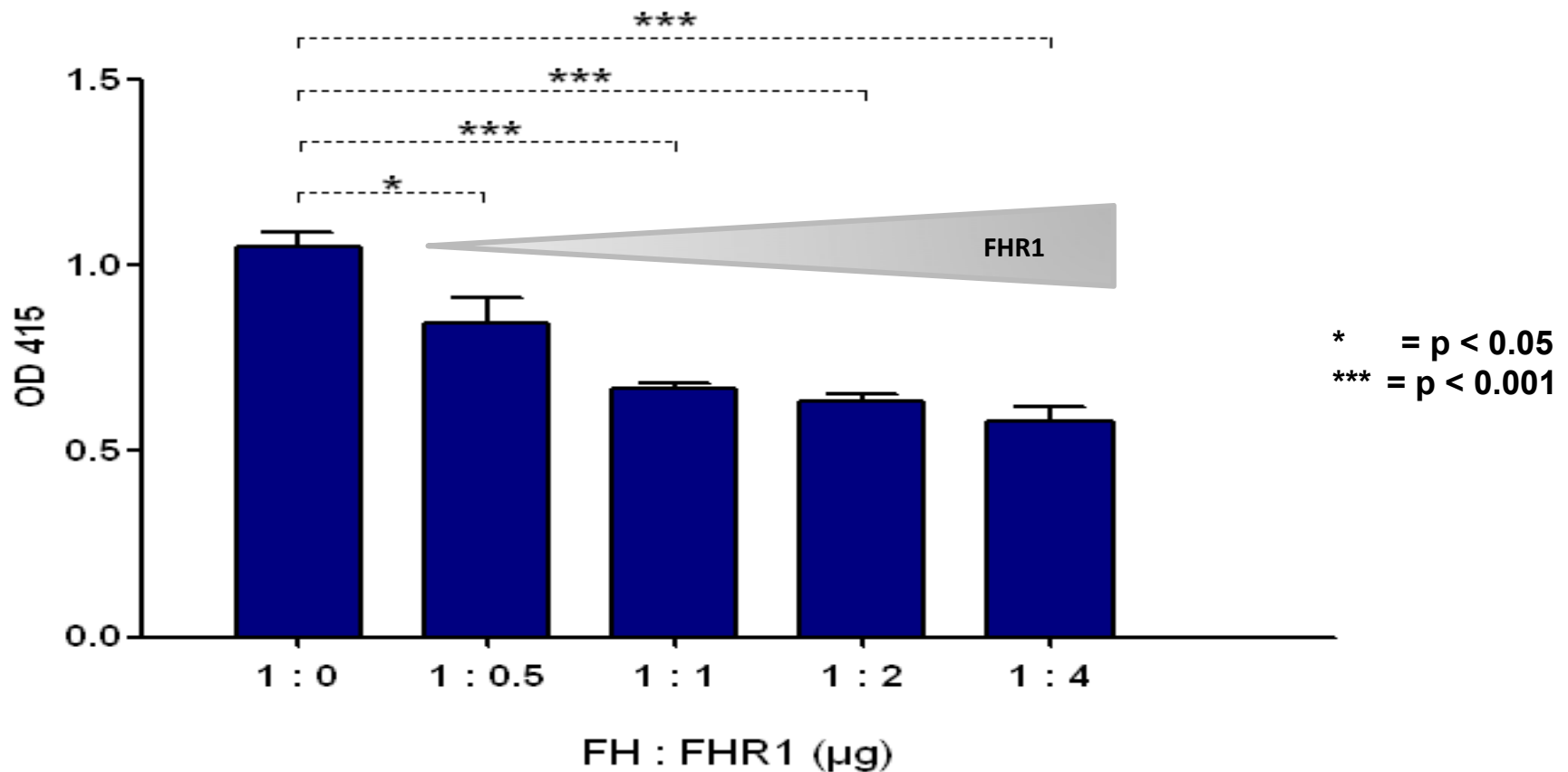


Infect Immun 2013;81:2678

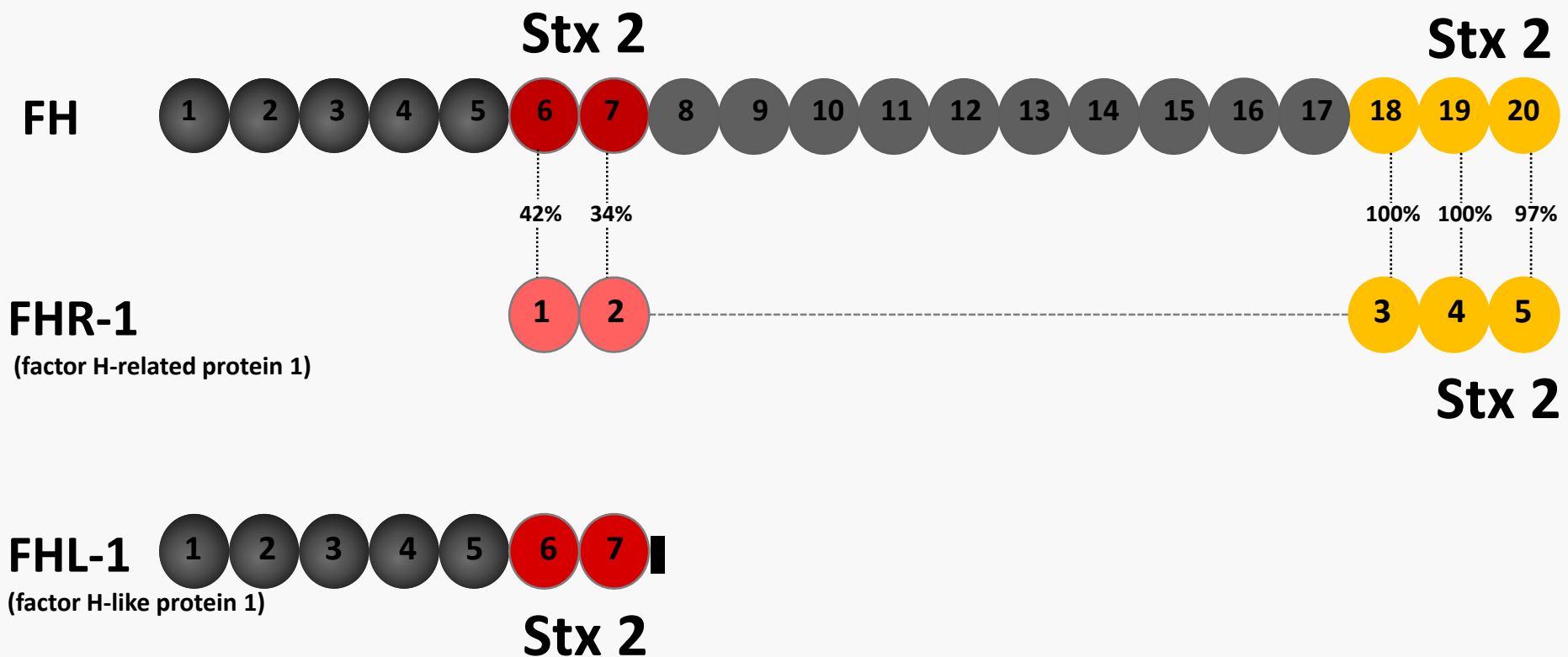
DOES Stx 2 ALSO BIND TO PROTEINS OF THE FH FAMILY?



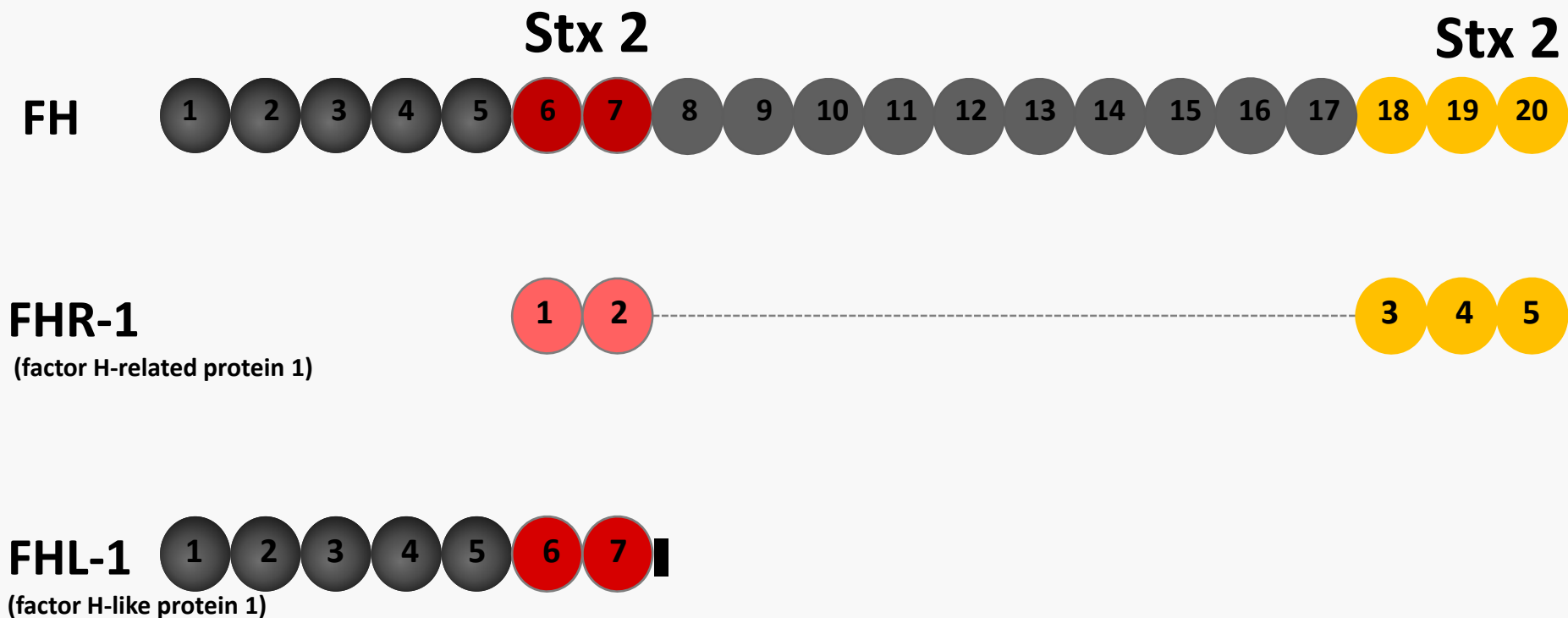
FHR-1 COMPETES WITH FH FOR Stx2 BINDING



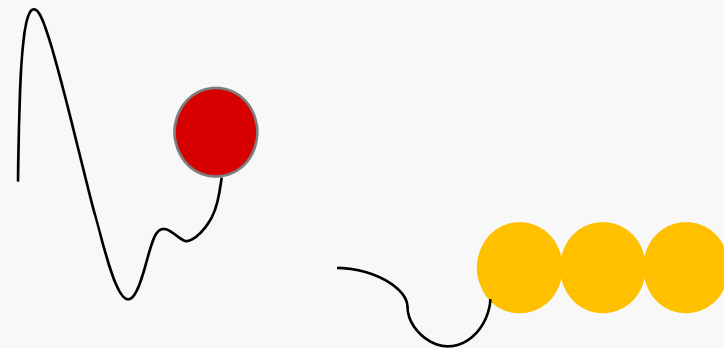
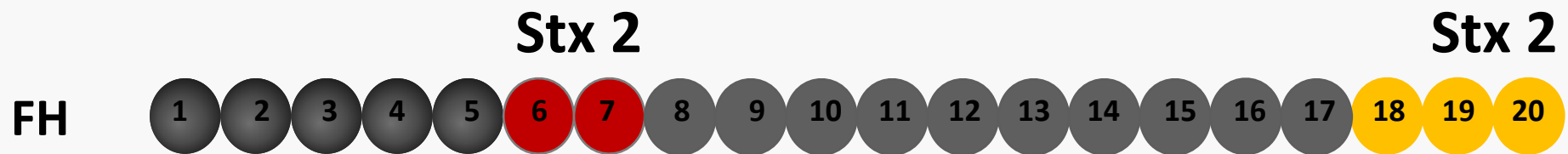
Stx 2 BINDING TO PROTEINS OF FH FAMILY



COMPETITION OF Stx 2 BINDING



FUTURE?



TAKE HOME MESSAGES

In addition to aHUS, complement also plays a role in eHUS

Stx 2 activates complement

Stx 2 binds to FH downmodulating its protective function

Stx 2 binds to proteins of the FH family – deficiency of these proteins may result in an even stronger FH binding

Stx 2 downregulates CD59 on the target cell

Sem Thromb Hemost 2014;40:503

Sem Thromb Hemost 2014;40:508



**Thomas
Giner**

**Johannes
Hofer**

**Anne Dettmar
Jun Oh
Elisabeth Binder
Gerhard Gstraunthaler
Werner Streif**

**Alejandra
Rosales**

**Magdalena
Riedl**

**Therese
Jungrathmayr**



Abdul-Basit Khan

www.hus-online.at

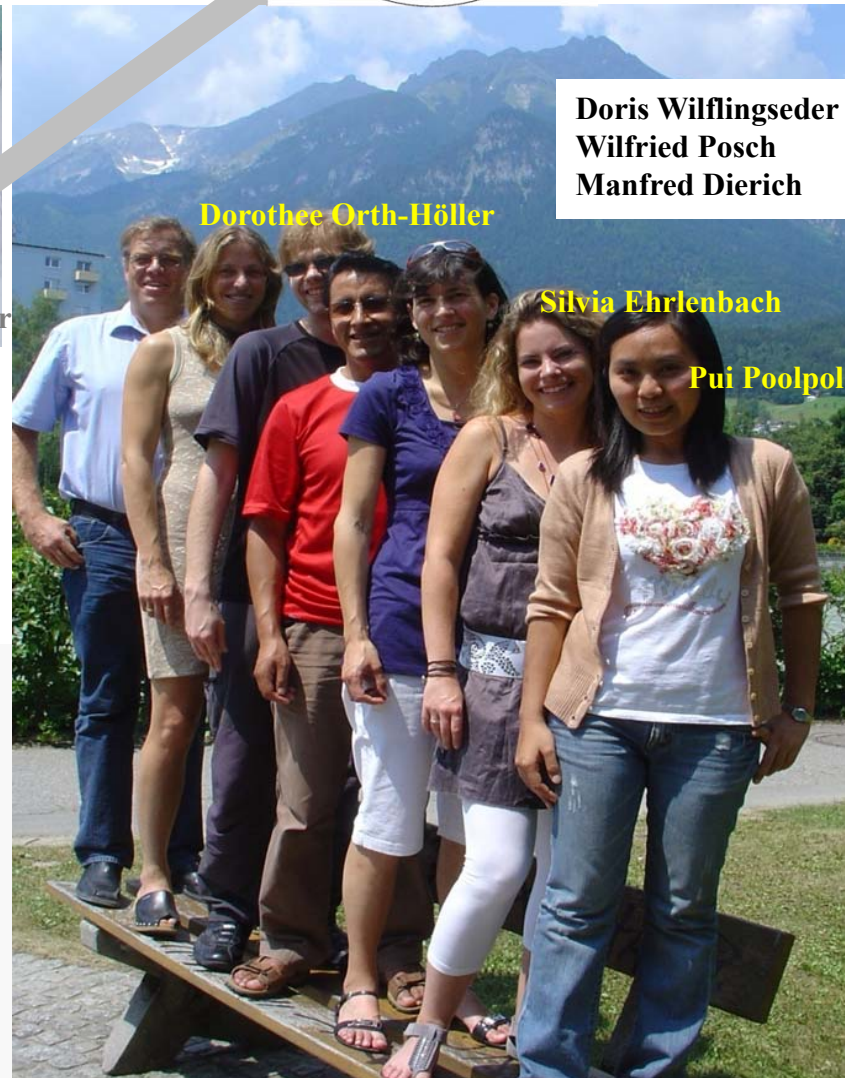


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Santiago Rodriguez de Cordoba
Christine Skerka
Peter Zipfel**



Dorothee Orth-Höller

**Doris Wilflingseder
Wilfried Posch
Manfred Dierich**

Silvia Ehrlenbach

Pui Poolpol



WWW.HOROS.AT



SAVE THE DATE

11 June – 13 June 2013

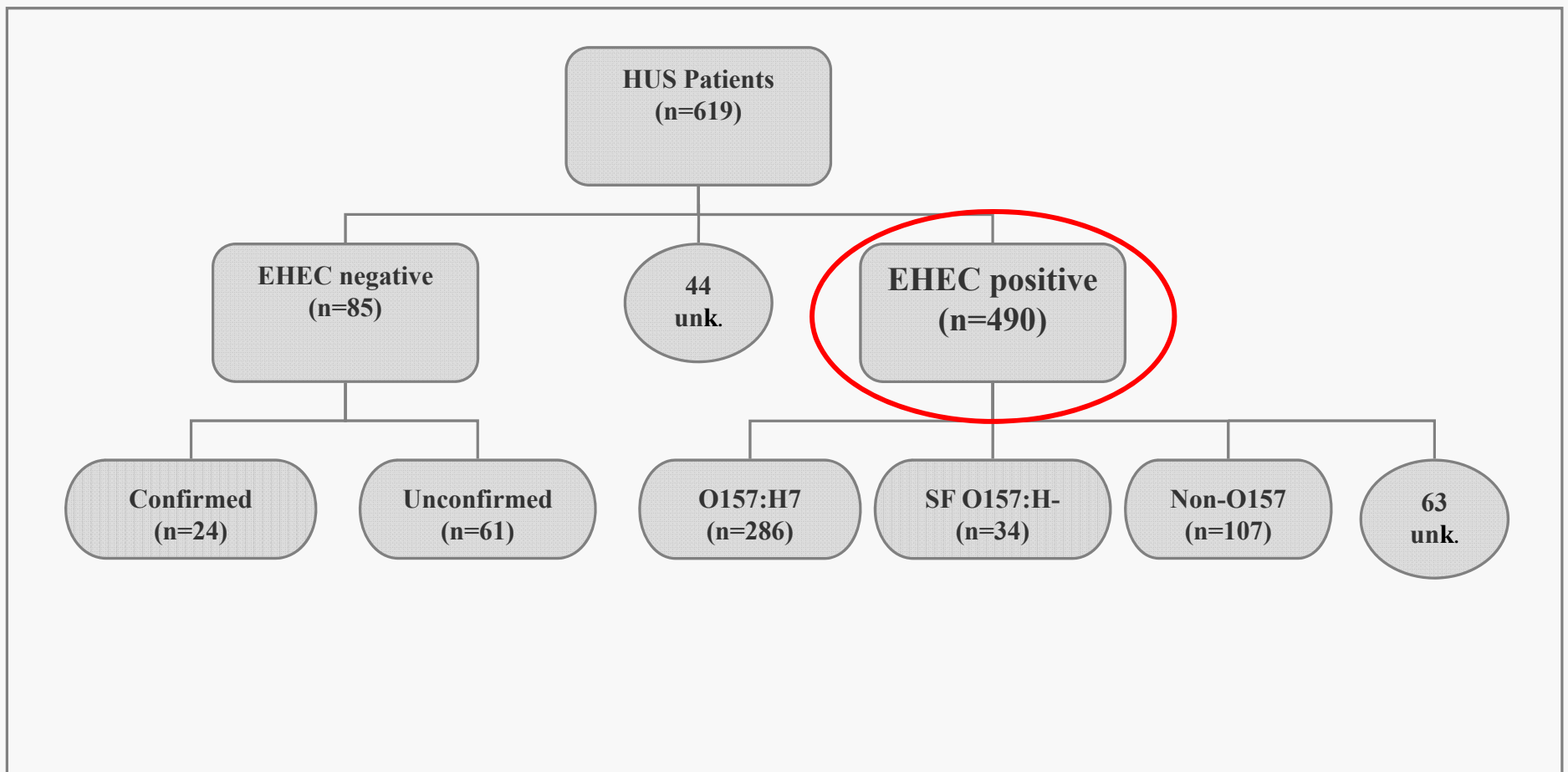
**6th international meeting
on HUS & related disorders
in Innsbruck, Austria**

DIAGNOSIS: STUDY POPULATION

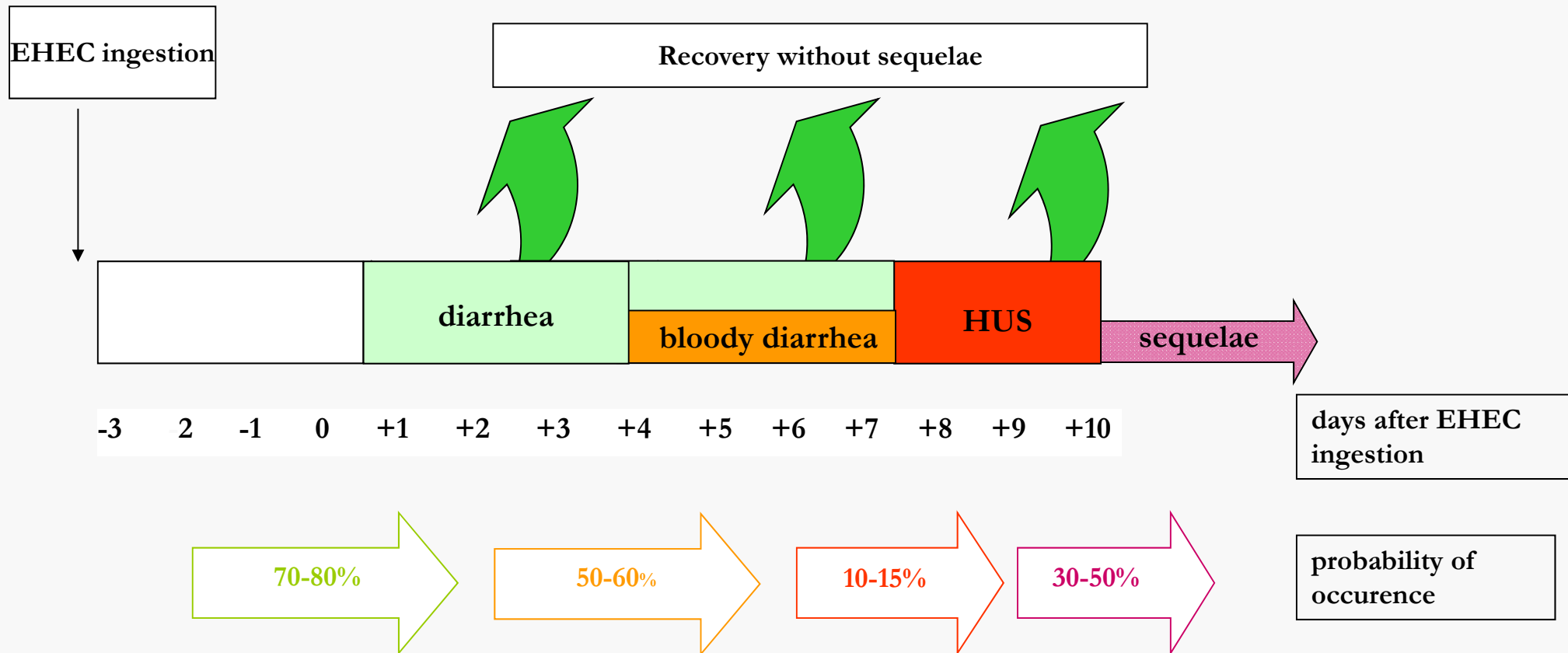
	Acute phase	One- year follow up	Two-year follow up	Three-year follow up	Five-year follow up
Number of all patients	619	449	354	306	274
Boys : Girls	0.89	0.78	0.81	0.78	0.82
Age, Median years (IQR)*	2.9 (1.4 - 5.2)	2.9 (1.6 - 5.4)	2.7 (1.5 - 5.0)	2.9 (1.5 - 5.7)	2.6 (1.4 - 6.1)
Number of EHEC+ patients	490	366	290	249	226
Boys : Girls	0.84	0.73	0.77	0.73	0.74
Age, Median years (IQR)*	2.7 (1.4 – 4.9)	2.7 (1.6 - 5.0)	2.7 (1.5 - 4.8)	2.9 (1.5 - 5.4)	2.5 (1.3 – 5.3)

*Time relapsed since acute phase was subtracted in order to allow direct comparison.

DISEASE CAUSE



Diagnostics & Clinical courses



Adapted from Phil Tarr

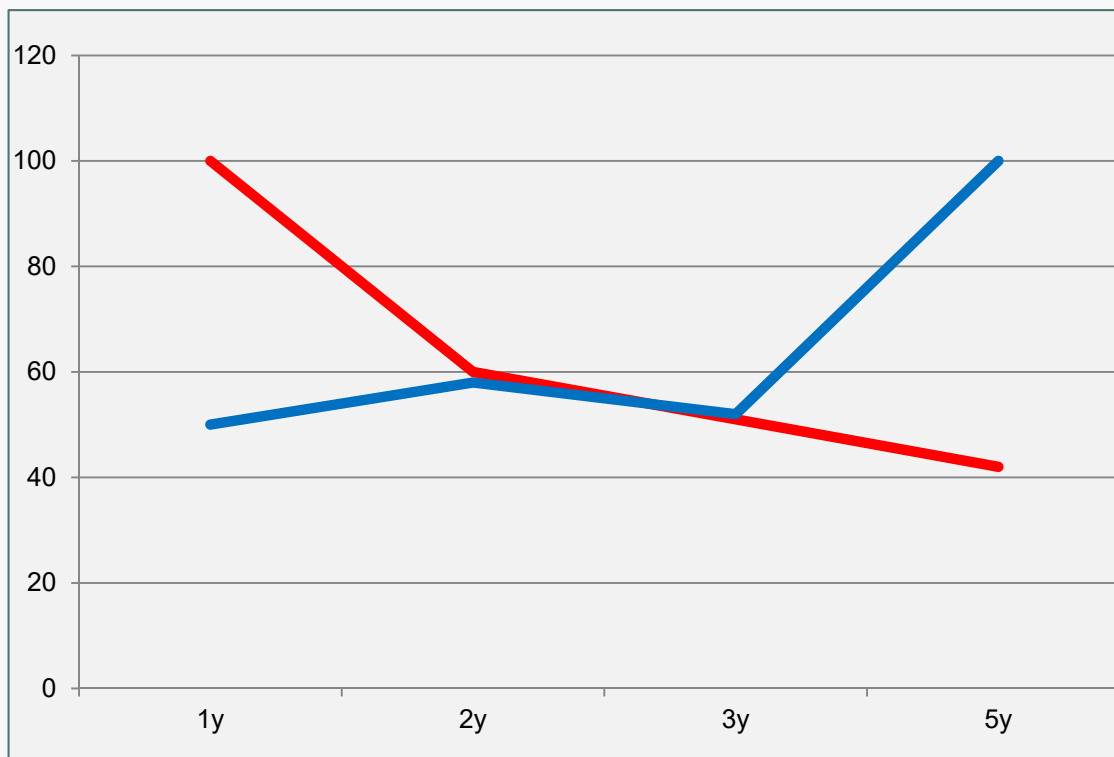
CLINICAL COURSES: LONG TERM

**70% of patients recovered completely after the acute phase,
30% presented with hypertension, neurolog. symptoms, renal
impairment**



CLINICAL COURSES: LONG TERM

**70% of patients recovered completely after the acute phase,
30% presented with hypertension, neurolog. symptoms, renal
impairment**



**In general,
only 54% of patients
with symptoms after 5 years
already presented
symptoms after 1 year!**

DIAGNOSIS: STUDY POPULATION

“eHUS” when:

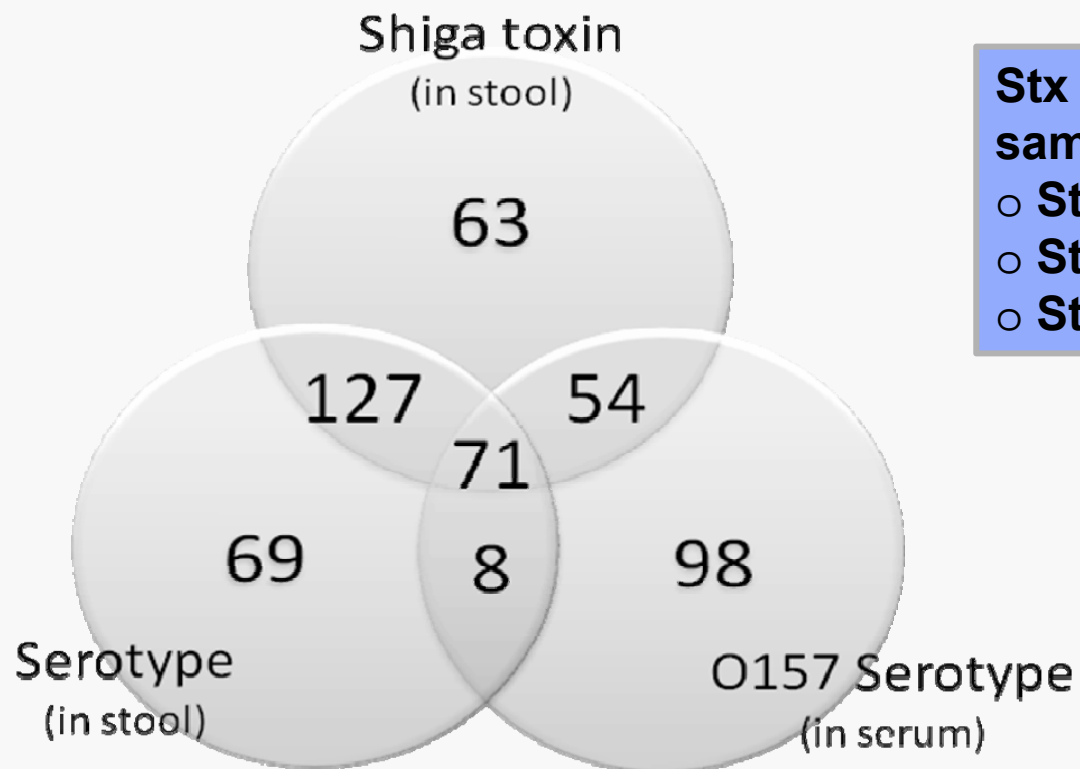
- 1. Isolation of EHEC from stool culture,**
- 2. Stx antigens in stool or *stx* genes
evidenced by ELISA or PCR, or**
- 3. IgG and IgM antibodies against
E. coli O157 lipopolysaccharide (LPS)
in serum, detected by immunoblot**

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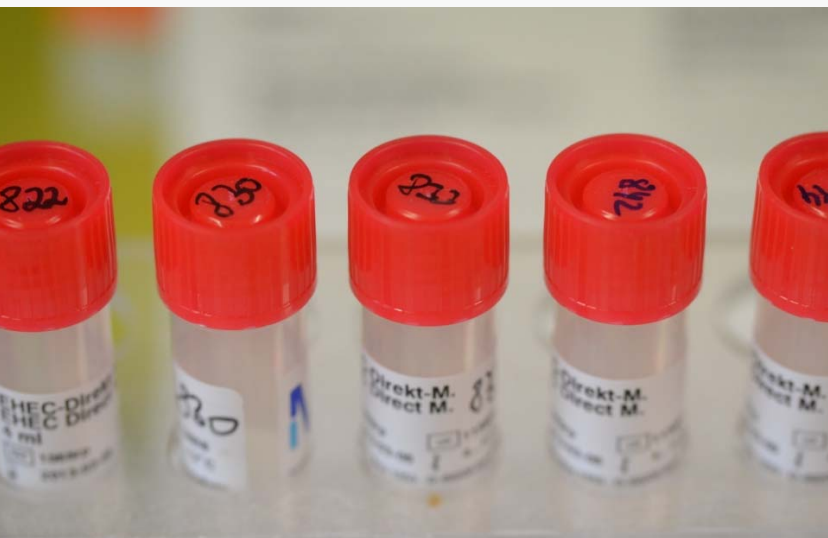
DIAGNOSIS: MICROBIOLOGICAL FINDINGS



Stx in 64% of stool samples

- **Stx 2** in 77%
- **Stx 1&2** in 13%
- **Stx 1** in 10%

SHIGATOXIN ELISA



Premier EHEC

Enzyme immunoassay for the Detection of the Toxins produced by Enterohemorrhagic *E. Coli* in stool specimens or culture isolates / Test immunoenzimatico per la ricerca delle Tossine prodotte da *E. Coli* enterohemorragico in campioni di feci o da ceppi isolati in coltura / Test enzimatique pour la détection des toxines produites par *E. Coli* entérohémorragique dans des échantillons de selles ou sur colonies / Enzaimunoanálisis para la detección de toxinas producidas por *E. Coli* Enterohemorrágica en muestras de heces o en aislamientos en cultivo / Enzymimmunoassay zum Nachweis von Toxinen, die von enterohämorrhagischen *E. Coli* in Stuhlproben oder Kulturen produziert werden.

96 antibody coated microwells / pozzetti con anticorpo / puits recouverts d'anticorps immobilisés / Pocillos revestidos de anticuerpo / Antikörper-beschichtete Mikrotiterkavitäten

18.8 mL	DIL	SPE	8605.097	2013-10-30
100 mL	BUF	WASH	20X	II
10.0 mL	CONJ	ENZ	8603.102	2013-12-26
12.5 mL	SUBS	II	8604.098	2013-12-25
10.0 mL	SOLN	STOP	1601.512	2014-05-07
3.4 mL	CONTROL	+	8601.119	2014-06-27
3.4 mL	CONTROL	-	1603.284	2014-05-08

96 transfer pipettes / pipette / pipettes / pipetas / Transferpipetten
3 microwell strip sealers / sigilli per pozzetti / films adhésifs / selladores de tiras de micropocillos / Klebefolien zum Verschließen der Platte.

REF 608096
IVD
LOT 608096C166
2013-10-30

Meridian
Bioscience, Inc.
Cincinnati, Ohio 45204

EC REP

MBE-Via dell' Industria, 7
20020 Villa Cortese (Milano)
Italy

2 C 8 C



SN 6392 4/01

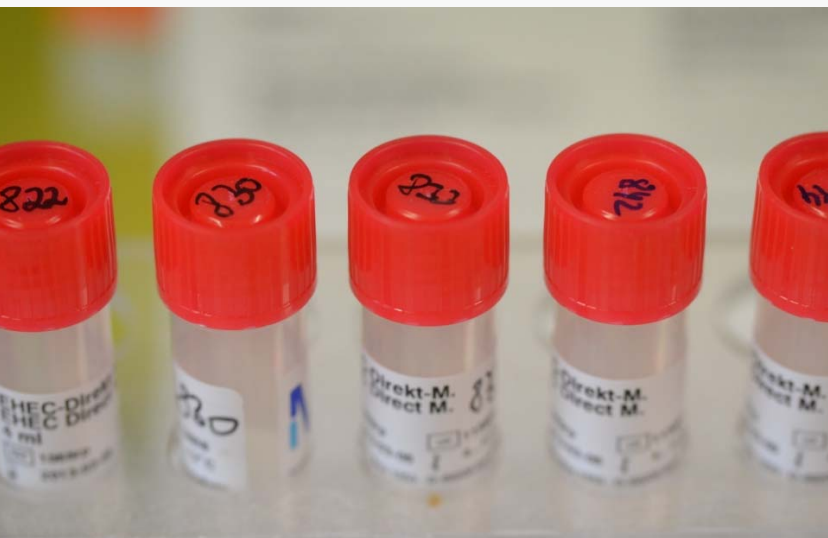
Premier EHEC Test Procedure

<p>1</p> <p>Measure 200µl of Sample Diluent into a test tube.</p>	<p>2</p> <p>Mix specimen thoroughly. Using a transfer pipet, add 50µl to the tube. Gently expel and withdraw the suspension several times.</p>	<p>3</p> <p>Vortex for 15 seconds.</p>
<p>4</p> <p>Detach microwells needed, place in holder and add 100µl of diluted specimen to the appropriate well.</p>	<p>5</p> <p>Add two drops of Positive or Negative Control to the appropriate wells. Shake firmly for 30 seconds. Seal the plate and incubate for 1 hour at room temperature.</p>	<p>6</p> <p>Wash 5 times with 1X Wash Buffer. See package insert for proper wash procedure.</p>
<p>7</p> <p>Add 2 drops of Detection Antibody to all wells. Seal the plate and incubate for 30 minutes at room temperature.</p>	<p>8</p> <p>Repeat wash procedure as in Step 6.</p>	<p>9</p> <p>Add 2 drops of Enzyme Conjugate to all wells. Seal the plate and incubate for 30 minutes at room temperature.</p>
<p>10</p> <p>Repeat wash procedure as in Step 6.</p>	<p>11</p> <p>Add 2 drops of Substrate to all wells. Incubate for 10 minutes at room temperature.</p>	<p>12</p> <p>Add 2 drops of Stop Solution to all wells and shake firmly for 30 seconds.</p>

Rev. 5/99

SN11152

SHIGATOXIN ELISA



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18.8 mL	DIL	SPE	8605.097	2013-10-30
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2013-10-30

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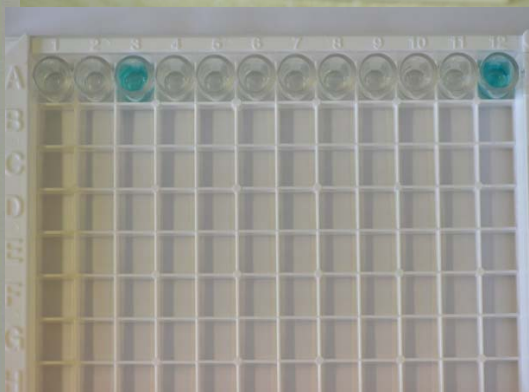
SN 6392 401

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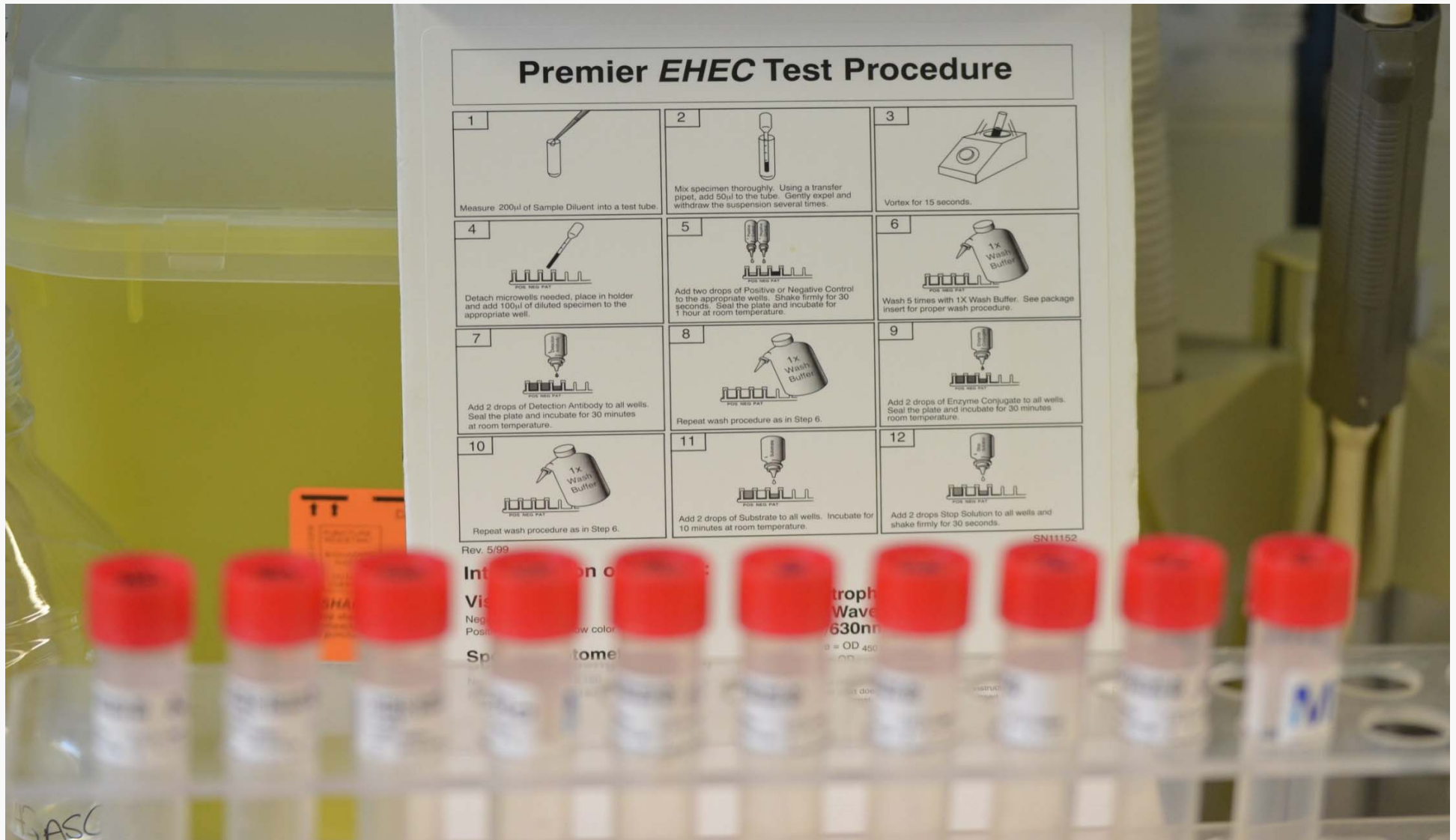
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Rev. 5/99

SN11152



EHEC ELISA



ECULIZUMAB IN TREATMENT OF PNH



ECULIZUMAB IN TREATMENT OF PNH

**Start of treatment:
07:00**



ECULIZUMAB IN TREATMENT OF PNH

**Start of treatment:
07:00**



Reports:

Hillmen et al. 2004, NEJM 350:552

Hillmen et al. 2006, NEJM 360:2142

Long term results

Dramatic increase of survival

7-times lower rate of thrombosis

(Kelly et al. 2011, Blood 117:6786)

TAKE HOME MESSAGES

Diagnosis: identify Shiga toxins in stool when HUS

Clinical courses: follow up patients for >5 years

**Therapy: as HUS is a
complement-dependent disease,
consider Eculizumab in severe clinical courses!**