Exploring complement in 2026: from biological assessment to in situ convertases (C3/C5), when and how to measure them?

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FHU COMET
COMplement Expertise and Therpeutics

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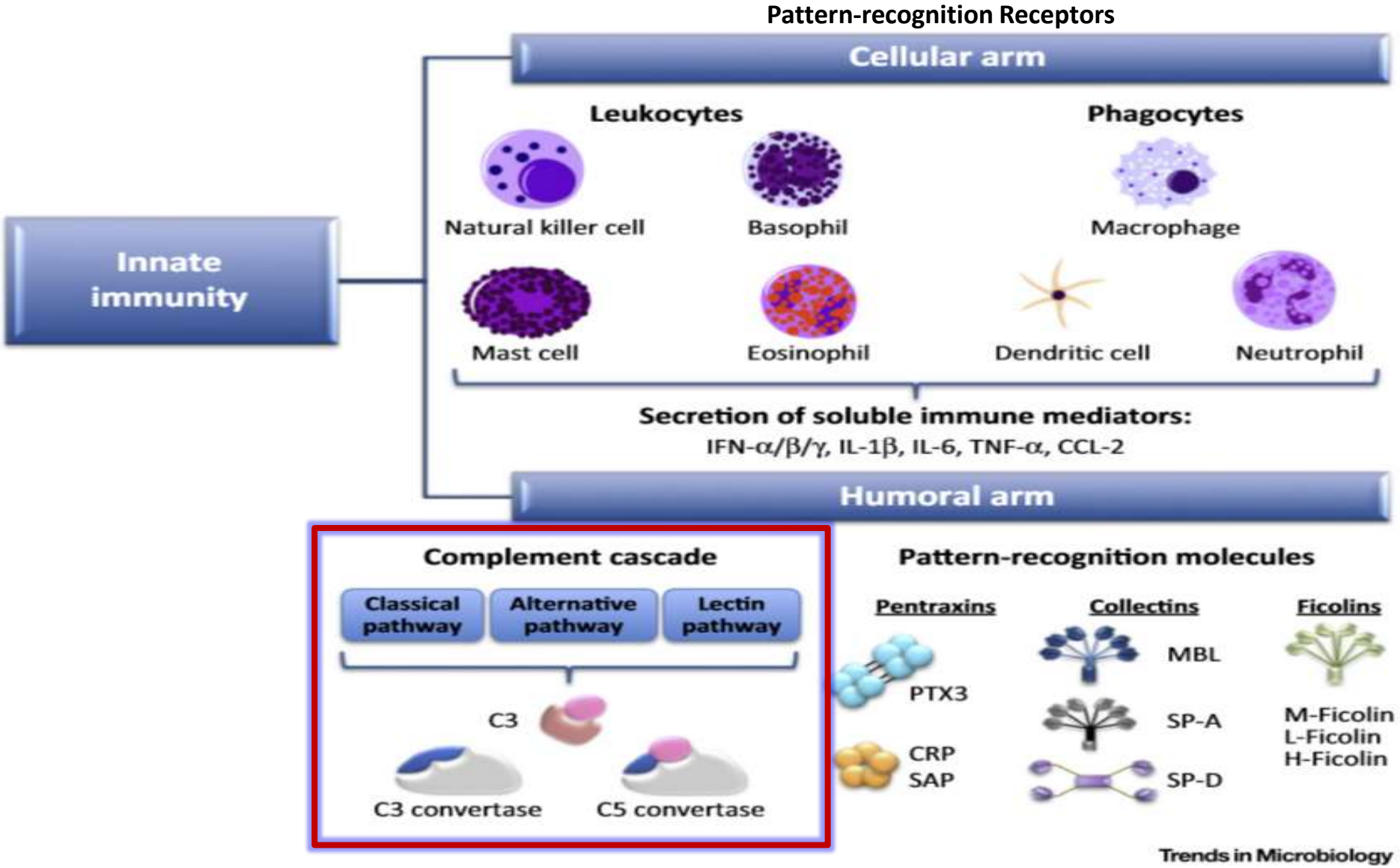
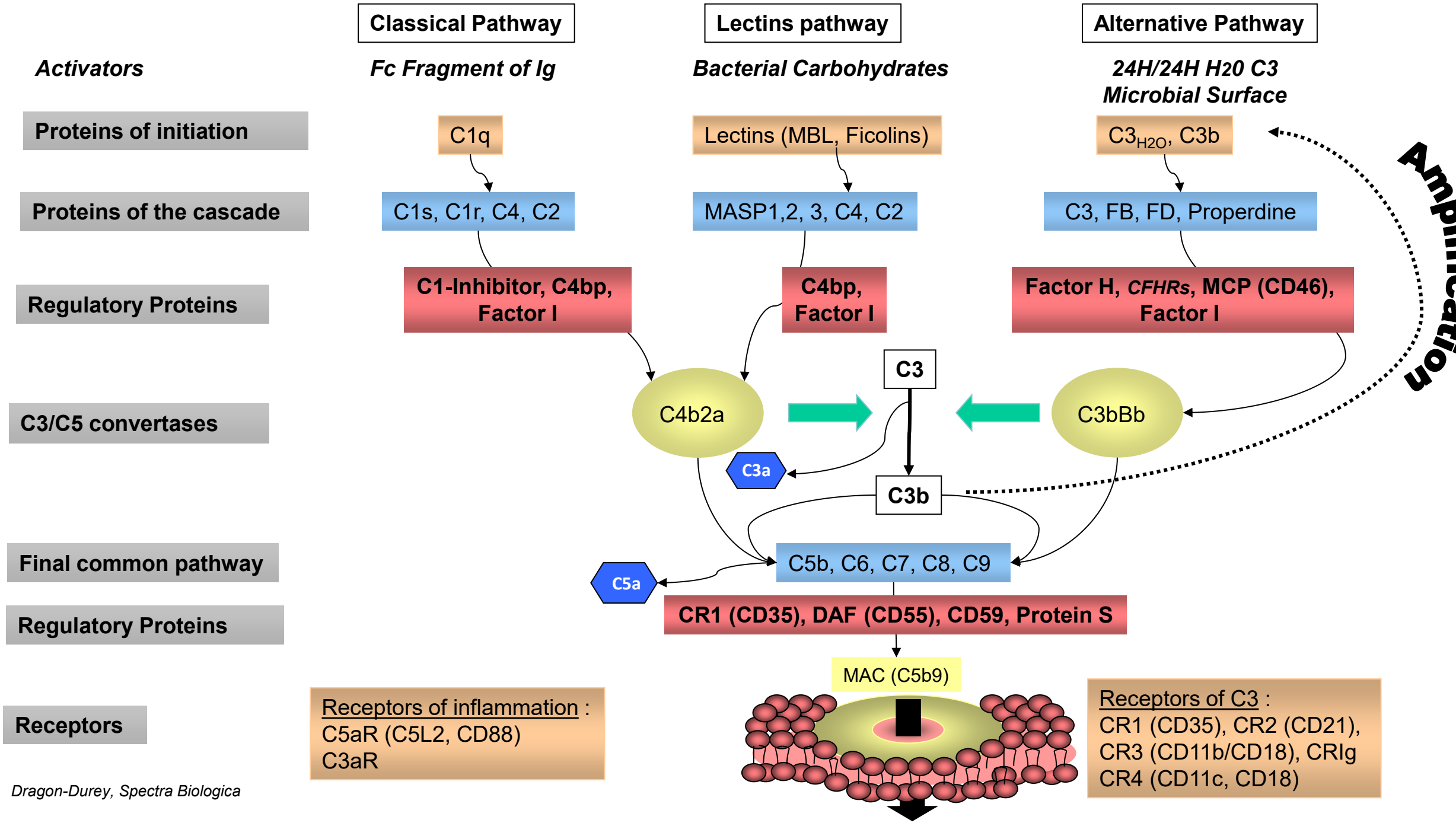
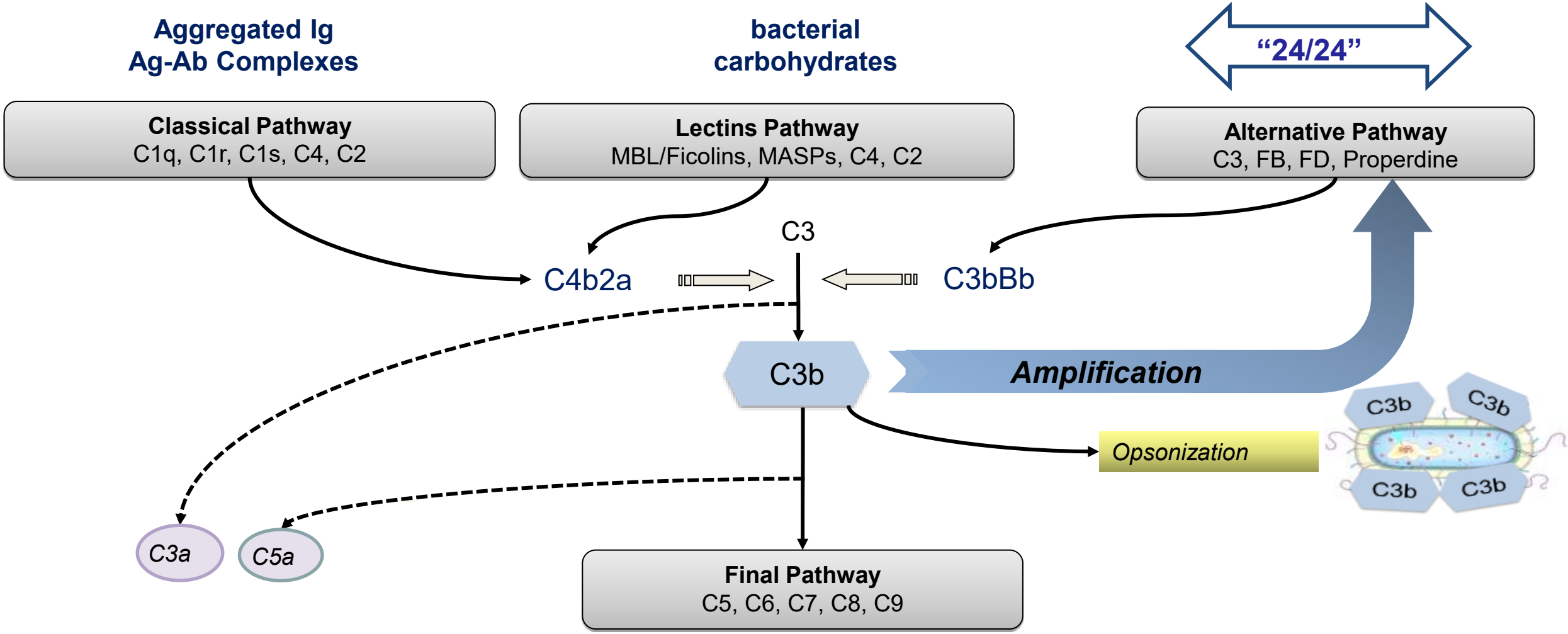
The complement system belongs to the innate immunity




Classical Pathway
Lectins pathway
Alternative Pathway
Activators
Fc Fragment of Ig
Bacterial Carbohydrates
24H/24H H20 C3
Microbial Surface
Proteins of initiation
Proteins of the cascade
Regulatory Proteins
C3/C5 convertases
Final common pathway
Regulatory Proteins
Receptors
C1q
Lectins (MBL, Ficolins)
C3H2O, C3b
C1s, C1r, C4, C2
MASP1,2, 3, C4, C2
C3, FB, FD, Properdine
C1-Inhibitor, C4bp, Factor I
C4bp, Factor I
Factor H, CFHRs, MCP (CD46), Factor I
C3
C4b2a
C3bBb
C3a
C3b
C5a
C5b, C6, C7, C8, C9
CR1 (CD35), DAF (CD55), CD59, Protein S
MAC (C5b9)
Amplification
Receptors of inflammation :
C5aR (C5L2, CD88)
C3aR
Receptors of C3 :
CR1 (CD35), CR2 (CD21),
CR3 (CD11b/CD18), CRIg
CR4 (CD11c, CD18)
Dragon-Durey, Spectra Biologica


Aggregated Ig
Ag-Ab Complexes
bacterial
carbohydrates
"24/24"
Classical Pathway
C1q, C1r, C1s, C4, C2
Lectins Pathway
MBL/Ficolins, MASPs, C4, C2
Alternative Pathway
C3, FB, FD, Properdine
C3
C4b2a
C3bBb
C3b
Amplification
Opsonization
C3b
C3b
C3b
C3b
C3a
C5a
Final Pathway
C5, C6, C7, C8, C9

Aggregated Ig
Ag-Ab Complexes

bacterial
carbohydrates

"24/24"

Classical Pathway
C1q, C1r, C1s, C4, C2

Lectins Pathway
MBL/Ficolins, MASPs, C4, C2

Alternative Pathway
C3, FB, FD, Properdine

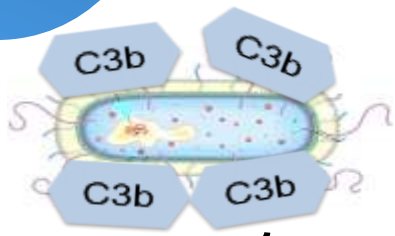
C3

C4b2a

C3bBb

C3b

Amplification



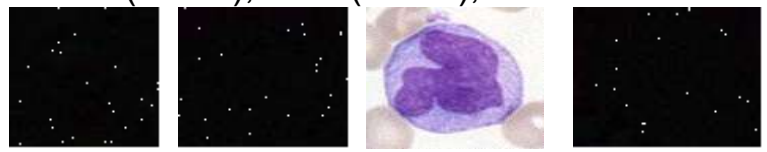
Opsonization

C3a

C5a

Final Pathway
C5, C6, C7, C8, C9

CR3 (CD11b/CD18),
CR4 (CD11c/CD18), **CRIg**
CR1 (CD35), **CR2** (CD21),



Phagocytosis
Immun complexes elimination
Adaptative immune response modulation

Aggregated Ig
Ag-Ab Complexes

bacterial
carbohydrates

"24/24"

Classical Pathway
C1q, C1r, C1s, C4, C2

Lectins Pathway
MBL/Ficolins, MASPs, C4, C2

Alternative Pathway
C3, FB, FD, Properdine

C3

C4b2a

C3bBb

C3b

Amplification

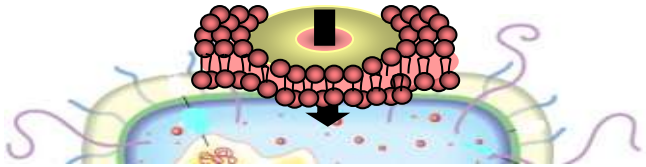
Opsonization

C3a

C5a

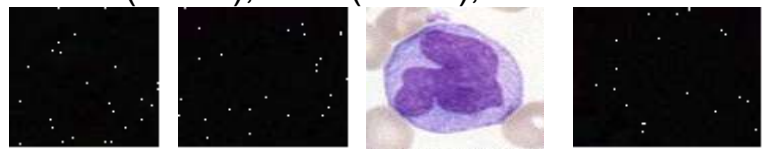
Final Pathway
C5, C6, C7, C8, C9

C5b-9 (MAC)



Ct Dependent Cytolysis

CR3 (CD11b/CD18),
CR4 (CD11c/CD18), **CRIg**
CR1 (CD35), **CR2** (CD21),



Phagocytosis
Immun complexes elimination
Adaptative immune response modulation

Aggregated Ig
Ag-Ab Complexes

bacterial
carbohydrates

"24/24"

Classical Pathway
C1q, C1r, C1s, C4, C2

Lectins Pathway
MBL/Ficolins, MASPs, C4, C2

Alternative Pathway
C3, FB, FD, Properdine

C3

C4b2a

C3bBb

C3b

Amplification

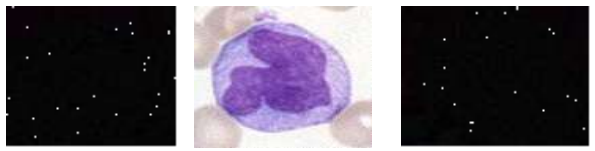
Opsonization

C3a

C5a

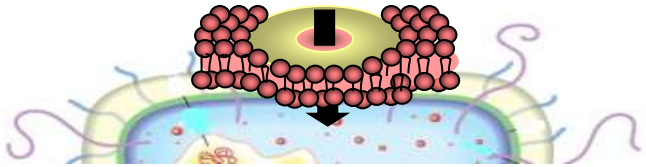
Final Pathway
C5, C6, C7, C8, C9

C3aR, C5aR (CD88, C5L2)



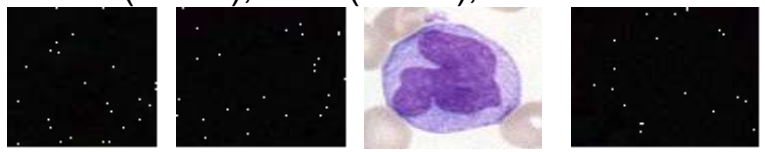
Chimiotactism
Activation
Inflammation

C5b-9 (MAC)



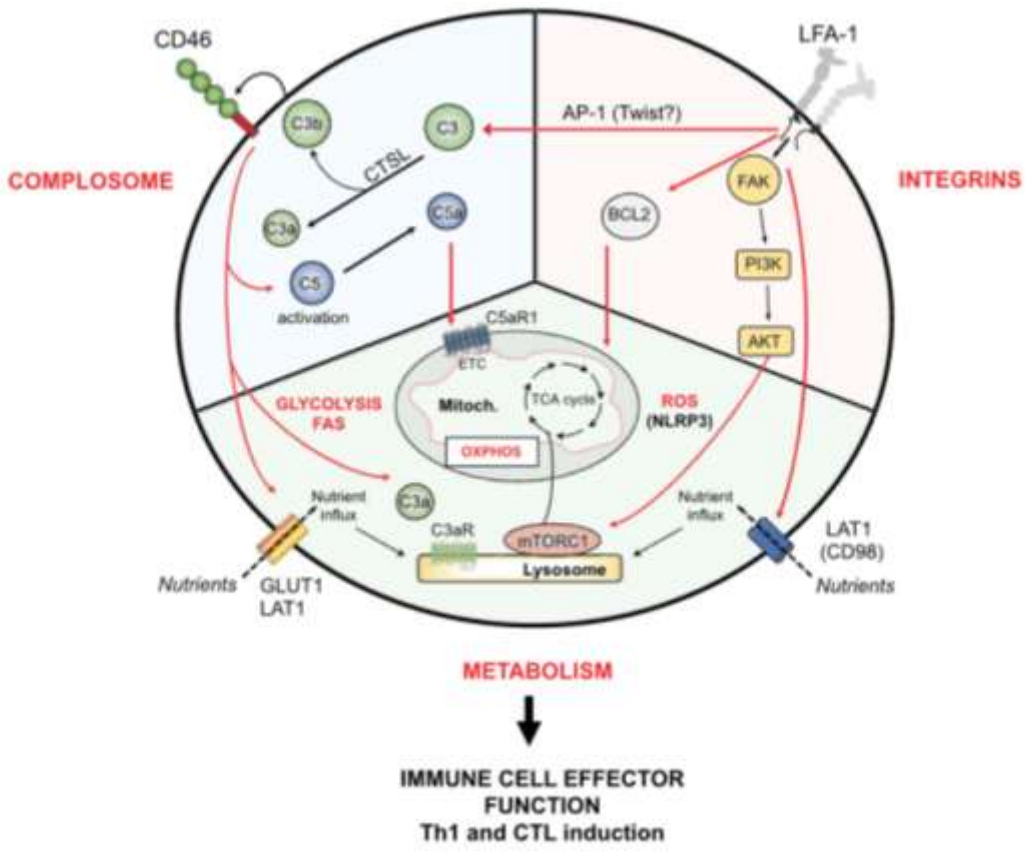
Ct Dependent Cytolysis

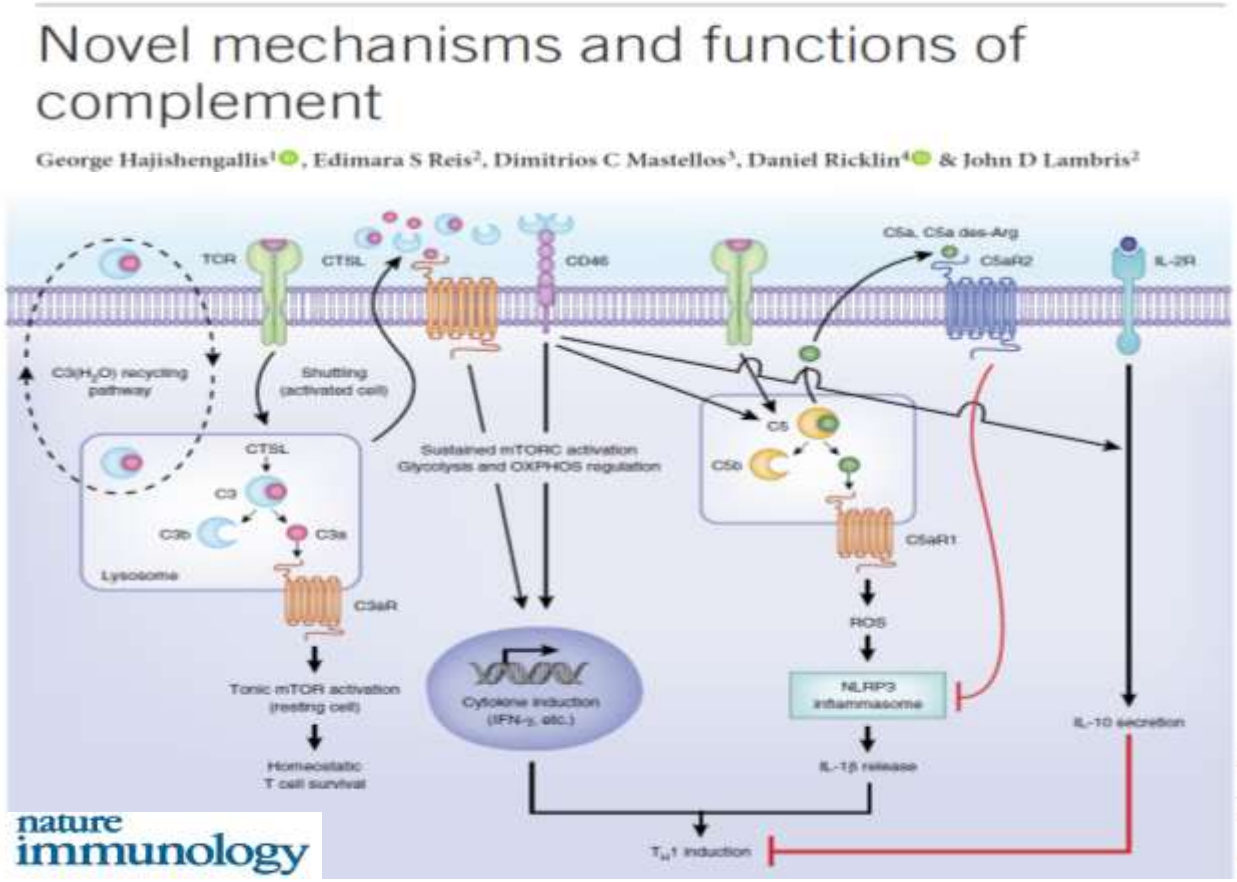
CR3 (CD11b/CD18),
CR4 (CD11c/CD18), **CRIg**
CR1 (CD35), **CR2** (CD21),



Phagocytosis
Immun complexes elimination
Adaptative immune response modulation

Non canonical functions of Complement: intra-cellular « complosome »





N. Merle et al., Br J Pharmacol (2021)

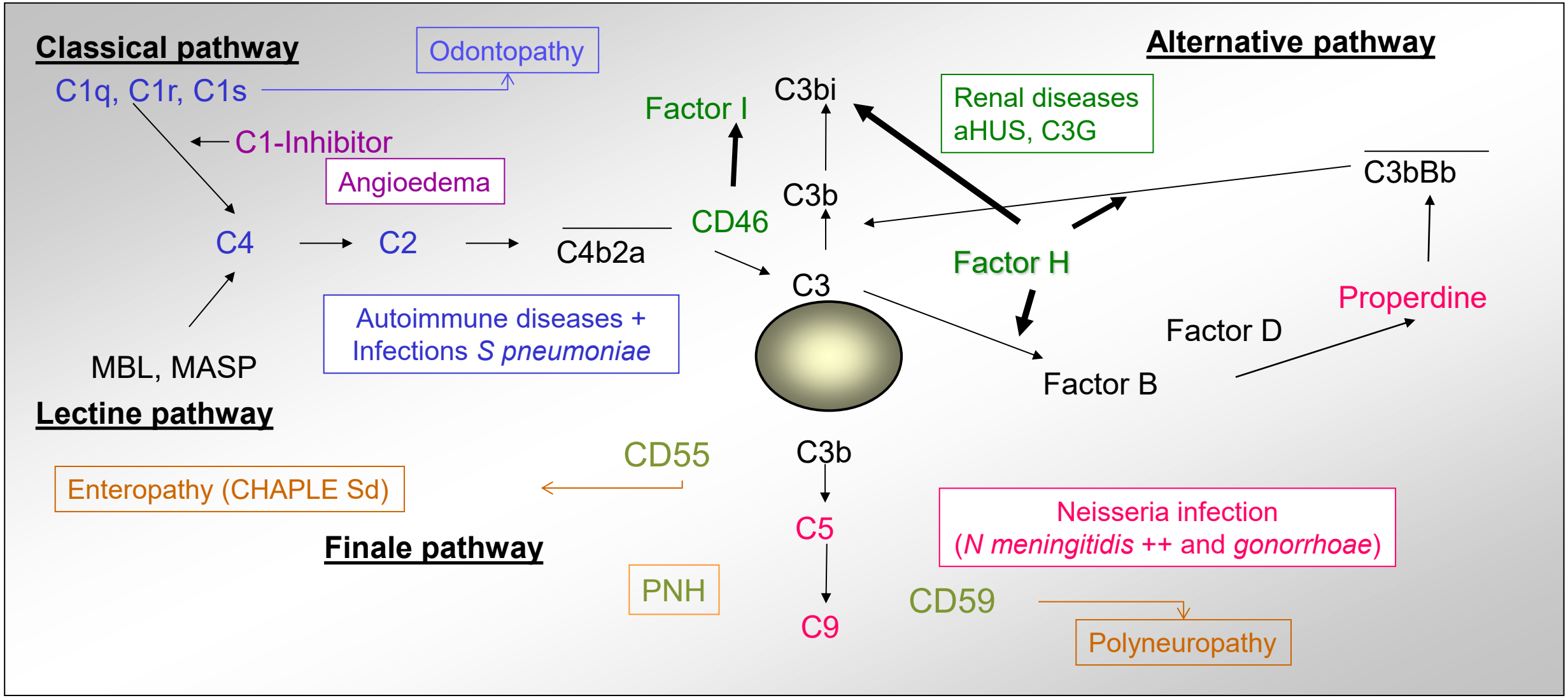
Role in :

Immune cells regulation+++,pregnancy, tissue haemostasis, lipid metabolism, coagulation, cancer…

Complement exploration in human diseases

- Complement component deficiencies : association with various diseases
- Role in inflammation : autoimmune diseases (SLE, myasthenia gravis…), ischemia-reperfusion injury, septic shock…
- Role in tissue injury of some diseases (complement-mediated /associated diseases: aHUS, C3G…)
- Complement components : targets of autoantibodies
- Target of new therapies

Diseases associated with complement component deficiencies



2 types of deficiencies

Deficiencies of one component of the cascade :

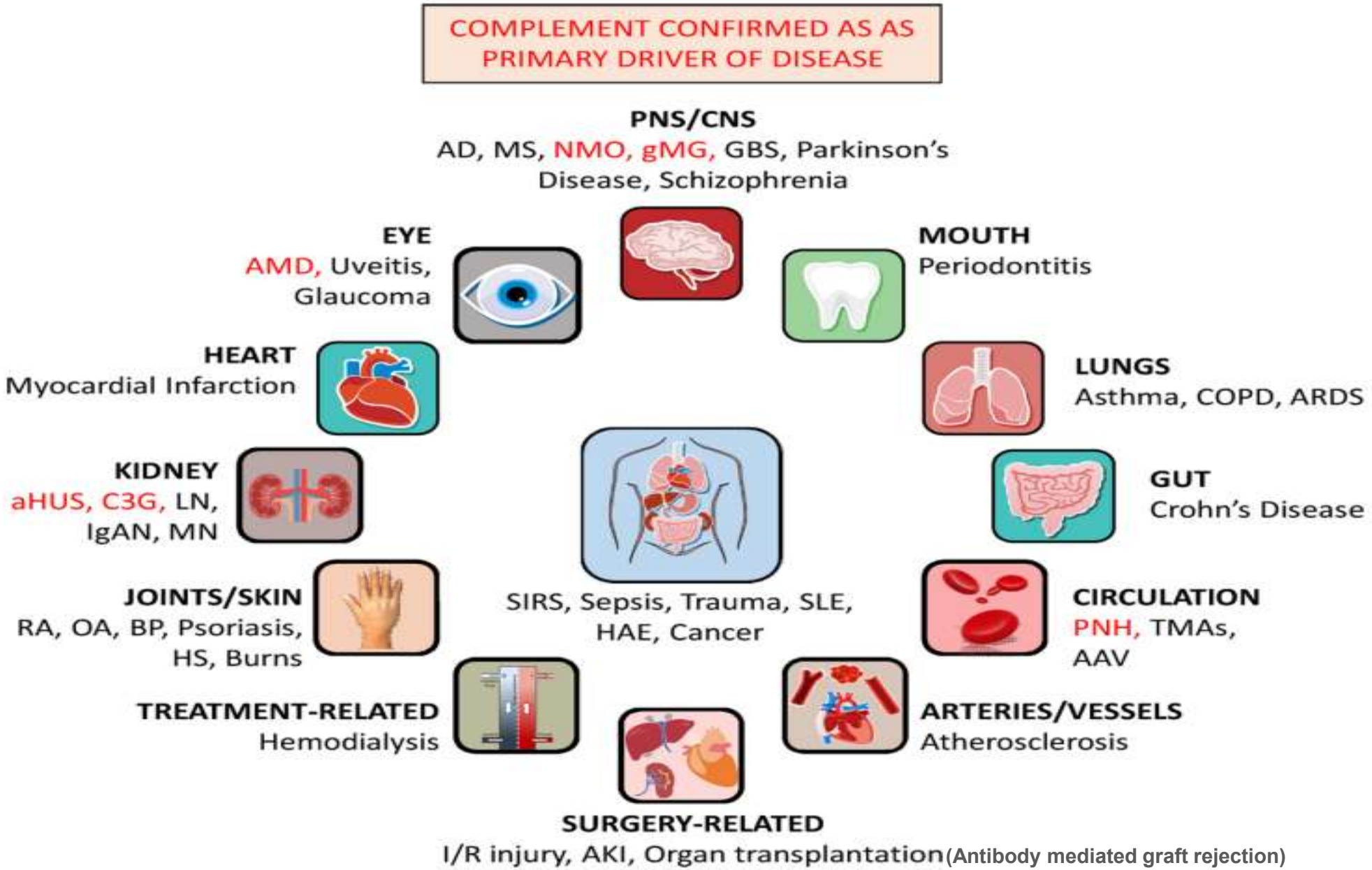
Phenotype linked to a **default of the complement system function** :

-Default of the CIC elimination and adaptative immunity regulation (AID),
-Default of pathogens elimination (PID)

Deficiencies of one regulator of the cascade

Phenotype linked to an **excess of the Complement system function**:

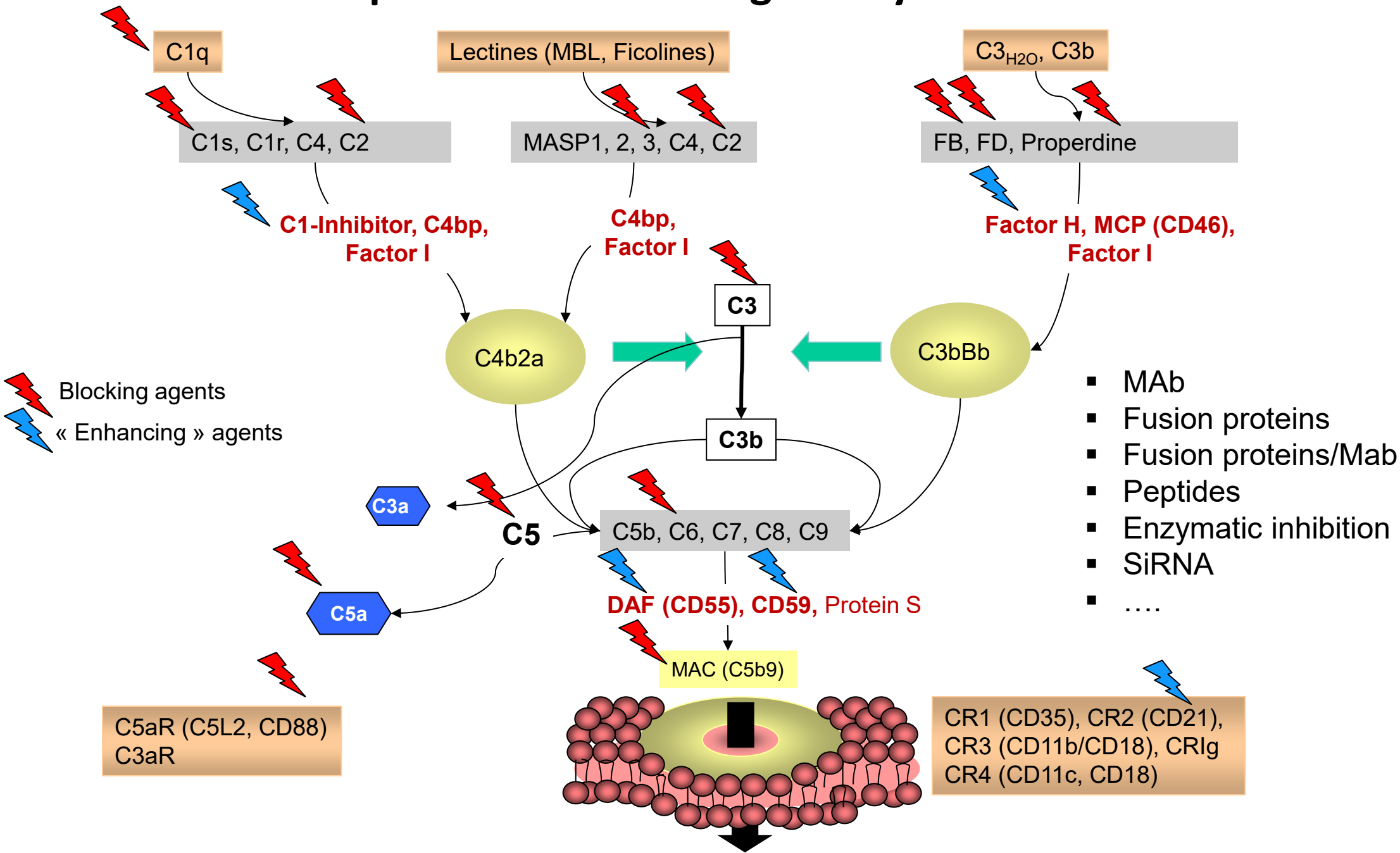
-Cell activation (endothelial ¢, Platelets, Lymphocytes, macrophages),
-Tissu depositions (C3b, C5b9: in kidneys…),
-Cellular destructions (endothelial ¢ , axones..)



Zelek, WM, Mol Immunol 2019

Frequent Diseases → potential market!

Complement: a multitargeted system



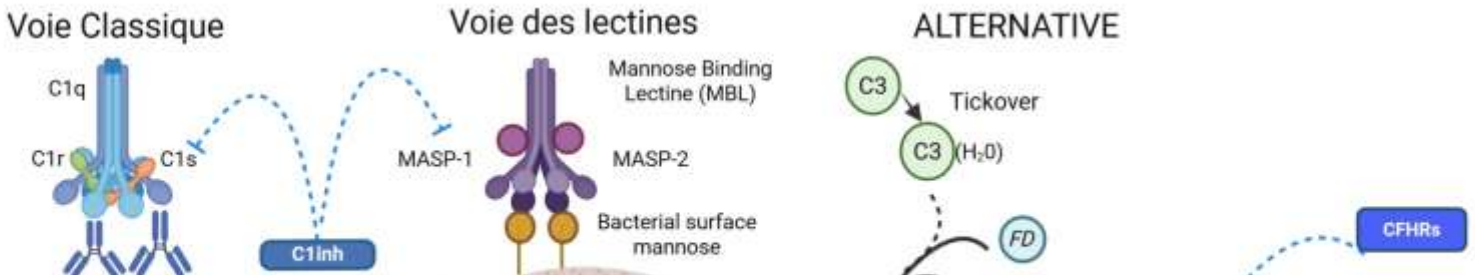
Complement exploration according to the clinical context

Context	Exploration	Objectives
SLE, Urticarial vasculatis	**First level**: CH50, C3,C4, sC5b9 **Secund level** : Factor B, C1 (q,r,s), C2, anti-C1q ab (lupus nephritis, Mac Duffy Sd)	Measure of classical pathway activation Look for hereditary or acquired classical pathway deficiency Look for anti-C1q ab for diagnosis (Mac Duffy) or pronosis (lupus nephritis)
aHUS, C3G	**First level**: CH50, C3, C4, Factor B, sC5b9, Bb, Factor H, Factor I, CD46 (SHUa), anti-FH ab + C3Nef, anti-C3b, anti-FB ab (if C3G) **Secund level** : Genetic study: *CFH,CFI,CD46,C3,FB,CFHRs*	Measure of alternative pathway activation Look for hereditary or acquired deficiency of alternative pathway regulation
Infections (recurrent, Menigococcus, pneumococcus)	**First level**: CH50, C3, C4, AP50 **Secund level** : C1 (q,r,s), C2 (pneumococcus), C5/C6/C7/C8/C9,properdine (meningococcus) Third level: FH, FI, (+/-MBL, Ficolins)	Look for protein deficiency of classical, terminal pathways and in properdin, Eventually deficiency in the alternative pathway regulation or in lectins pathway
Angioedema	**First level**: CH50, C3, C4, C1 Inh Ag et fonction **Secund level** : anti-C1 Inh ab; C1q, Genetic study: C1 INH +/-bradykinines (Factor XII)	Look for hereditary or acquired C1 inhibitor deficiency
Hemolysis/ thrombosis **Polyneuropathy** **Enteropathy**	**First level**: membrane expression of CD59, CD55 +/- CD14, CD16, CD24 on RBC, PNN and monocytes Same + genetic study Same + genetic study	Look for PNH clone (lack of GPI-anchored proteins) (CD55 neg/ CD59 neg) CD59 neg/ CD55 pos CD55 neg/ CD59 pos

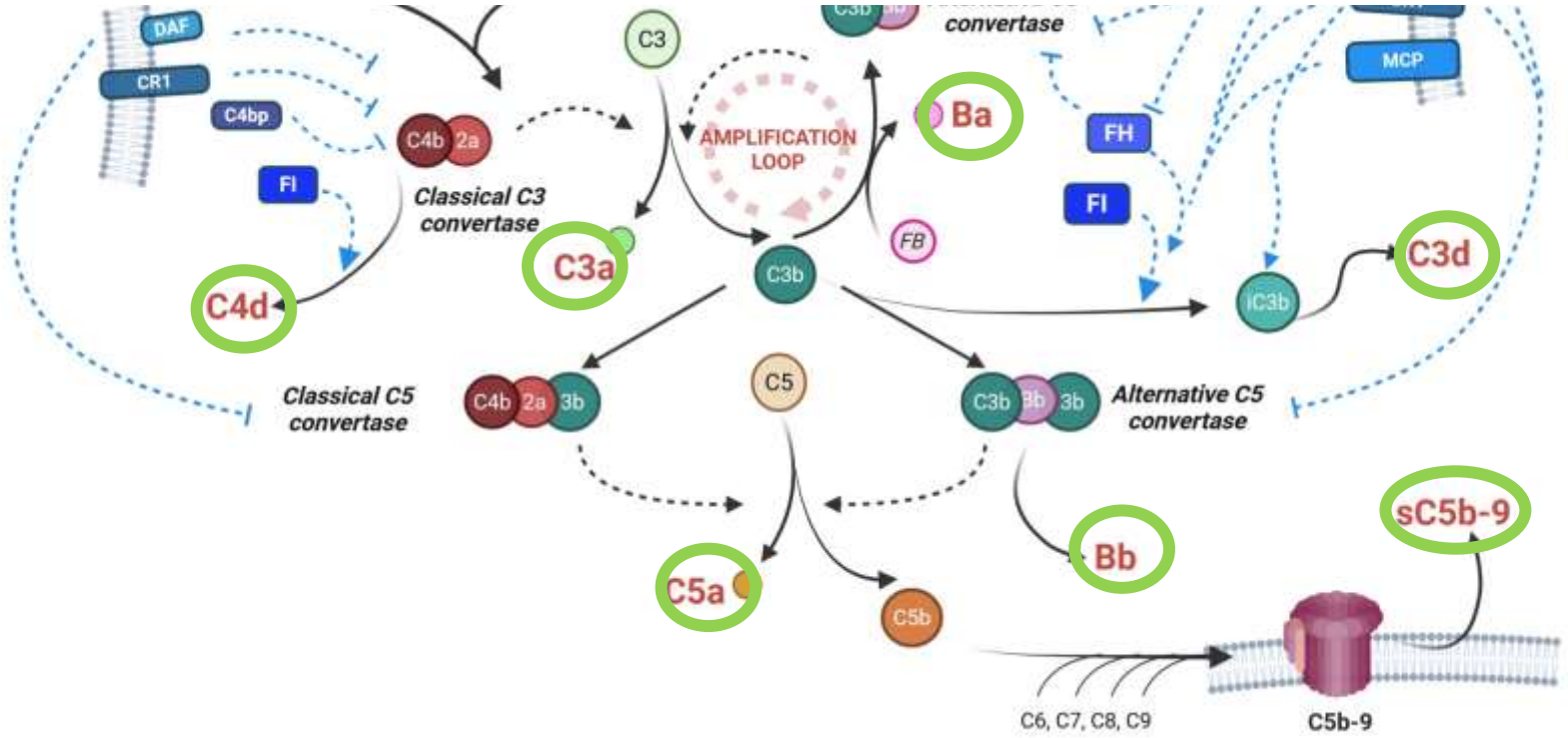
Complement exploration according to the treatment

Context	Exploration	Objectives
Treatment by distal blockers (C5)	CH50, C3,C4, sC5b9 (+/-C5a?) Measurement of the drugs (eculizumab, ravulizumab)	Evaluation of biological efficacy: -Measure of final pathway activation. Drug dose adaptation.
Treatment by AP proximal blockers	CH50, C3, C4, Bb, sC5b9 (+/- C3a, C5a?)	Evaluation of biological efficacy : -Measure of alternative and final pathways activation.
Treatment by C5a/C5aR1 axis blockers	C5a, granulocytes/ monocytes phenotyping Cytokines measurement	Evaluation of biological efficacy : -Measure of complement activation -Measure of granulocytes/monocytes activation

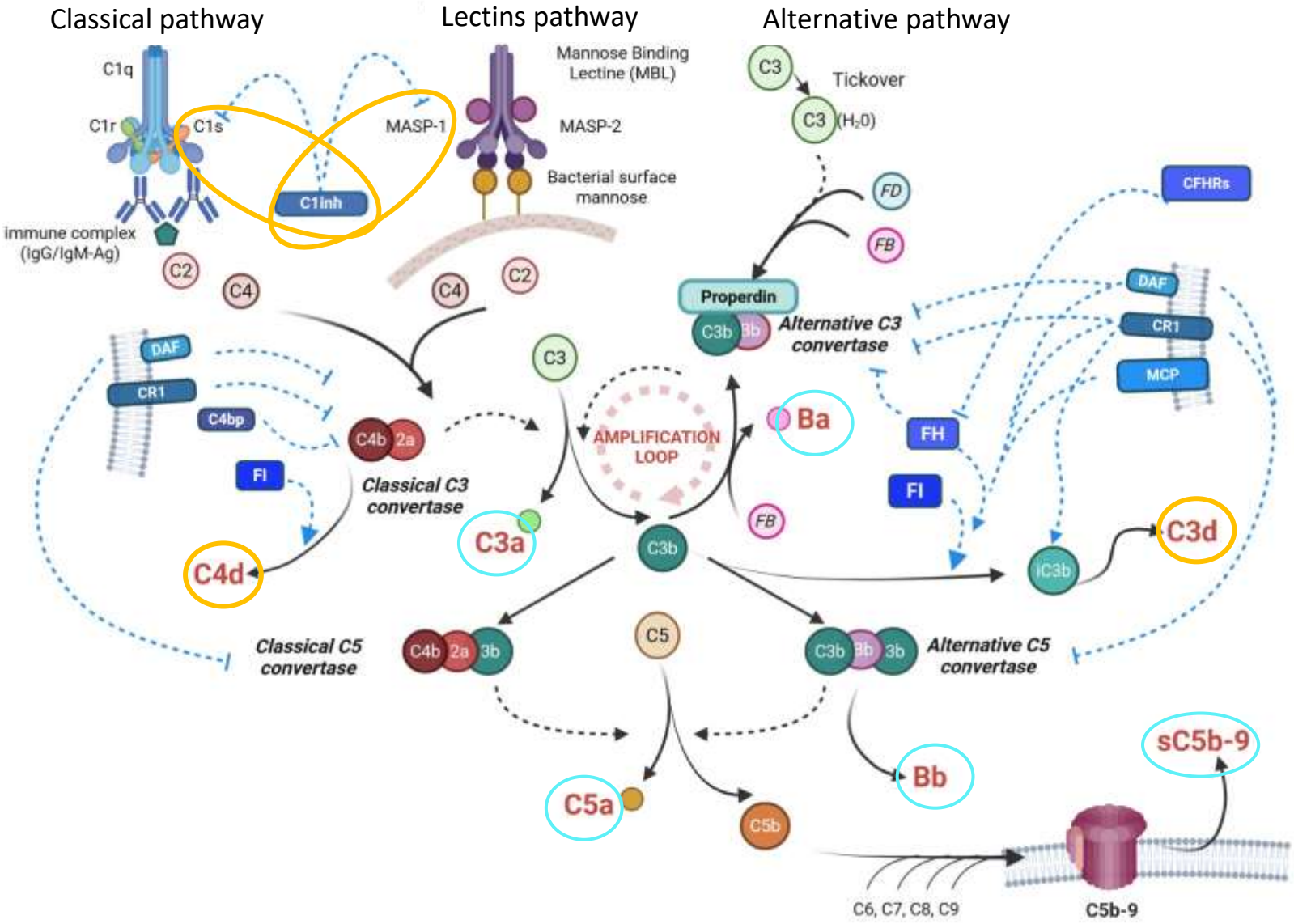
Assessment of Complement activation state in body fluids



Only measurement at the proteic level of products generated by the cascade allows determining the activated pathway(s) and its intensity



Assessment of Complement activation state in body fluids

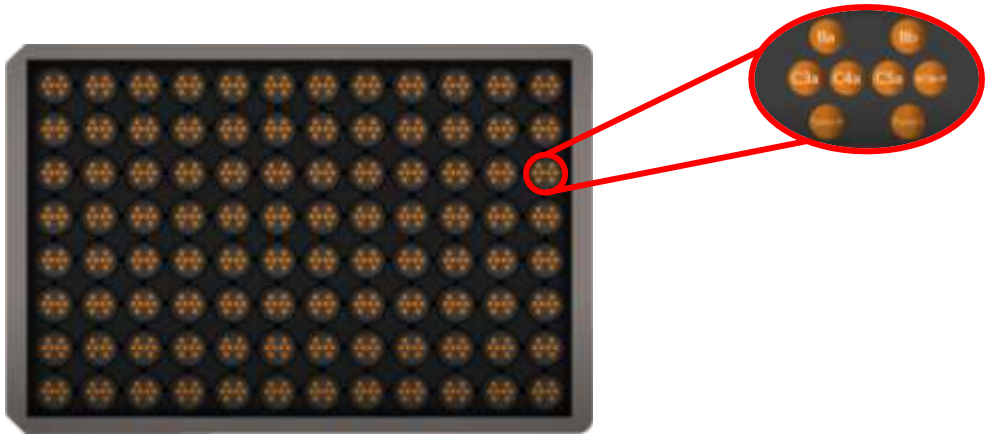


- Single ELISA (Svar®, Hycult®, Quidel®)
- Multiplexed ELISA (Quidel®)

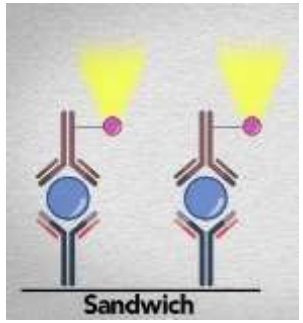
+/- measurement of intact proteins (C4, C3, FB, C5)-> ratios (multiplexed ELISA (Quidel®) or turbidimentry (The Binding site®))

+/- inflammation markers (in plasma) : CRP : IL-6 stimulation, Calprotectin : granulocytes activation (SvarCalpro®)

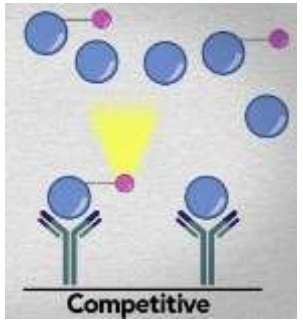
MicroVue Complement Multiplex



Panel 1: “fragment” (Complement Activation)

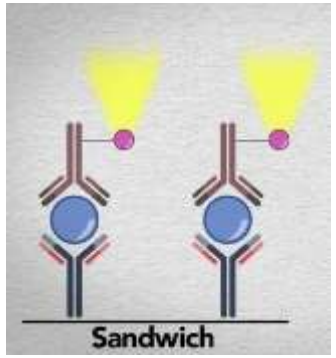


Ba, Bb, C3a, C4a, C5a, sC5b-9,



Factor H, Factor I

Panel 2: “complete” (Complement components levels)

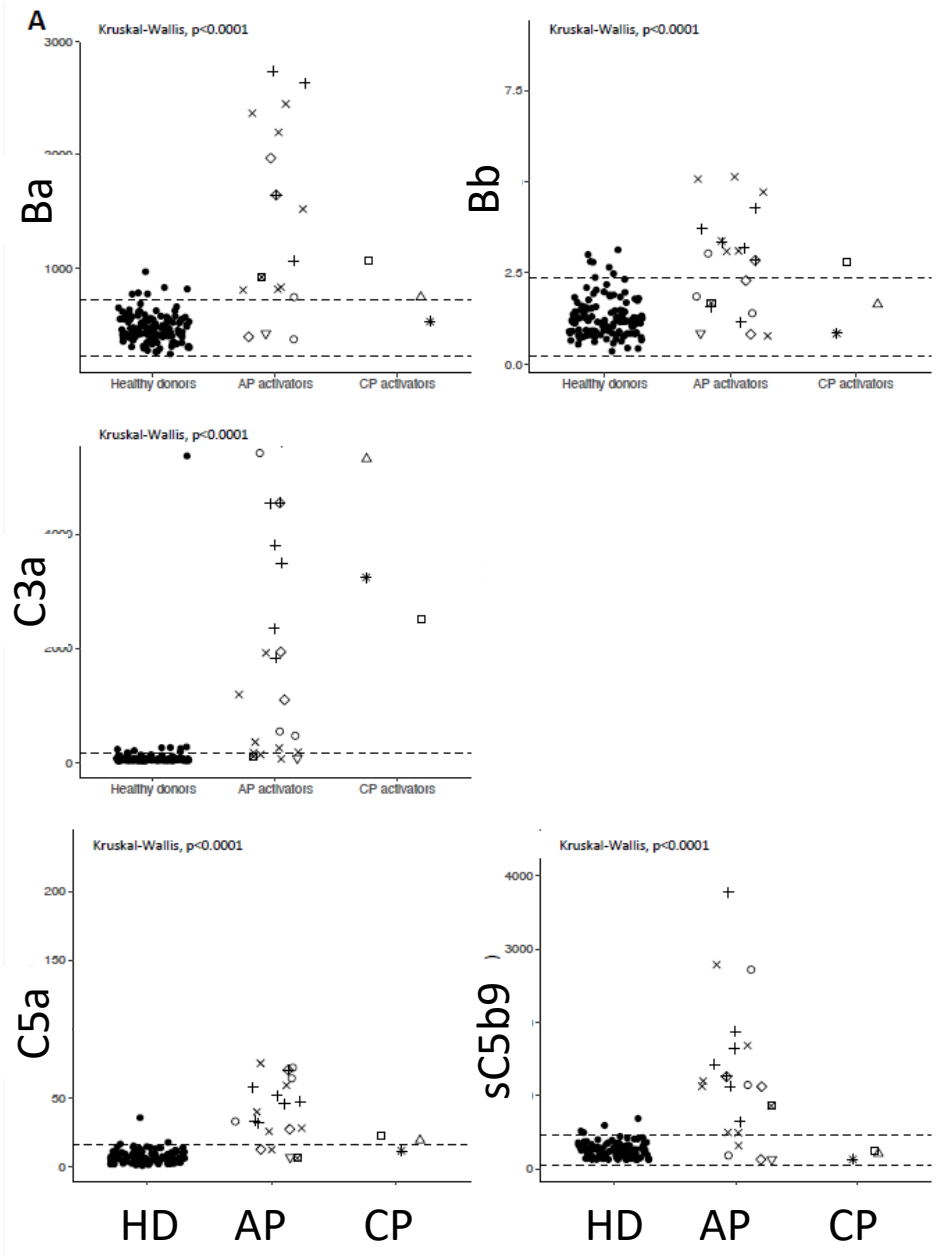


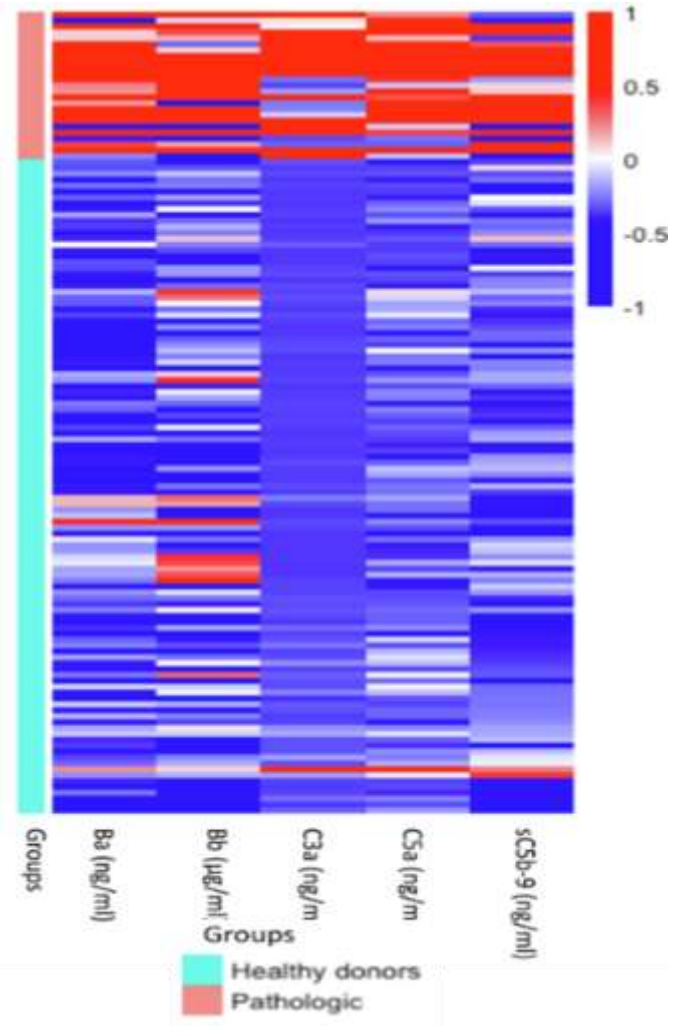
C1q, C2 Intact, C3 Intact, C4 Intact, C5 Intact, Factor D, Factor P

Panel routine: FH, FI, sC5b9, Bb

QuidelOrtho™

Complement activation profile assessed in plasma from patients with various complement-mediated disorders.

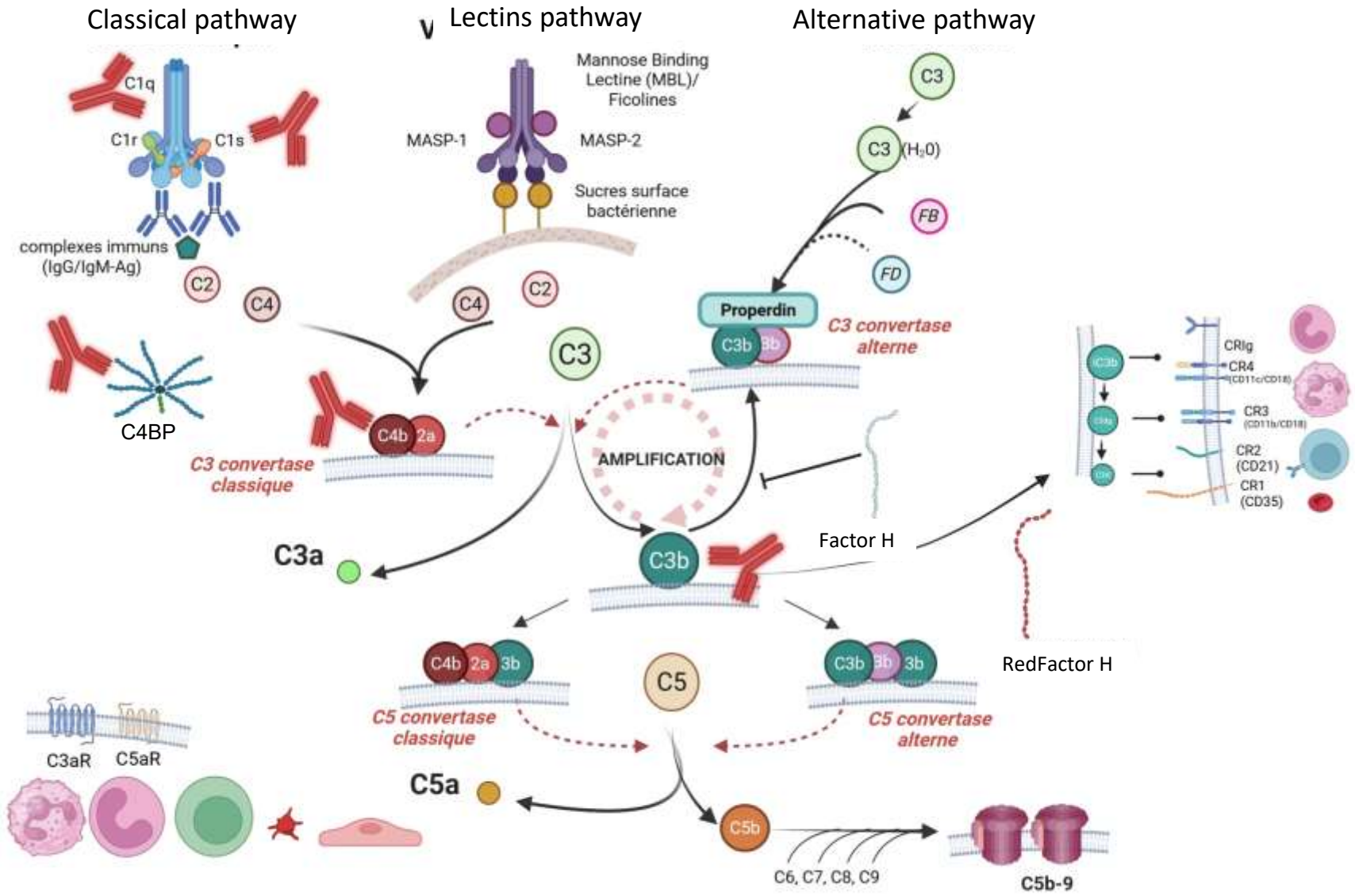




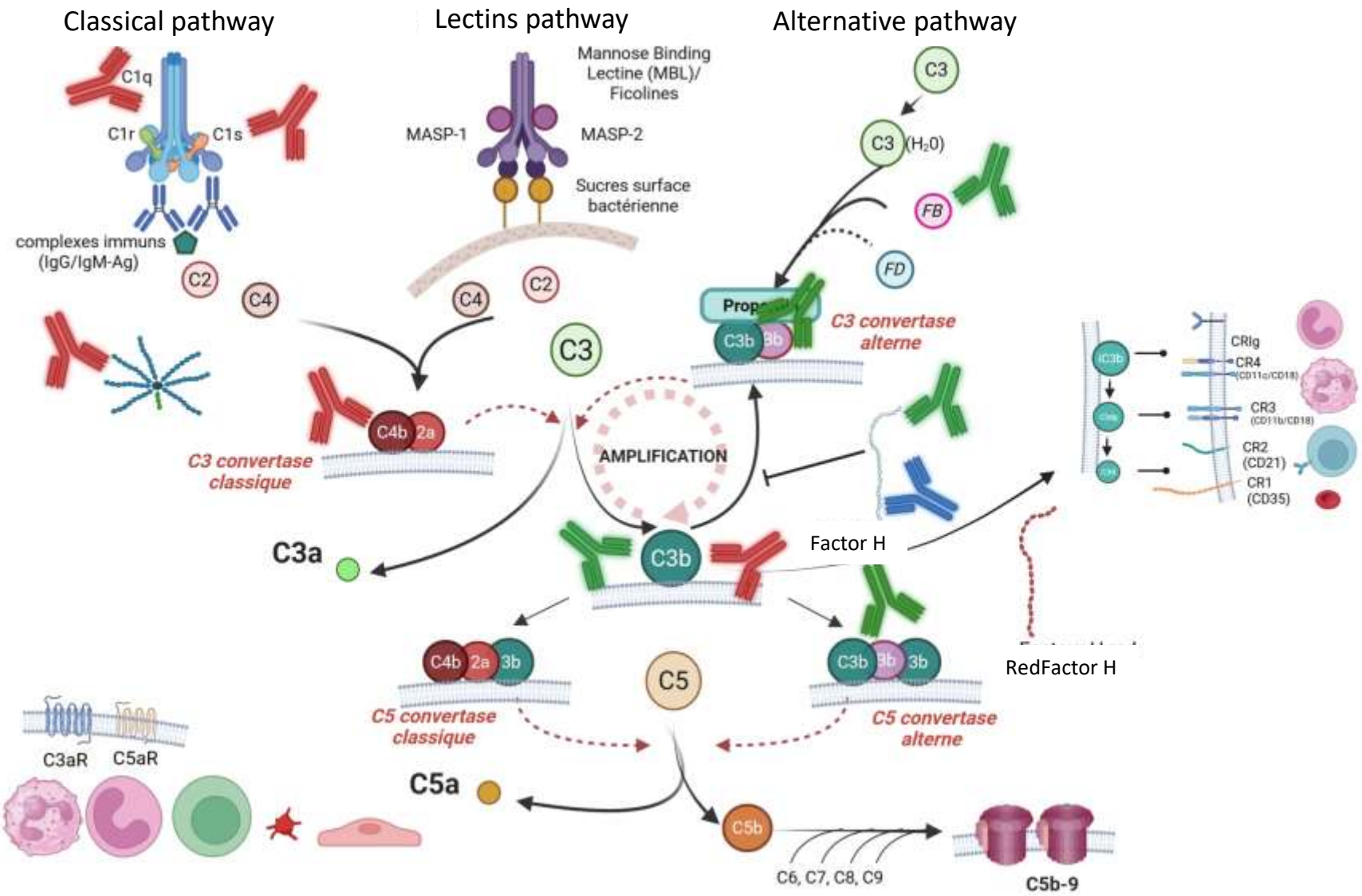
M.-S. Meuleman, Clin Chimica Acta (2024)

Anti-Complement proteins autoantibodies

SLE/APS



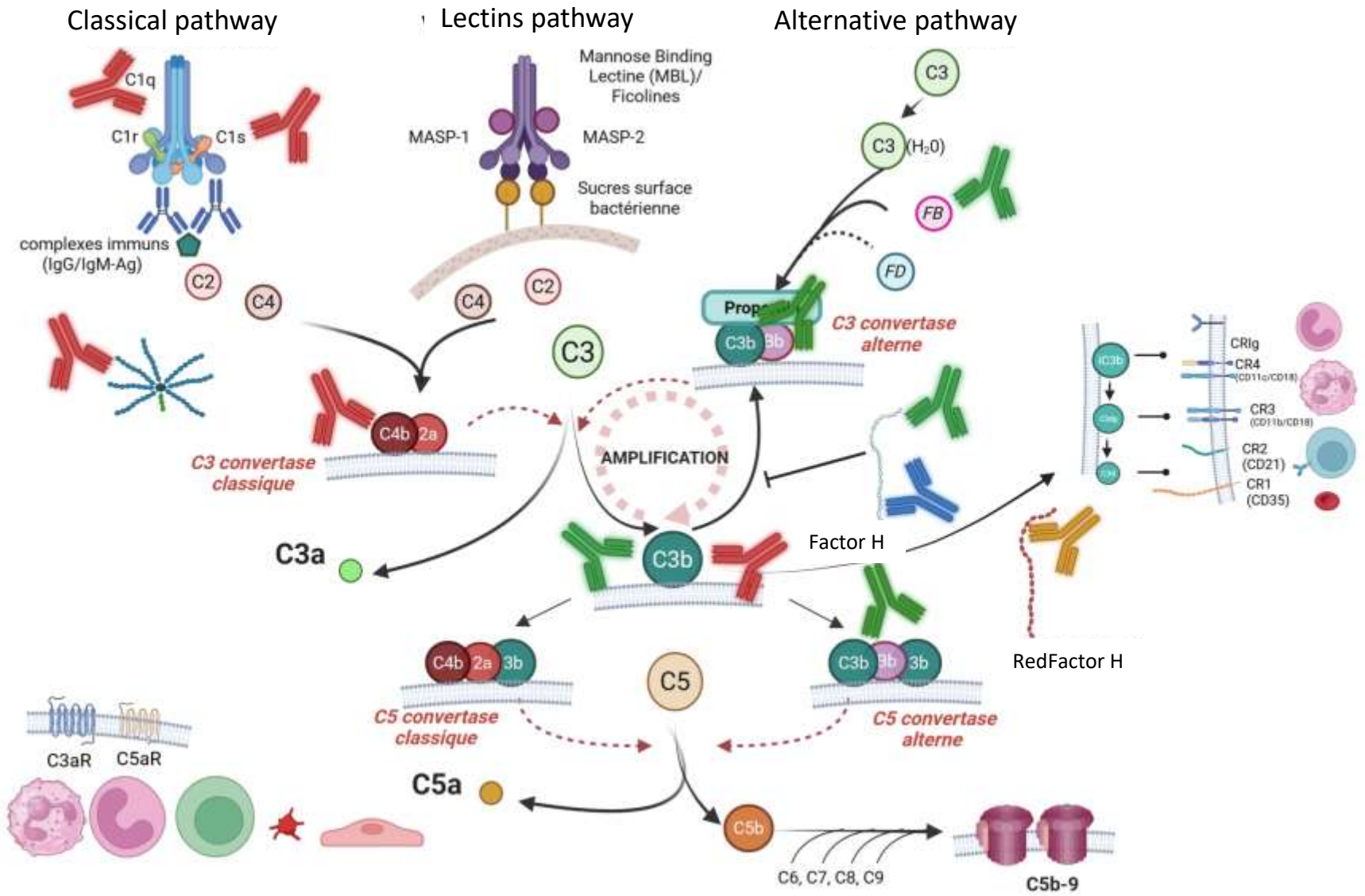
Anti-Complement proteins autoantibodies



SLE/APS
HUS
C3G

H

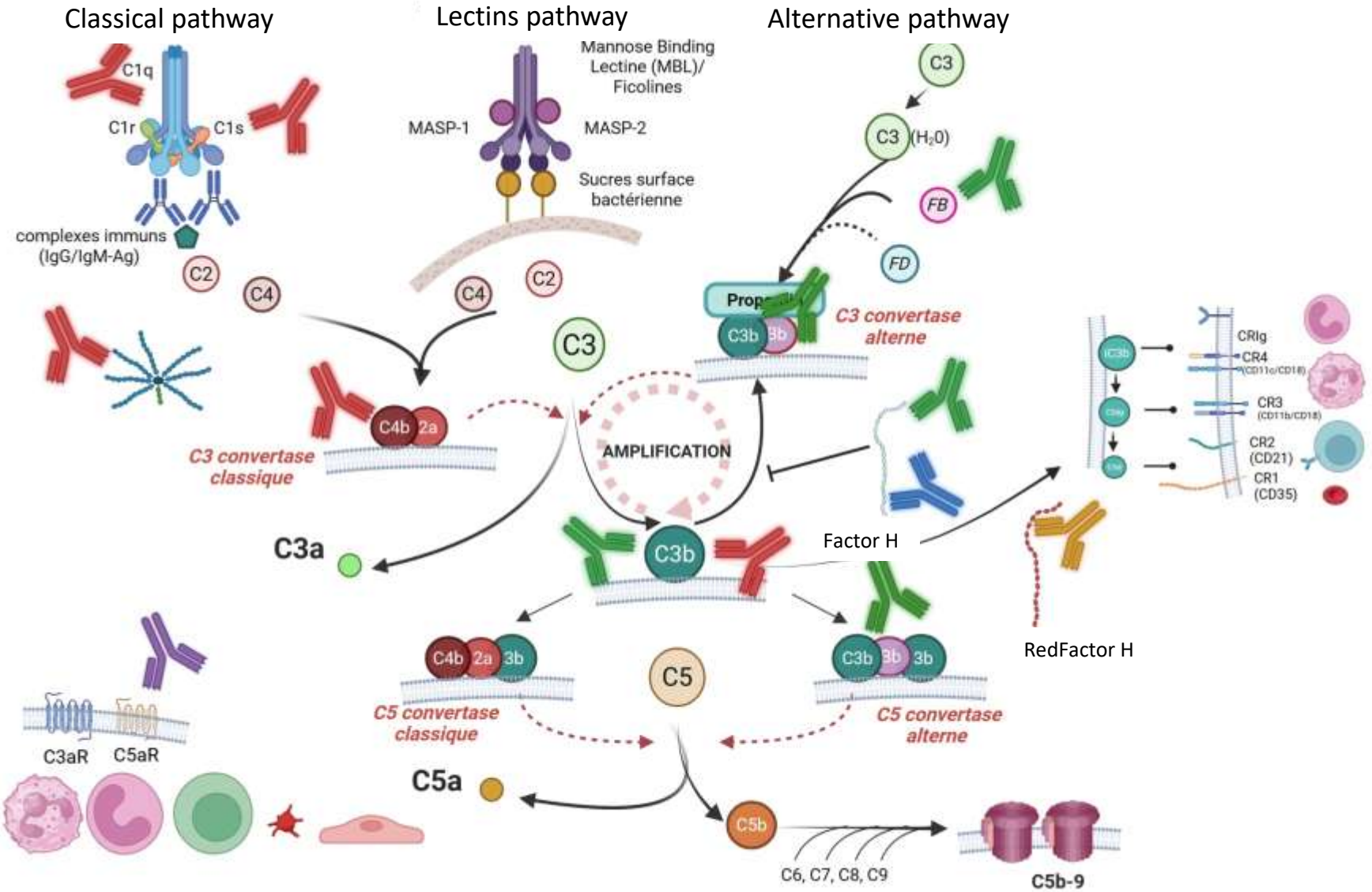
Anti-Complement proteins autoantibodies



SLE/APS
C3G
HUS
Cancers

H

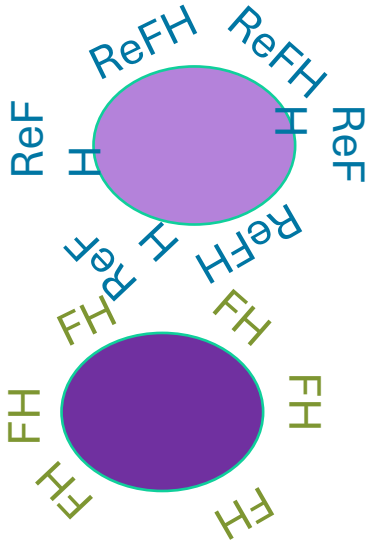
Anti-Complement proteins autoantibodies

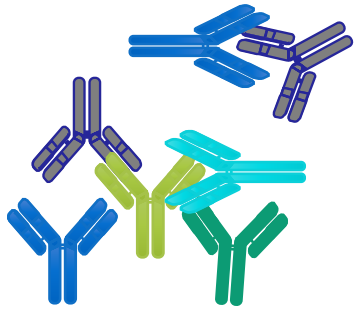


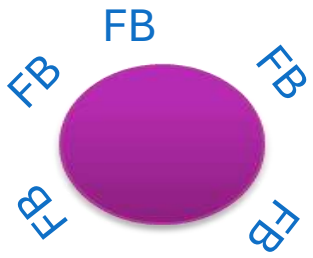
SLE/APS
C3G
HUS
Cancers
AAV?

H

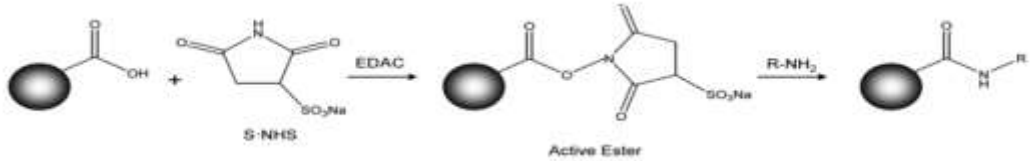
Multiplexed detection of anti-complement protein autoantibodies

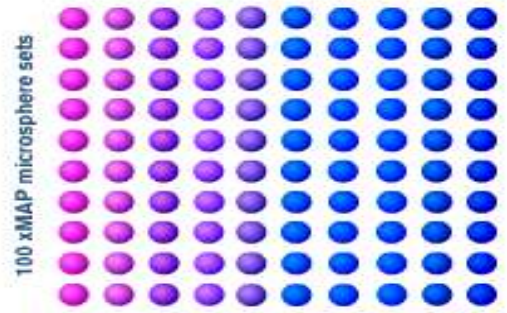




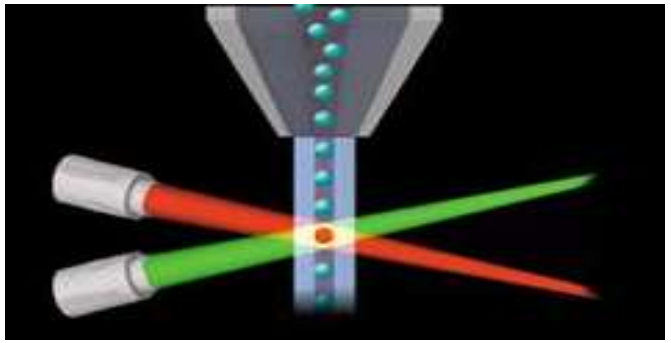


Covalent coupling of native proteins on different fluorescent beads



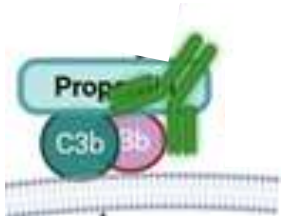


+ anti-Hum IgG/IgA or IgM **PE**



R Philip, Eur J Immunol, 2024
Revel, Artero et al. Oncoimmunol, 2024

Nephritic factors in C3G and MPGN : Enhancing effect of autoantibodies targeting the Convertases

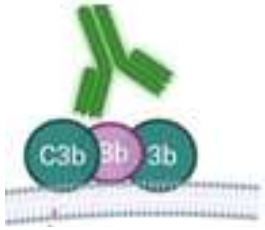


C3 Nef

AP C3 convertase

C4 Nef

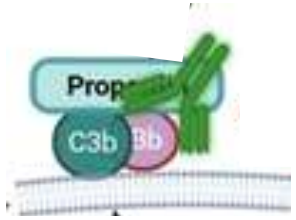
CP C3 convertase



C5 Nef

AP C5 convertase

Nephritic factors in C3G and MPGN: Enhancing effect of autoantibodies targeting the Convertases

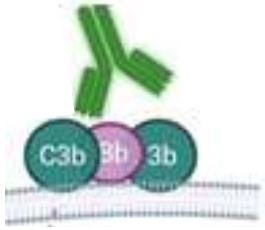


C3 Nef

AP C3 convertase

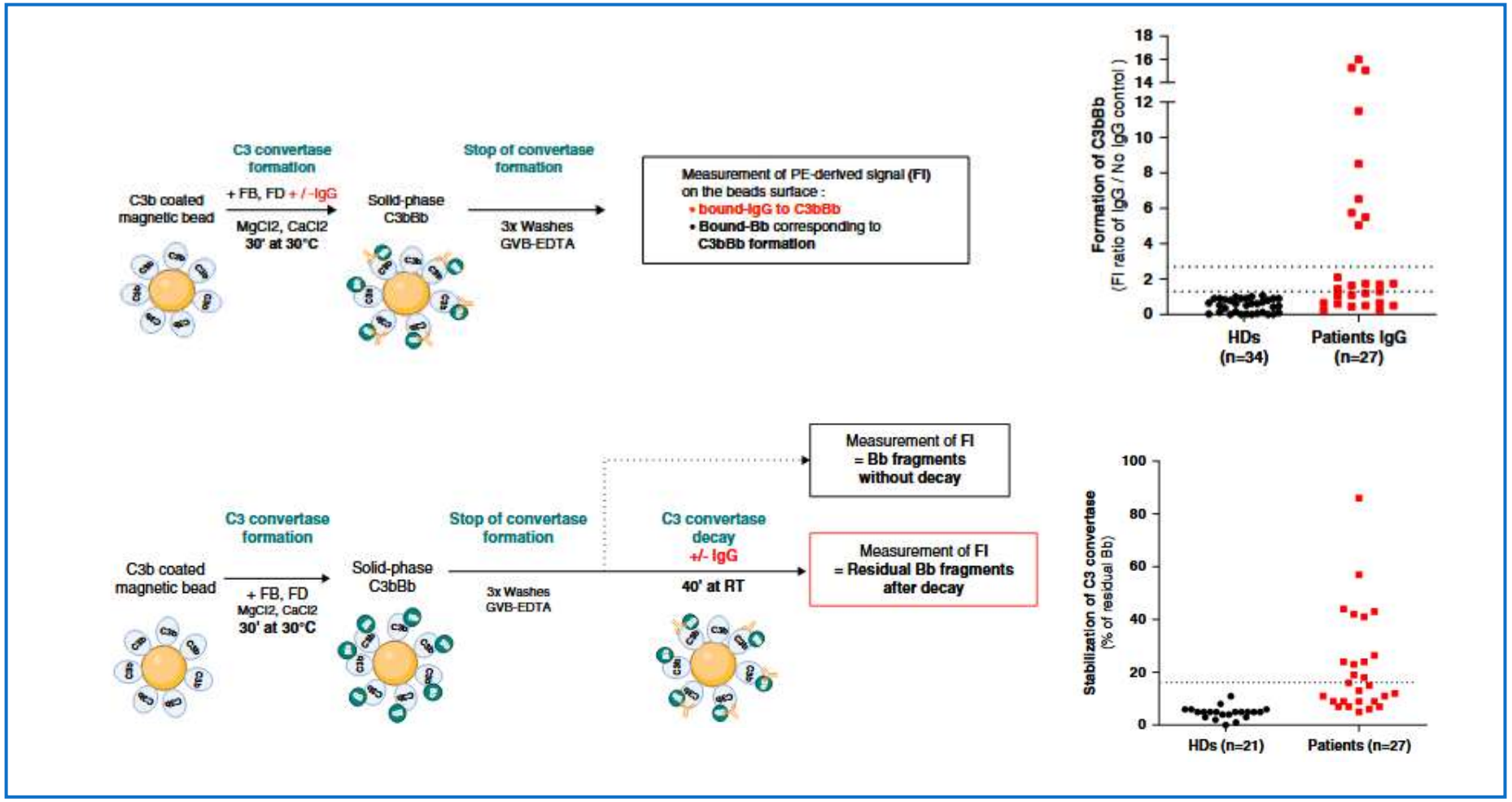
C4 Nef

CP C3 convertase



C5 Nef

AP C5 convertase



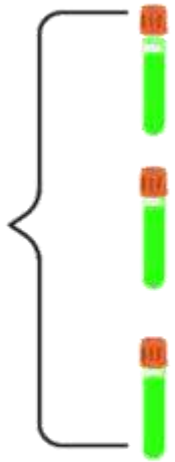
Roquigny J, JASN, 2025

Integrated complementomics

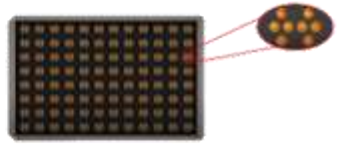
Patients' cohort

Controls' cohort

Plasma EDTA

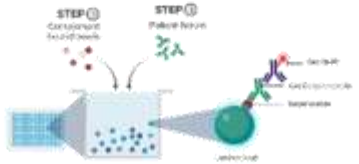


Complement Multiplex



C4d, Calprotectin, C1s-C1INH, CRP, C3d

Anti-Complement Ab detection by Luminex



Humoral complementomics

Validated tools

QuidelOrtho

SVAR

HycultBiotech

Hôpital européen Georges-Pompidou AP-HP

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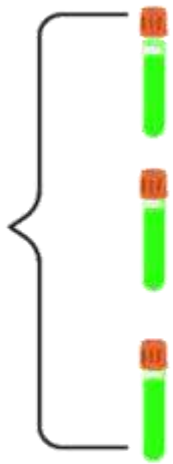

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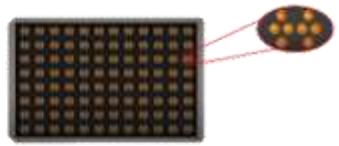

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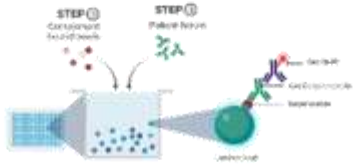

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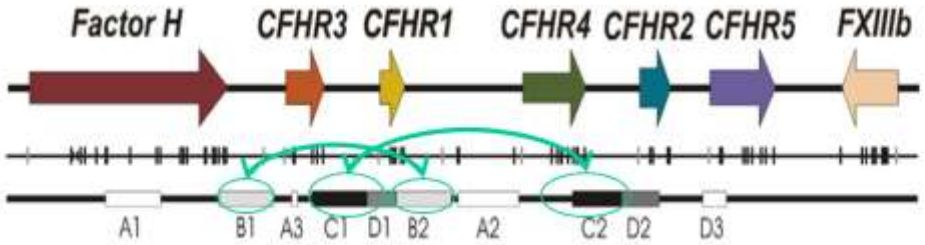
Complome



Factor H/CFHR gene cluster

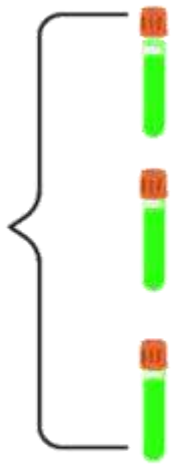
Exons

Recombination domains

Integrated complementomics

Patients' cohort

Plasma EDTA



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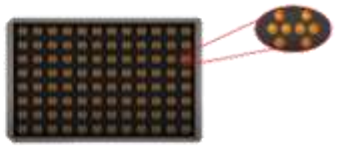

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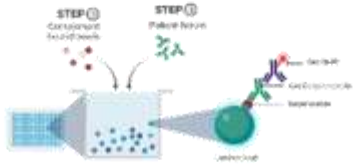

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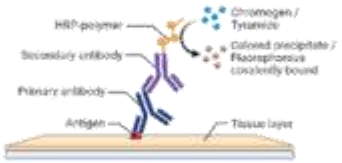
Complome

Tissue

FFPE

IHC/IFA



Validated tools

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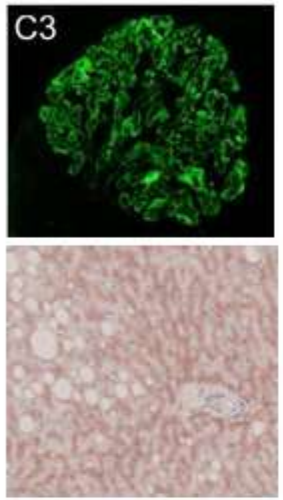
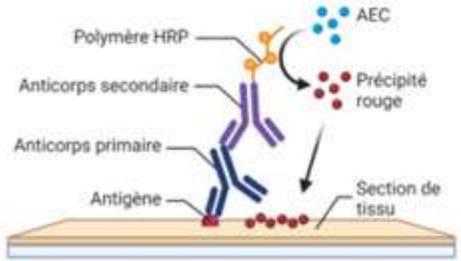
Lunaphore technologies

Antibody-based techniques are the most widely used

Monoplex

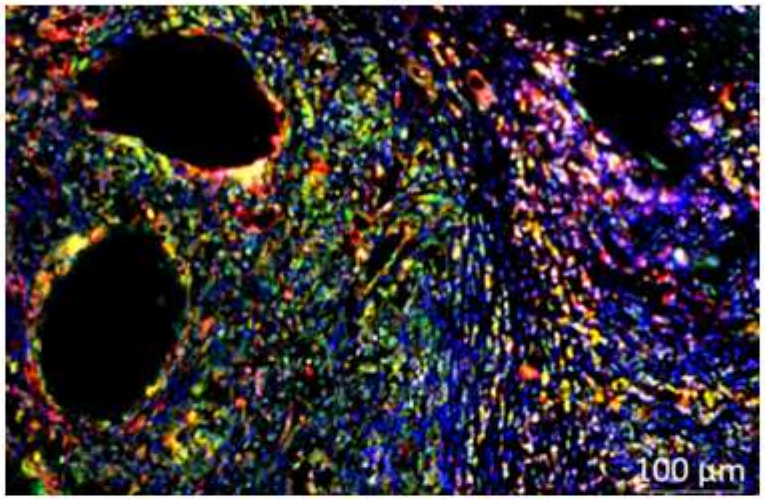


IHC or IF : 1 marker + nucleus (hematoxylin)

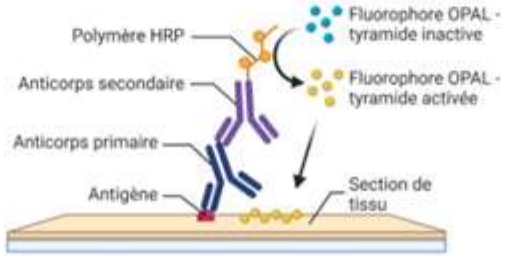


Possibility of combining 2 markers (IHC)
4 markers (IF with 4 different channels).

Multiplex

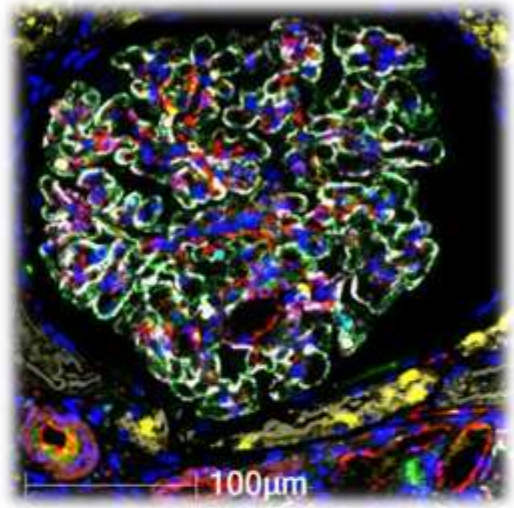


Tyramide /OPAL:
8 markers + nucleus (DAPI)



Detection of up to 6 markers without signal demixing
Detection of up to 8 markers with signal demixing

Hyperplex

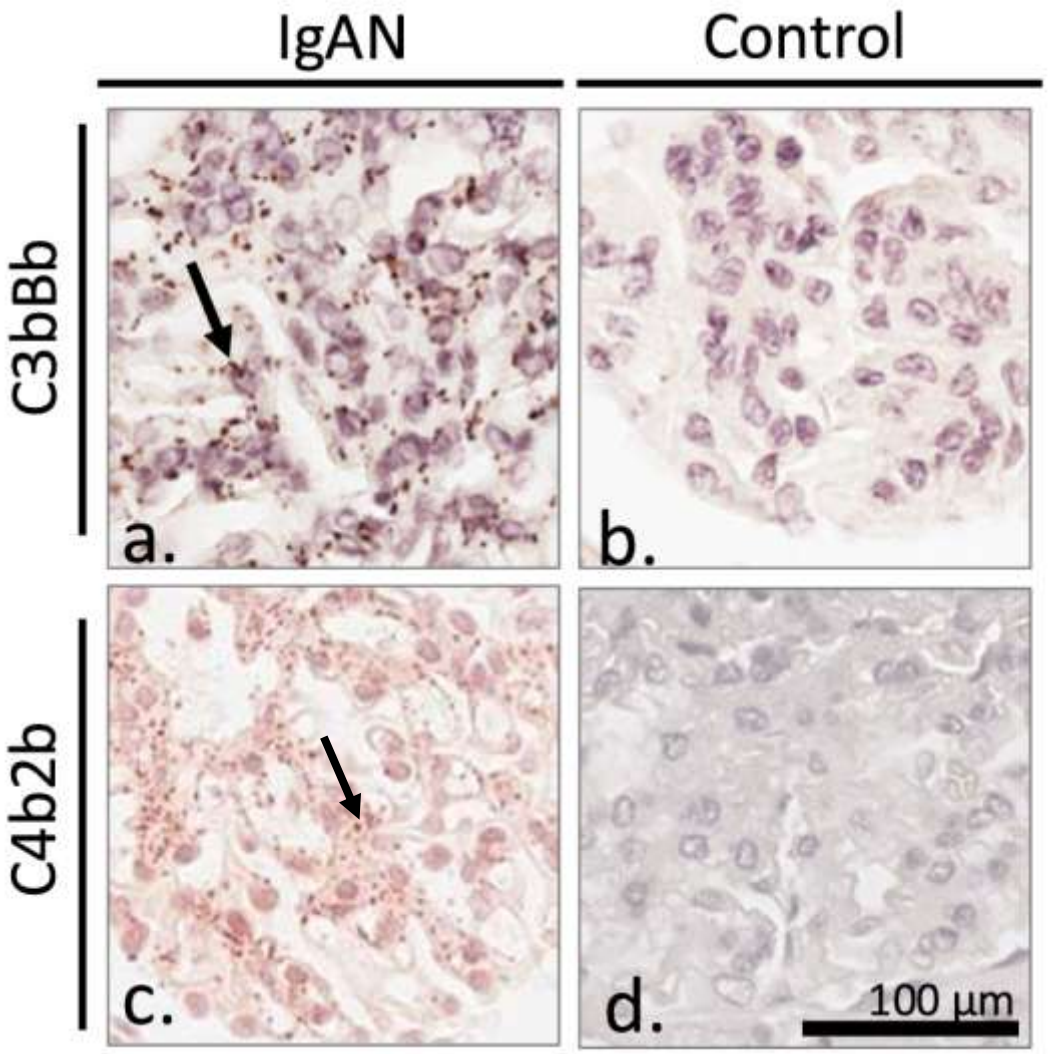


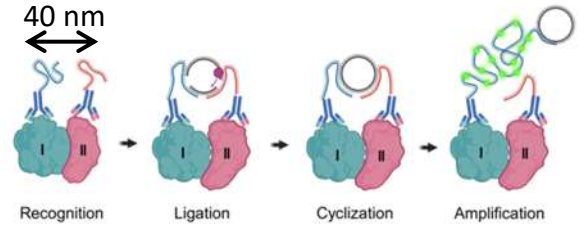
COMET (Lunaphore)
40-plex

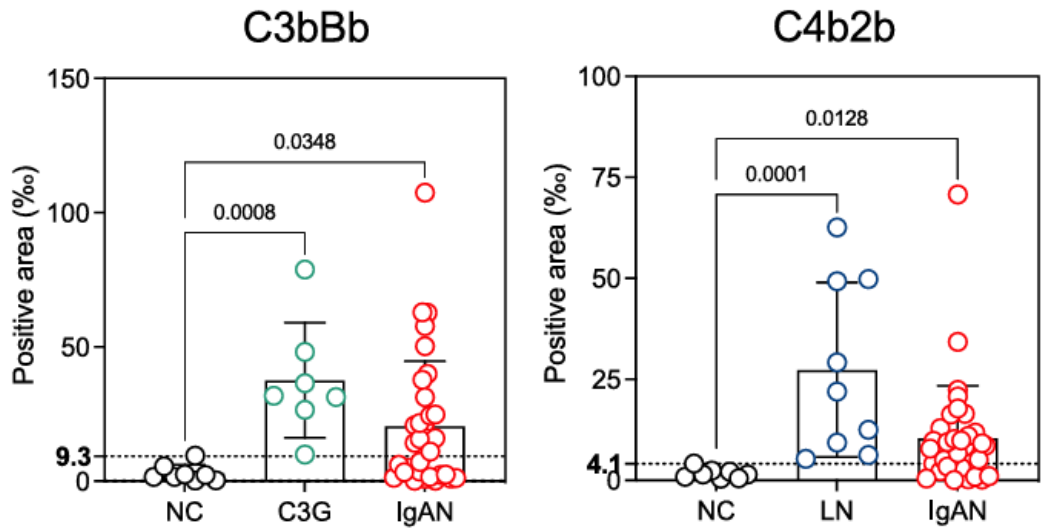
COMET (Lunaphore)
Phenocycler (Akoya)
MACSima (Miltenyi Biotec)
Cell DIVE (Leica Microsystems)
Orion / Orion HT (RareCyte)
Hyperion XTi (Standard BioTools) etc...

Thanks to Idris Boudhabhay

In situ Convertases quantification by proximity ligation Assay







Obrecht A, Nephrol Dial Transplant , 2026

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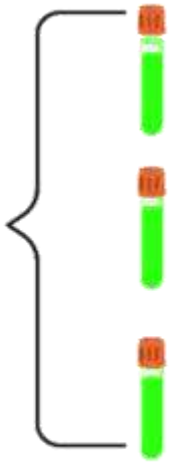

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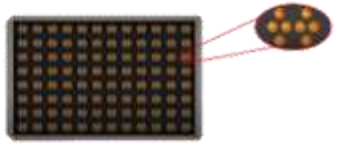

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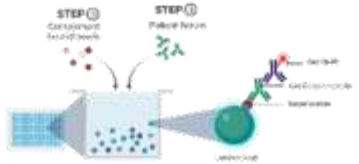

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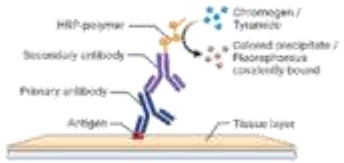


Lunaphore technologies

***In situ* Complementomics**

Take home messages

- ✓ Ct is involved in :
  - ✓ Protection and cleansing (elimination of **pathogens,** of **dying cells,** of **immune complexes),**
  - ✓ Sensoring **immune cells** and cross talk between innate and adaptative immunity

! Prevention of infections when using Ct blockers!

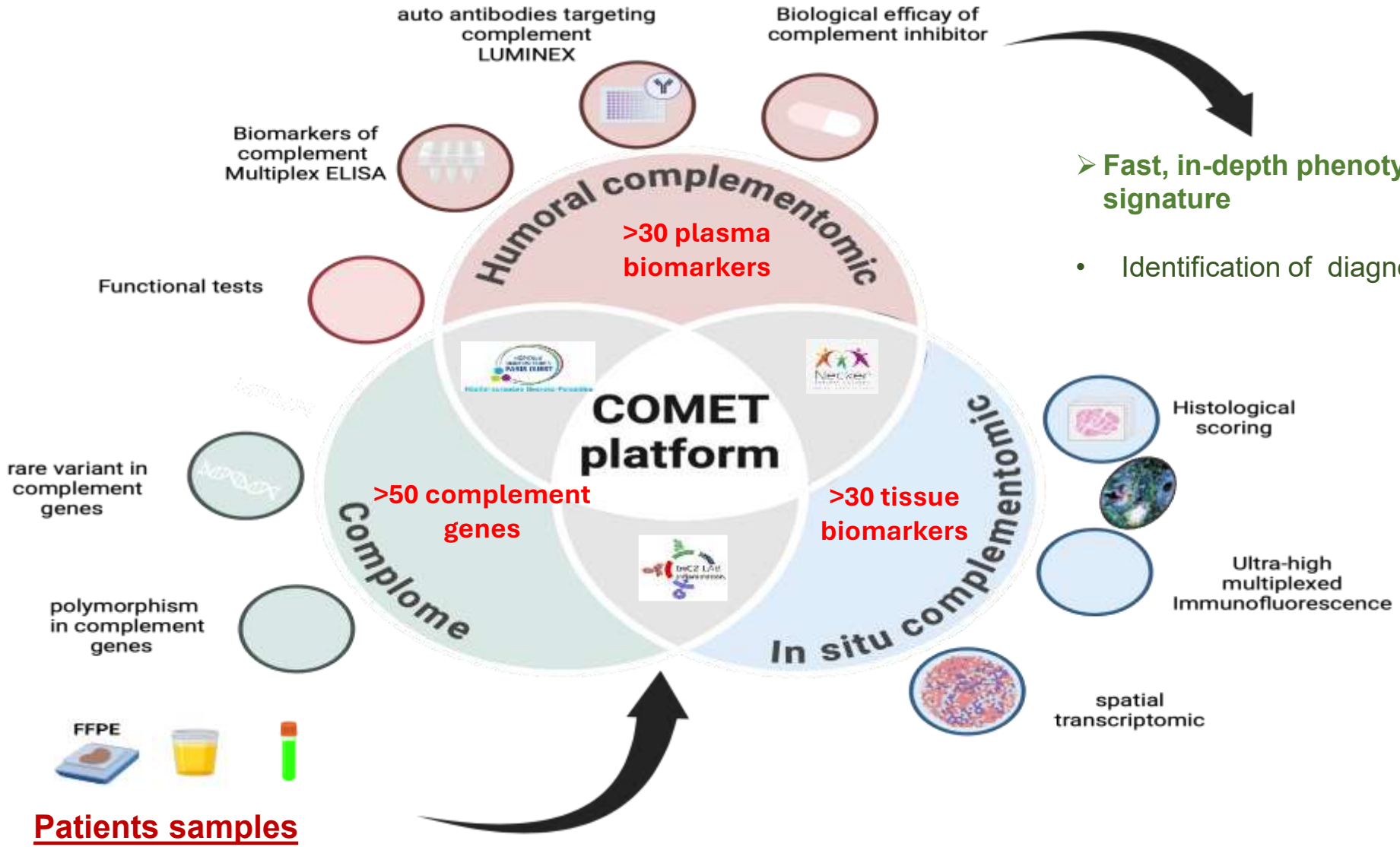
- ✓ There are hereditary or acquired **deficiencies**, **genetic polymorphisms** involved in different **rare and common diseases**

- ✓ **Complement activation** is involved in numerous **pathological situations**

- ✓ The **complement activation blockade is a therapeutical approach** using different targets and molecules **already in the market or in the pipeline**

The use of these molecules must/should be based on well **characterized physiopathological mechanisms** of the diseases
and accompanied by performant and standardized **biological exploration** to allow a personalized medicine and treatment monitoring

Integrated « complementomics » for precision medecine



➢ **Fast, in-depth phenotyping of the complement signature**

- Identification of diagnosis/prognosis biomarkers

Certification FHU Hospital University Federation, 2025

Acknowledgments

All members of the Inflammation, Complement and Cancer team and of HEGP Laboratory of Immunology

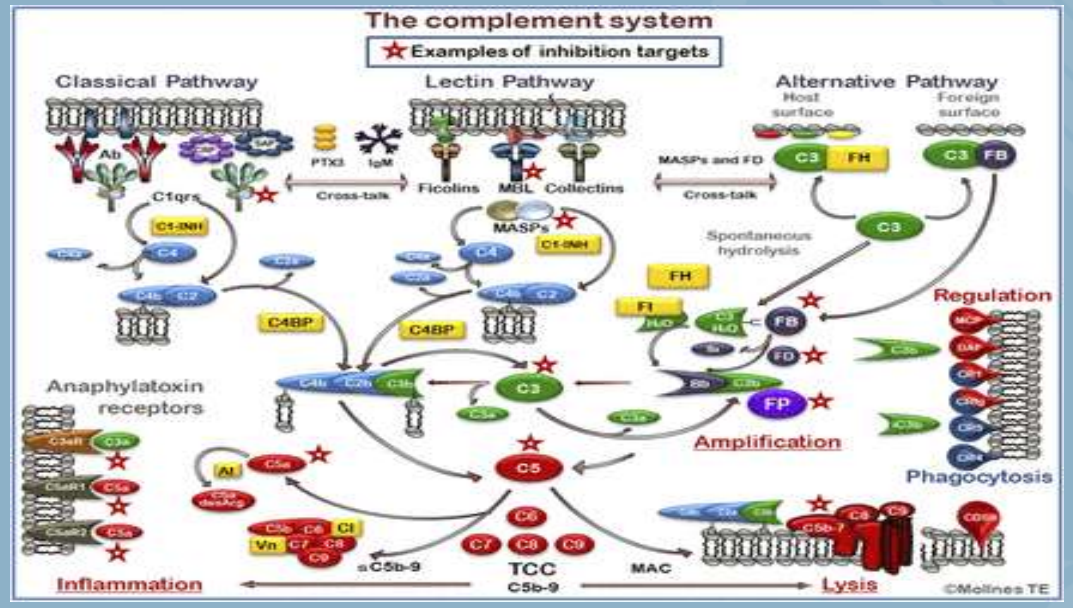
Complement group (Paris)

- **LT Roumenina**
- **H Hamidi**
- **I Boudhabhay**
- **A Grunenwald**
- **S Chauvet**
- **A Duval**
- **MS Meuleman**
- **J Roquigny**
- **P Martins Veira**
- **C El Sissy**
- **V Fremeaux-Bacchi**

CENTRE DE RECHERCHE DES CORDELIERS

Thank you for your attention!

P Garred, A tenner, T Mollnes, Pharmacological Rev 2021,



FHU COMET
COMplement Expertise and Therpeutics

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

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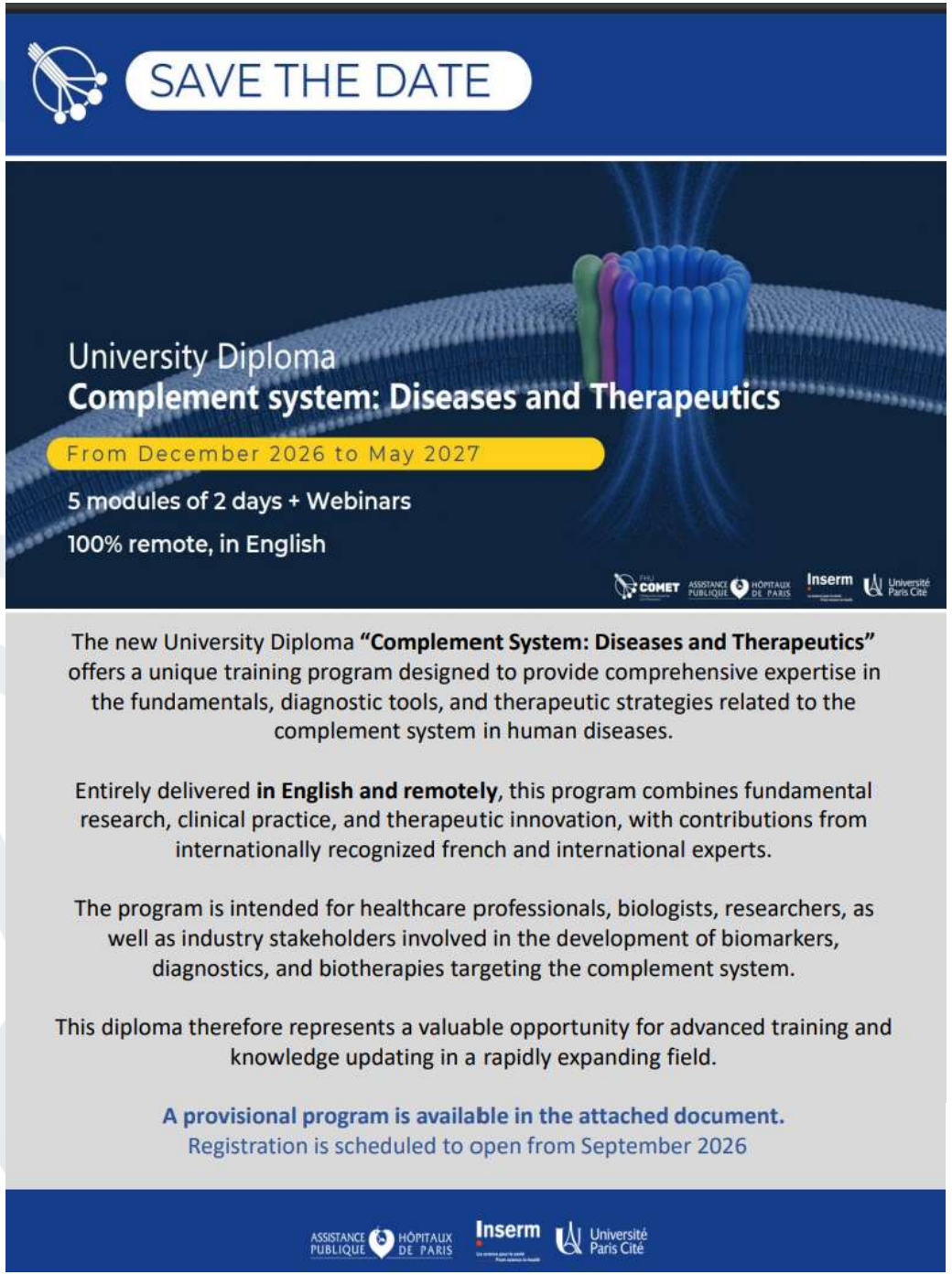
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University Diploma
Complement system: Diseases and Therapeutics
From December 2026 to May 2027
5 modules of 2 days + Webinars
100% remote, in English
The new University Diploma "Complement System: Diseases and Therapeutics" offers a unique training program designed to provide comprehensive expertise in the fundamentals, diagnostic tools, and therapeutic strategies related to the complement system in human diseases.
Entirely delivered in English and remotely, this program combines fundamental research, clinical practice, and therapeutic innovation, with contributions from internationally recognized french and international experts.
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