

Disclosure belangen spreker bijeenkomst  
Consortium Transfusiegeneskundig Onderzoek d.d. 22-11-2019

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Geen (potentiële) belangenverstrengeling

Voor bijeenkomst mogelijk relevante relaties

- Sponsoring of onderzoeksgeld
- Honorarium of andere (financiële) vergoeding
- Aandeelhouder
- Andere relatie, namelijk ...

Bedrijfsnamen

- Sanquin Research

# Complement Activation by anti-HLA antibodies in platelet refractoriness

Thijs van Osch, PhD Candidate, 2nd Year

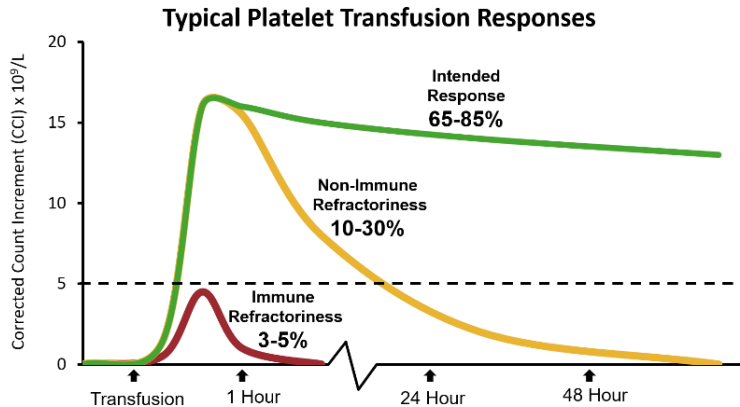
Department of Experimental Immunohematology (IHE), Immunoglobulin Research

Supervisors: Gestur Vidarsson and Jan Voorberg



## Platelet Transfusion

- To prevent or treat bleeding
- Mainly patients with oncological diseases receiving chemotherapy or stem cell transplant
- Significantly reduce mortality and hemorrhagic complications



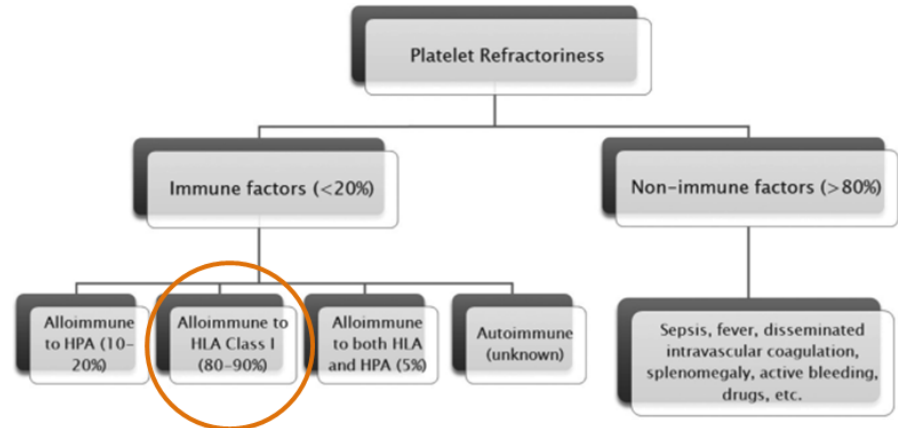
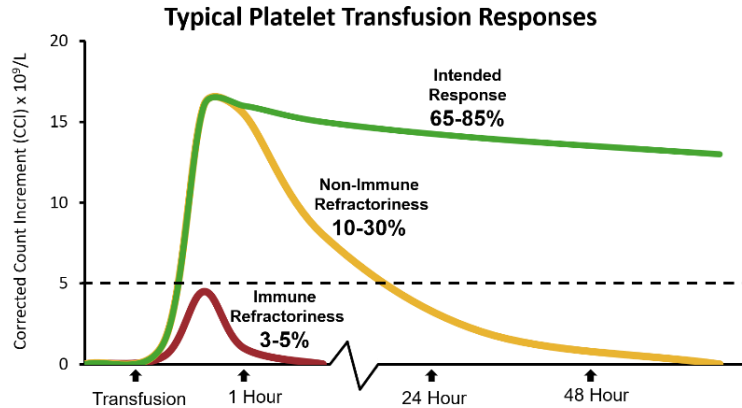
# What is platelet refractoriness?

- Clinically suspected when patients do not respond as expected to platelet transfusion
- The effectiveness of a platelet transfusion is evaluated using the post-transfusion platelet increment (PPI), which is also known as the count increment (CI) or the corrected count increment (CCI)

PPI = Post-transfusion platelet count minus pre-transfusion platelet count

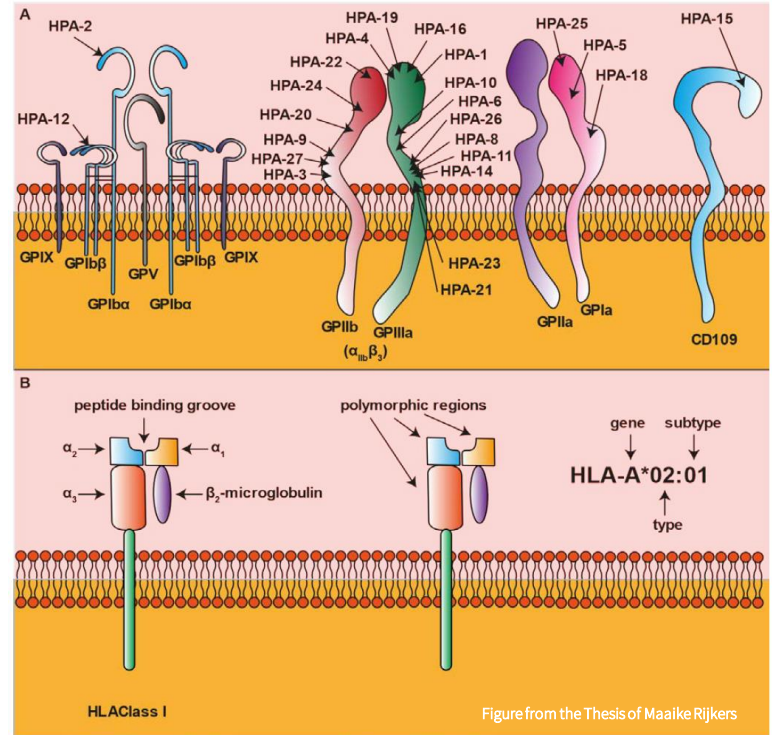
CCI = PPI ( $\mu\text{L}$ )  $\times$  body surface area ( $\text{m}^2$ ) / Number of platelets transfused ( $10^{11}$ )

- When after two consecutive platelet transfusions the expected increase in platelet count is not attained, the patient's condition is considered refractory to platelets. (CCI < 5000 per  $\text{m}^2$  per  $\mu\text{L}$ )



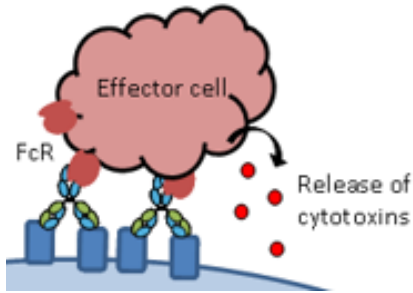
# Alloimmunization mediated platelet Clearance

- Can be induced by previous blood transfusions or pregnancy
- Alloimmunization against Human Platelet Antigens (HPA)
- 33 HPA's expressed on different Glycoproteins (GPs)
- Alloimmunization against Human Leukocyte Antigens (HLA) Class I
- Major Histocompatibility Complex (MHC), recognition between self and non-self
- Extremely Polymorphic; 14.800 different HLA Class I Alleles
- Mismatched platelets in subsequent transfusion will be opsonized and cleared from circulation via antibody-mediated platelet clearance

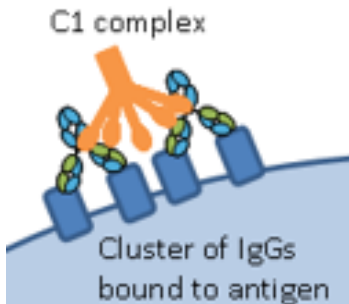


## Alloimmunization mediated platelet Clearance

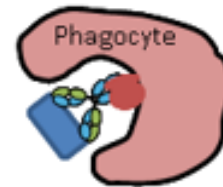
Antibody Dependent Cellular Cytotoxicity (ADCC)



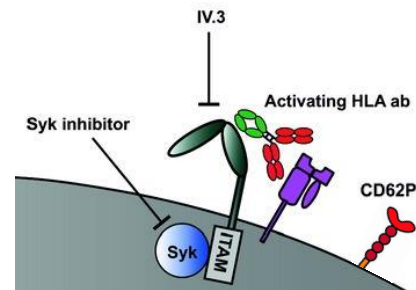
Classical Complement Pathway



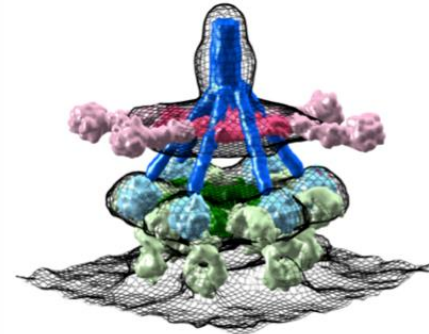
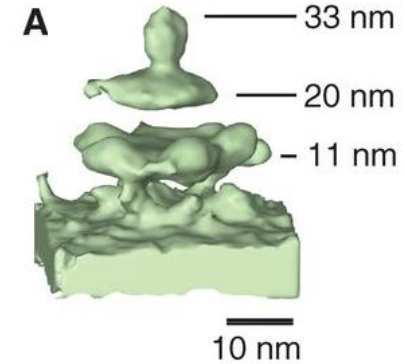
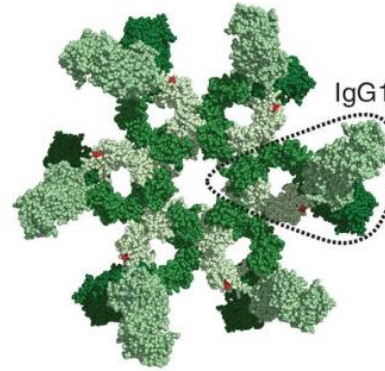
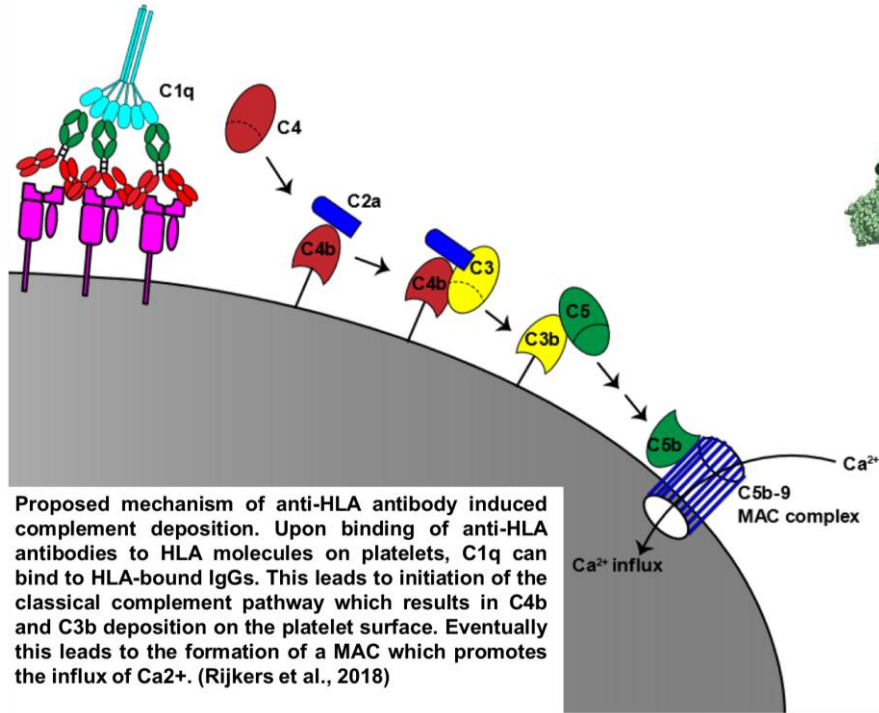
Antibody Dependent Cellular Phagocytosis (ADCP)



Platelet Activation

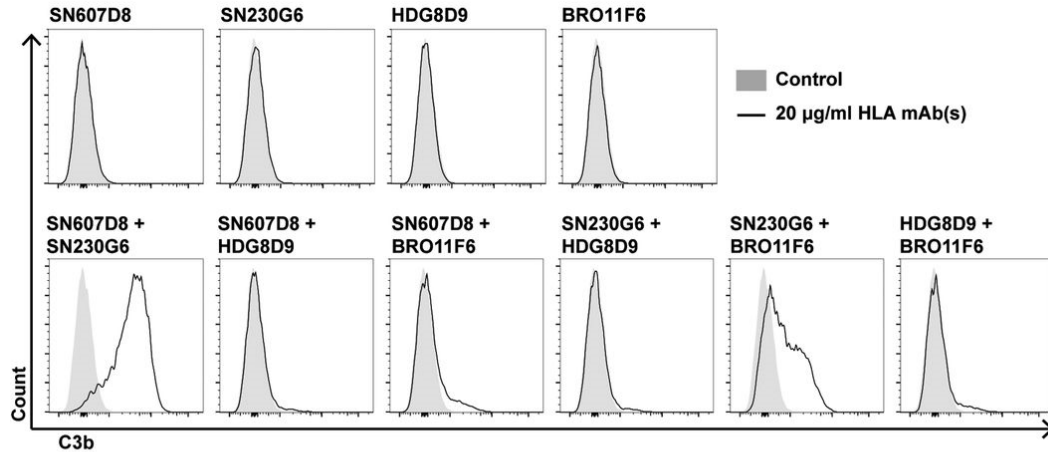


## Involvement of the complement system in platelet clearance

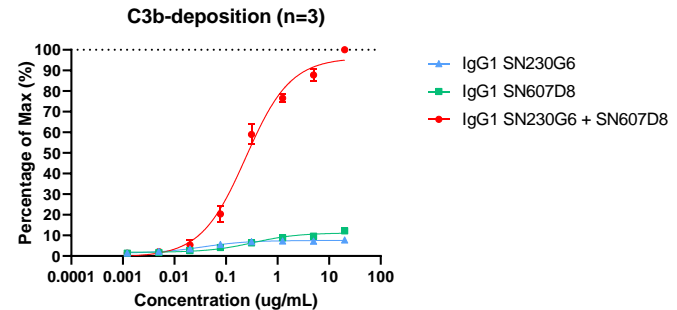
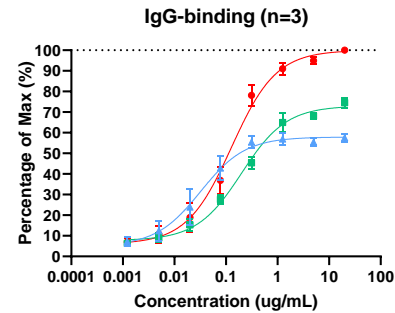


Diebold, Christoph A., et al.  
*Science* 343.6176 (2014):  
1260-1263.

# Synergistic properties on complement deposition by two anti-HLA mAbs



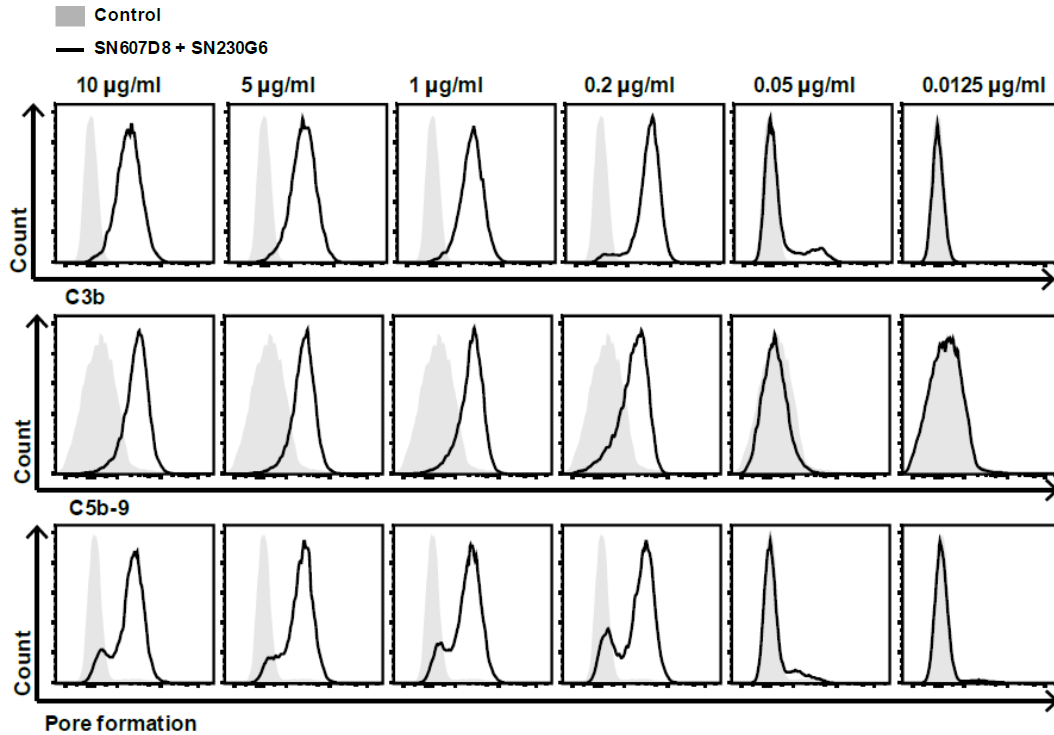
Antibody name	HLA specificity (determined by CDC on large (n>230) panels of HLA typed peripheral blood lymphocytes)
WIM8E5	A1/A10 (25/26/34/43/66)/A11/A9 (23+24)/A29/A30/A31/A33/A28(68+69)
SN607D8*	A2/A28(A68/A69)
SN230G6*	A2/B57/B58
HDG8D9	B51/B35
BRO11F6	A3/A11/A24
DK7C11	B12 (B44/B45)
OK8F12	B46/B62/B72



Rijkers, Maaike, et al.  
Haematologica, 2019,  
104.2: 403-416.



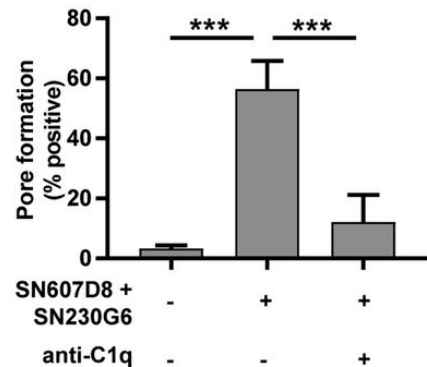
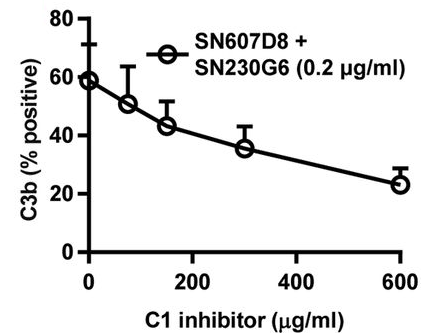
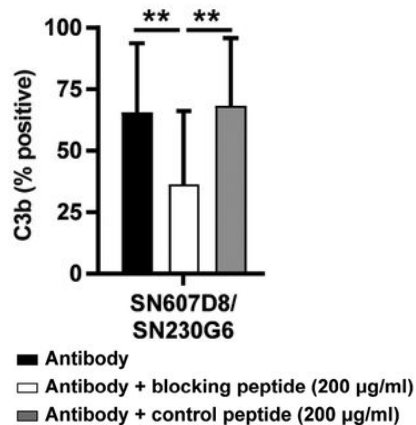
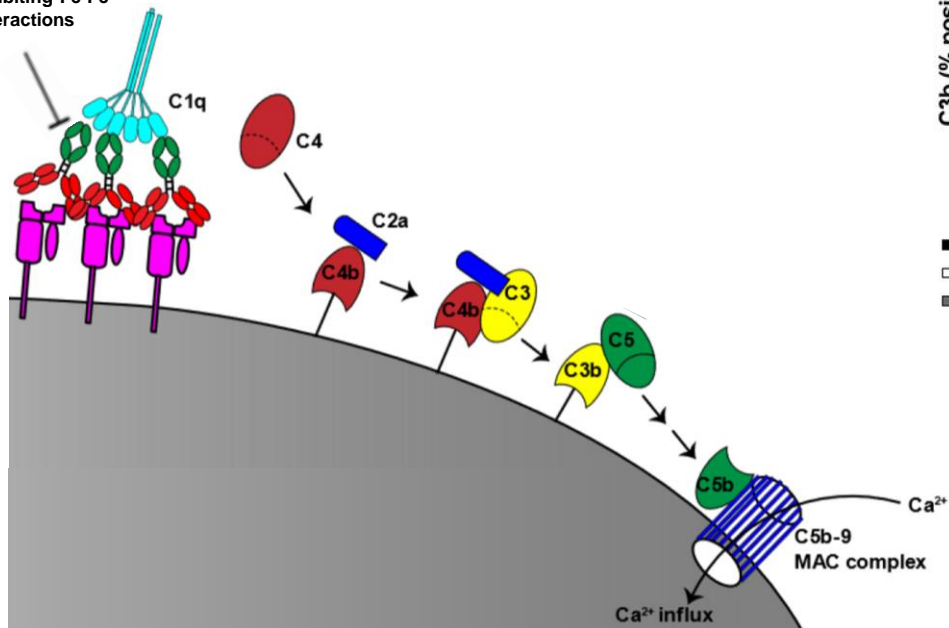
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Rijkers, Maaik, et al.  
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# Complement Inhibitors

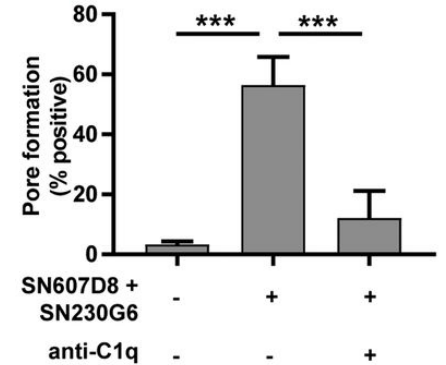
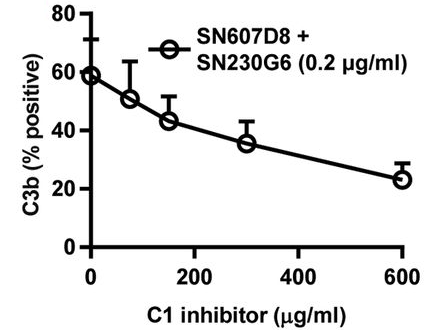
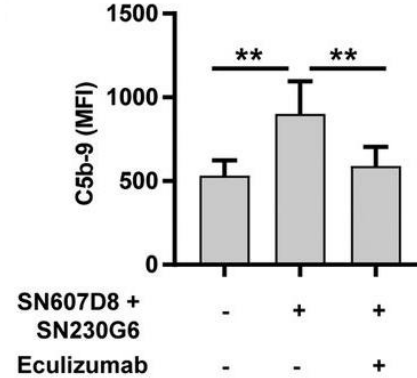
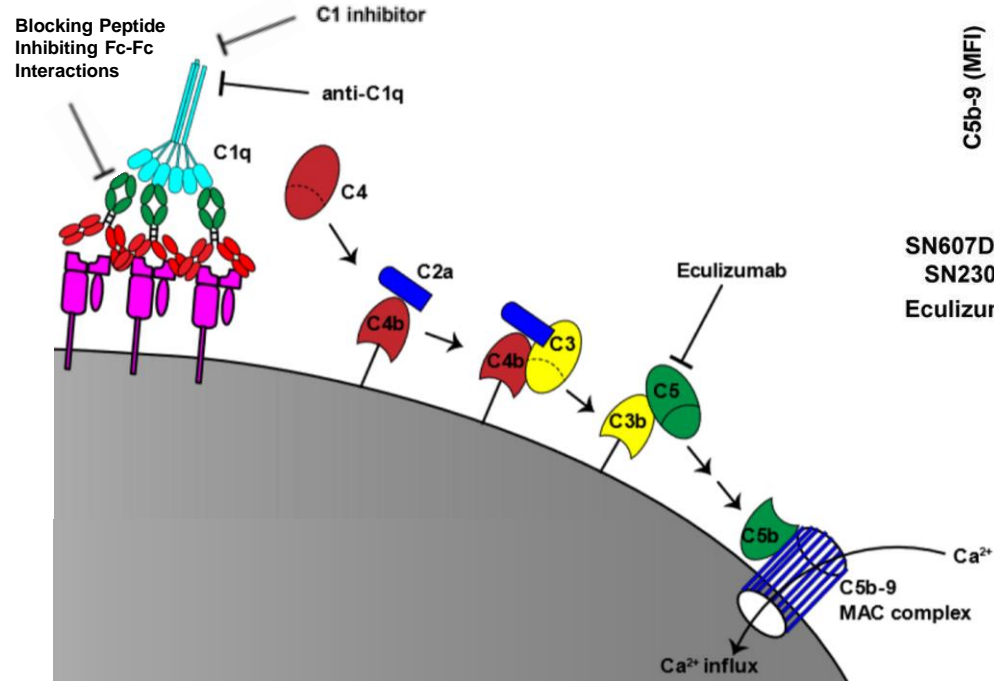
Blocking Peptide  
Inhibiting Fc-Fc  
Interactions



Rijkers, Maaik, et al.  
Haematologica, 2019,  
104.2: 403-416.

10 µg/mL SN607D8 + SN230G6  
50 µg/mL anti-C1q

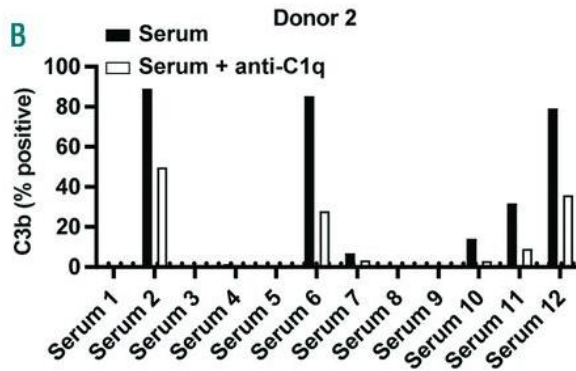
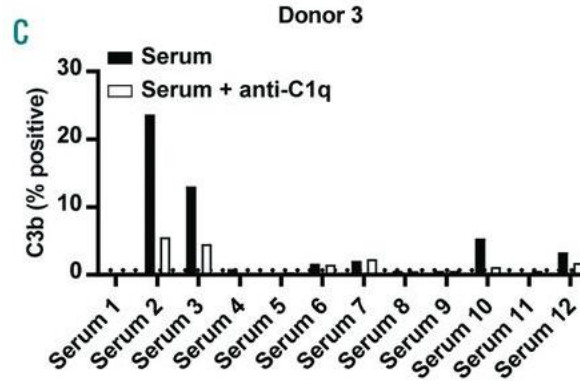
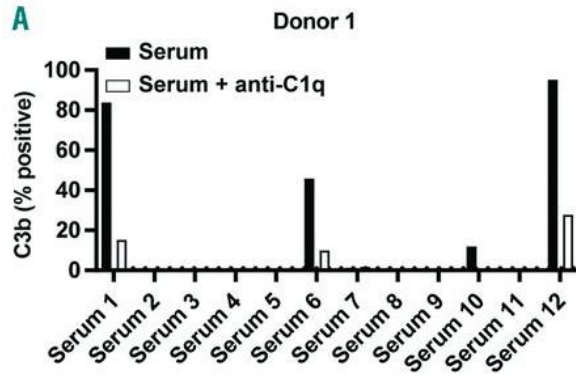
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Rijkers, Maaik, et al.  
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10 µg/mL SN607D8 + SN230G6  
50 µg/mL anti-C1q

# Complement Activation induced by anti-HLA Abs in Sera from patients with platelet refractoriness



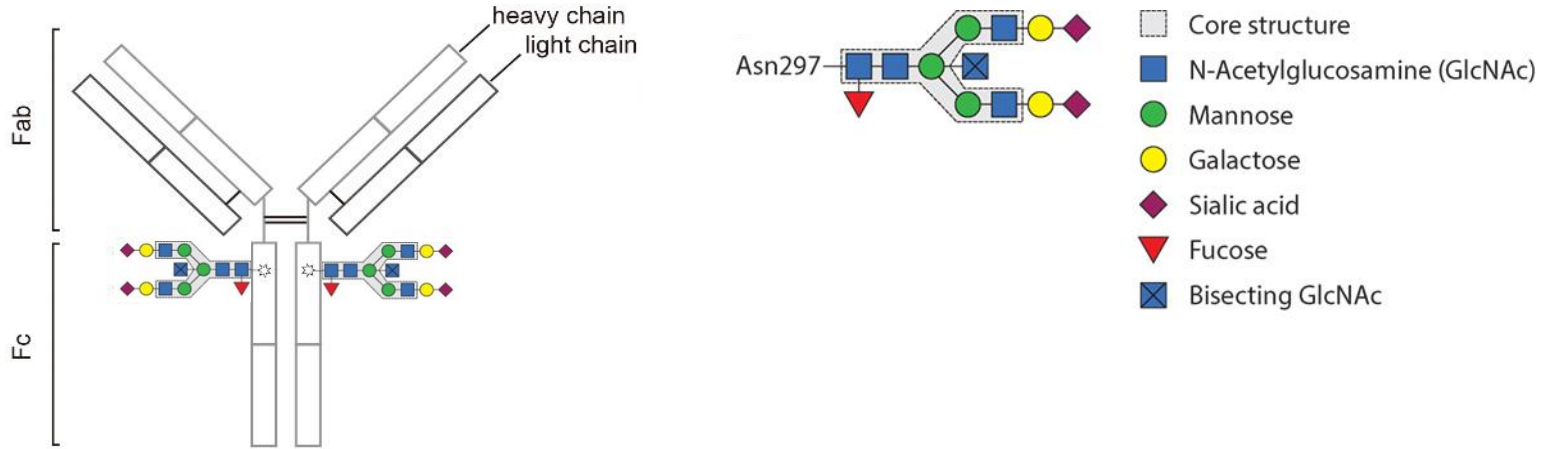
	donor 1	donor 2	donor 3
Serum 1	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 2	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 3	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 4	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 5	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 6	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 7	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 8	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 9	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 10	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 11	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38
Serum 12	A2 A3 B7	A1 A2 B15:01 B35	A11 A24 B35 B38

Rijkers, Maaik, et al.  
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## Conclusions

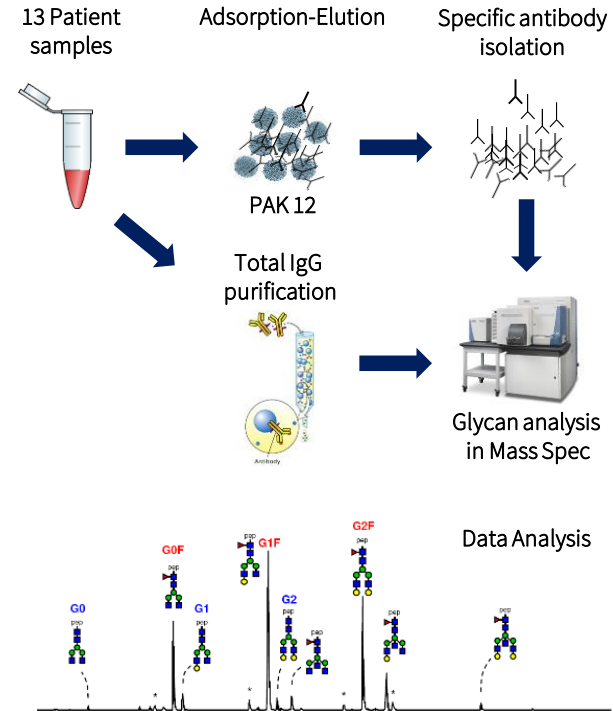
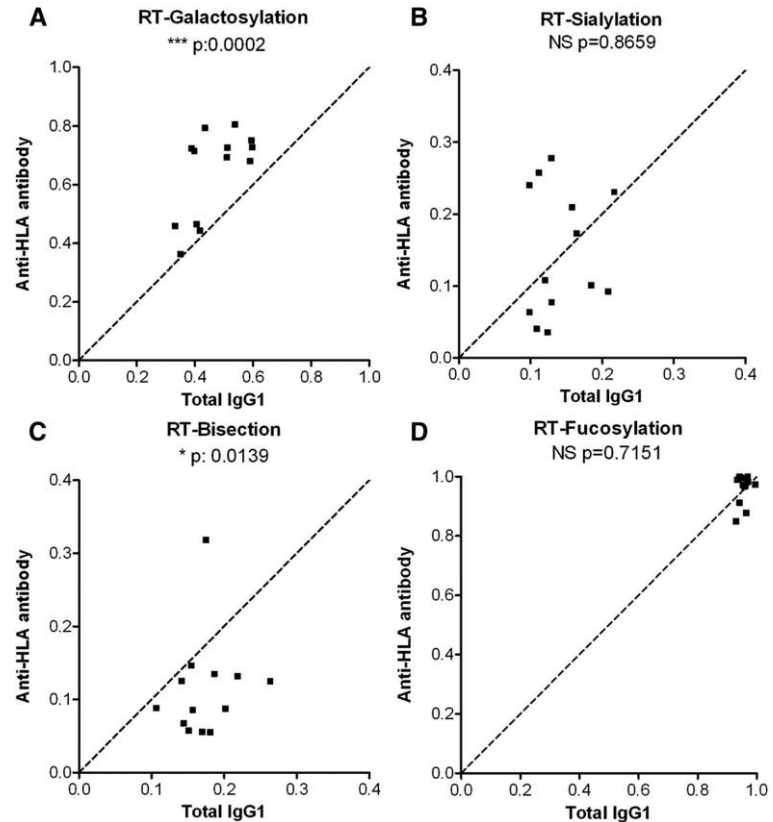
- Anti-HLA Antibodies are capable of inducing complement activation on platelets
  - Via the Classical Complement Pathway
- Synergistic effects when mAbs are used together suggesting oligomeric IgG Complexes
- Complement activity can be inhibited by blocking Fc-Fc interactions or C1/C5 inhibitors

## Fc-glycosylation of antibodies changes effector functions

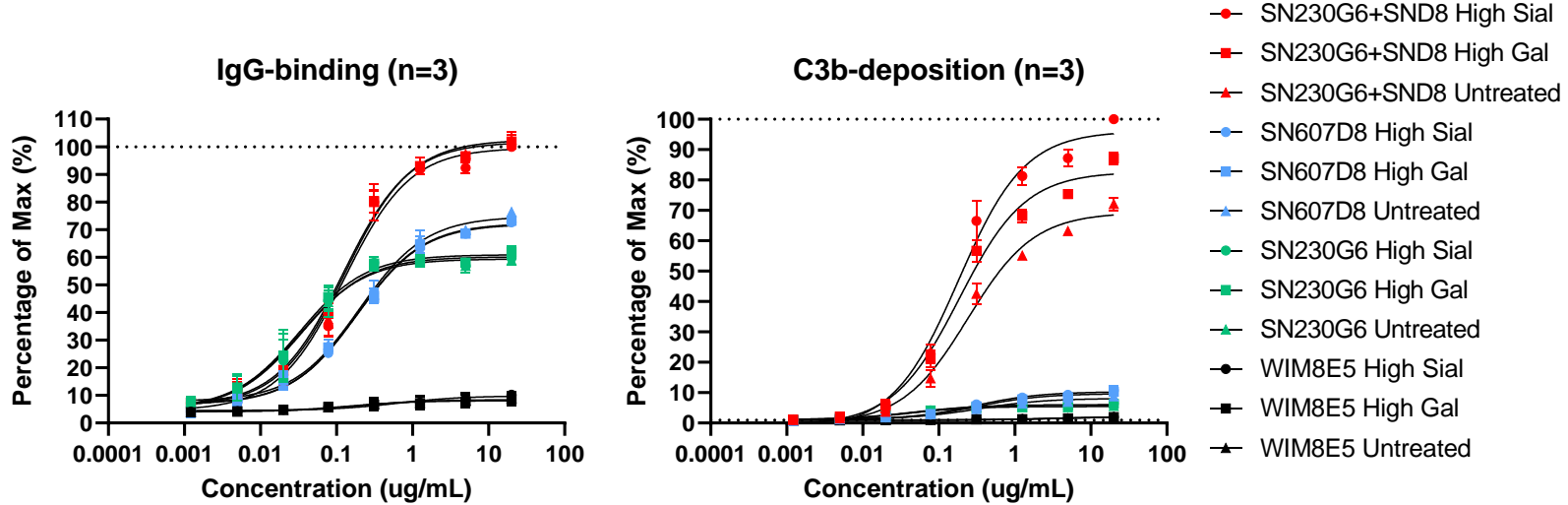


- Core structure of N-acetylglucosamine (GlcNAc) and mannose residues
- Additional extensions incl. Galactose, sialic acid, core fucosylation and bi-sected GlcNAc
- Glycosylation affects affinity to FcγR and C1q

# Increased Fc-galactosylation of anti-HLA antibodies observed in patients with platelet refractoriness



# Increased levels of Fc-galactosylation and -sialylation results in more complement deposition





## Conclusions

- Anti-HLA Antibodies are capable of inducing complement activation on platelets
    - Via the Classical Complement Pathway
  - Synergistic effects when mAbs are used together suggesting oligomeric IgG Complexes
  - Complement activity can be inhibited by blocking Fc-Fc interactions or C1/C5 inhibitors
- 
- Increased Fc-Galactosylation of anti-HLA Abs was observed in patients with platelet refractoriness
  - Both Fc-Galactosylation and Fc-sialylation results in more complement activity

## Acknowledgements

Maike Rijkers  
Rick Kapur  
Gillian Dekkers  
Tamas Pongracz  
Arthur Bentlage

Gestur Vidarsson  
Jan Voorberg  
Ellen van der Schoot  
Masja de Haas  
Leendert Porcelijn

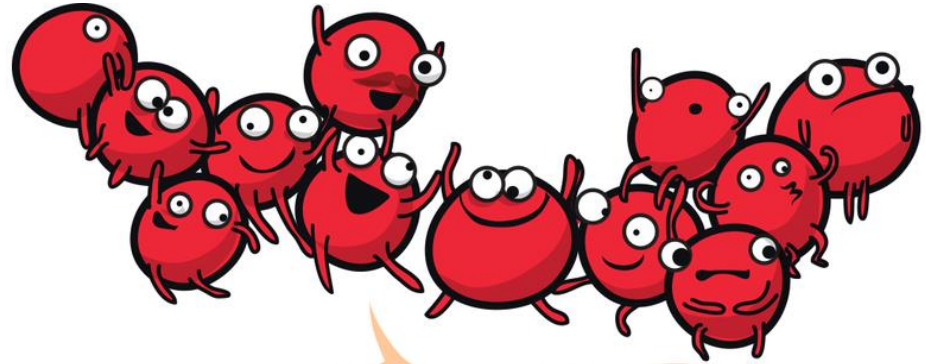
Zoltan Szittner  
David Schmidt  
Steven de Taeye  
Robin Temming  
Erik de Graaf  
Maximilian Brinkhaus  
Mads Larsen  
Remco Visser  
Suzanne Lissenberg-Thunnissen

Juulke Steuten  
Yasmin de Wit  
Anno Saris  
Anja ten Brinke  
Marieke van Ham

Manfred Wuhrer  
Sebastiaan Heidt  
Arend Mulder

Wim van Esch  
Giso Brassler

Martijn Nolte  
Mark Hoogenboezem



Thank You!