

Paroxysmale Nachtelijke Hemoglobinurie

Dr. Willemijn Hobo

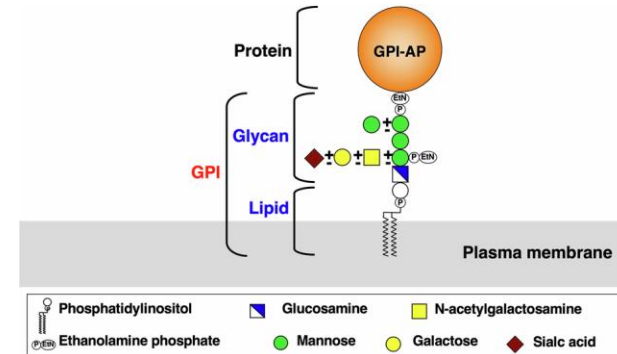
Dept. of Laboratory Medicine – Laboratory of Hematology

NVC-BSAC 2026

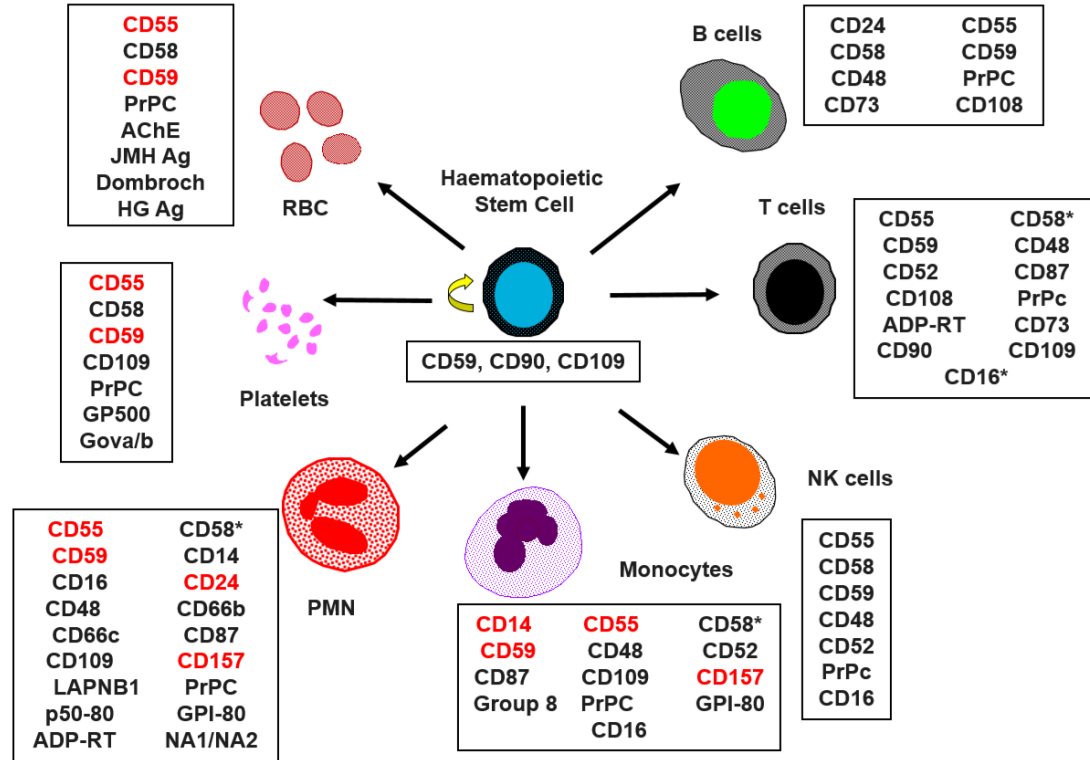
Radboudumc

PNH

- Aanvalsgewijs (nachtelijk) bloedplassen als gevolg van afbraak van de rode bloedcellen.
- Zeldzame verworven aandoening, 1-2/1.000.000 nieuwe patiënten per jaar.
- Oorzaak: somatische mutatie in PIGA gen op X-chromosoom (>100 mutaties bekend).
 - verstoord de vorming van glycosylfosfatidylinositol (GPI)-ankers
 - geen/verminderde expressie van GPI-verankerde eiwitten op bloedcellen.

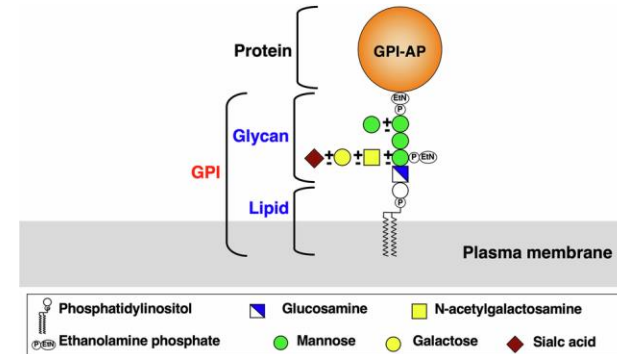


GPI-verankerde eiwitten op mature bloedcellen

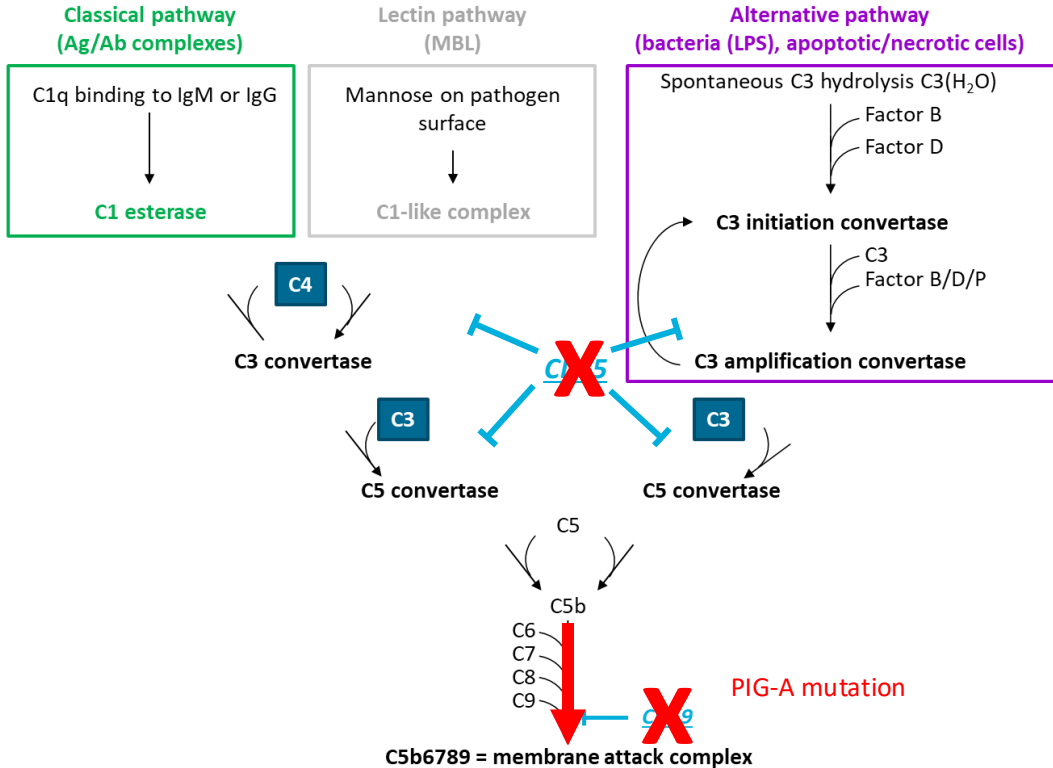


PNH

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- Oorzaak: somatische mutatie in PIGA gen op X-chromosoom.
 - verstoord de vorming van glycosylfosfatidylinositol (GPI)-ankers
 - geen/verminderde expressie van GPI-verankerde eiwitten op bloedcellen.
- Klinische kenmerken:
 - Hemolyse
 - Trombose
 - Beenmergfalen

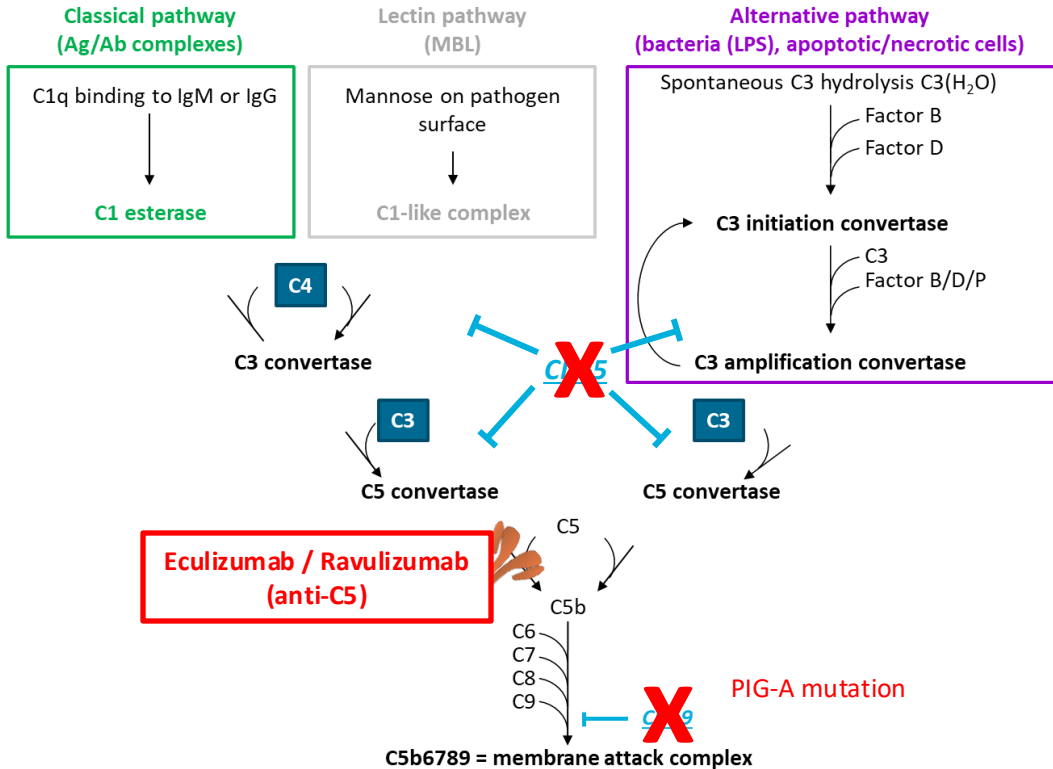


PNH – intravasculaire hemolyse

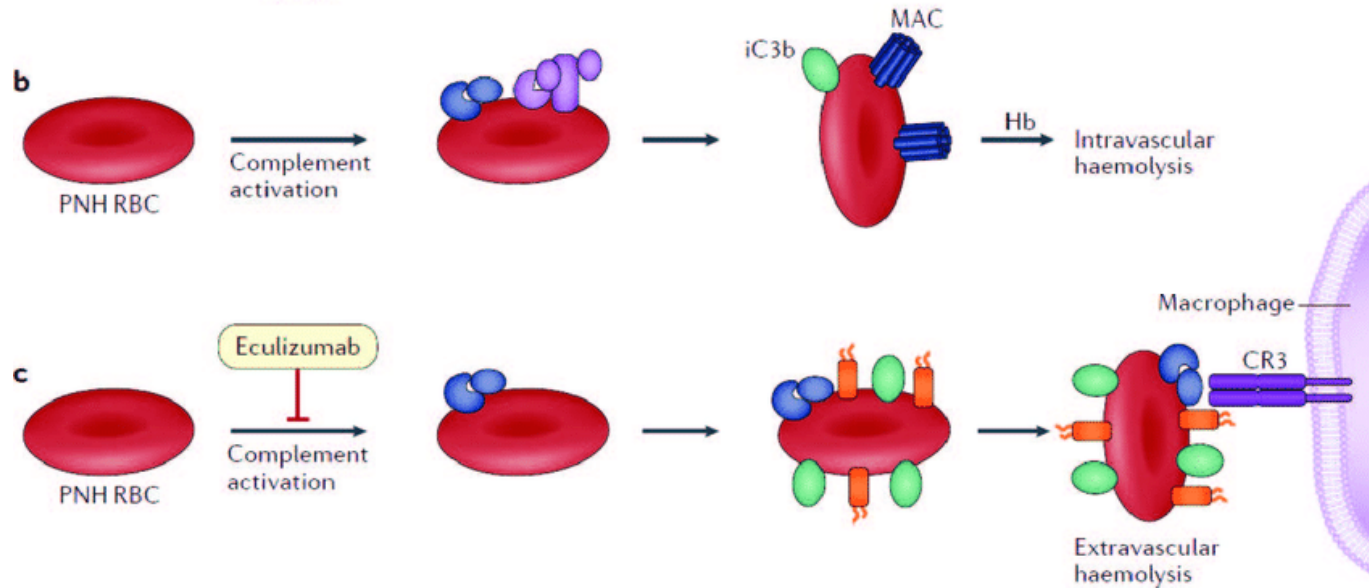


Er is altijd enige complementactivatie via de alternatieve route!

PNH – intravasculaire hemolyse



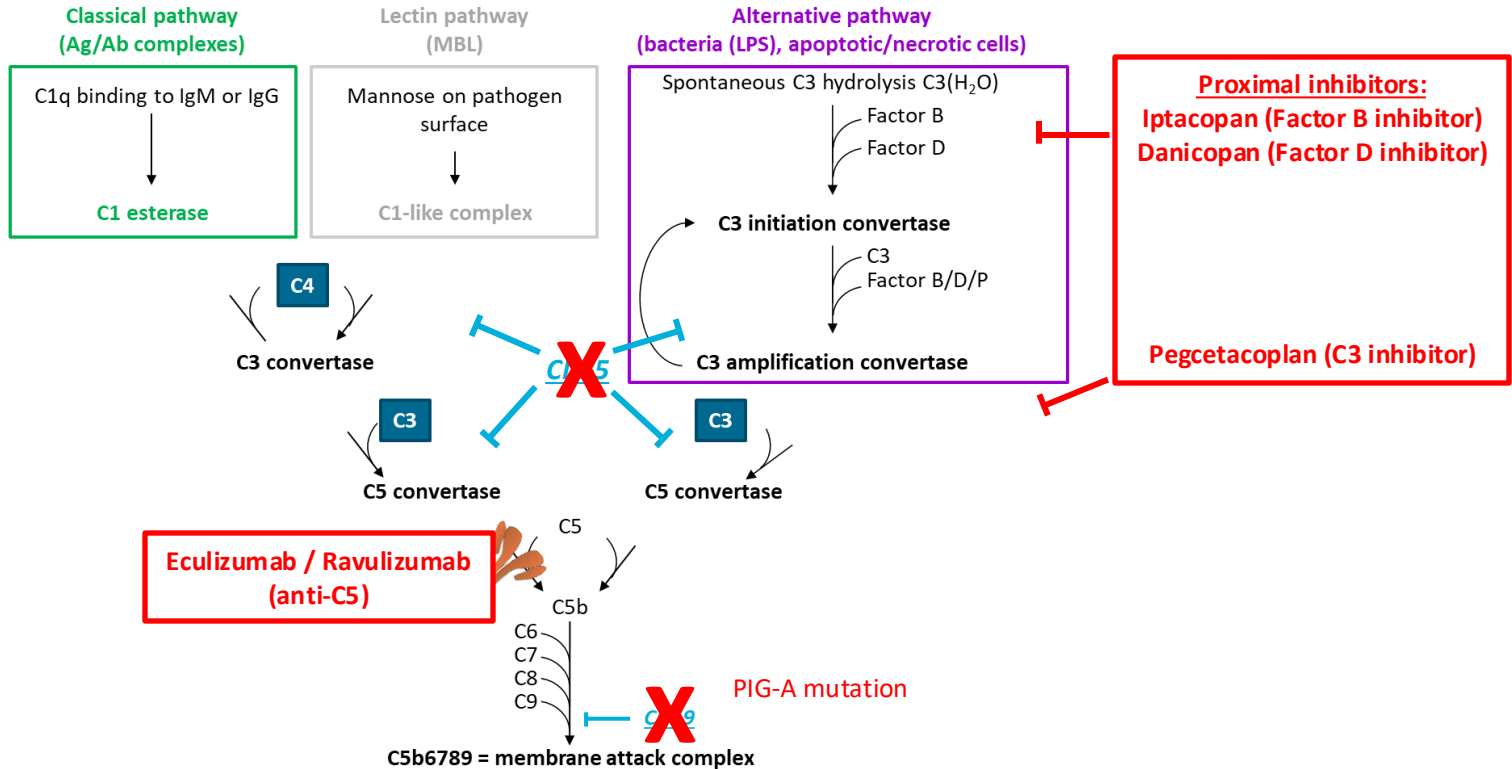
PNH – extravasculaire hemolyse



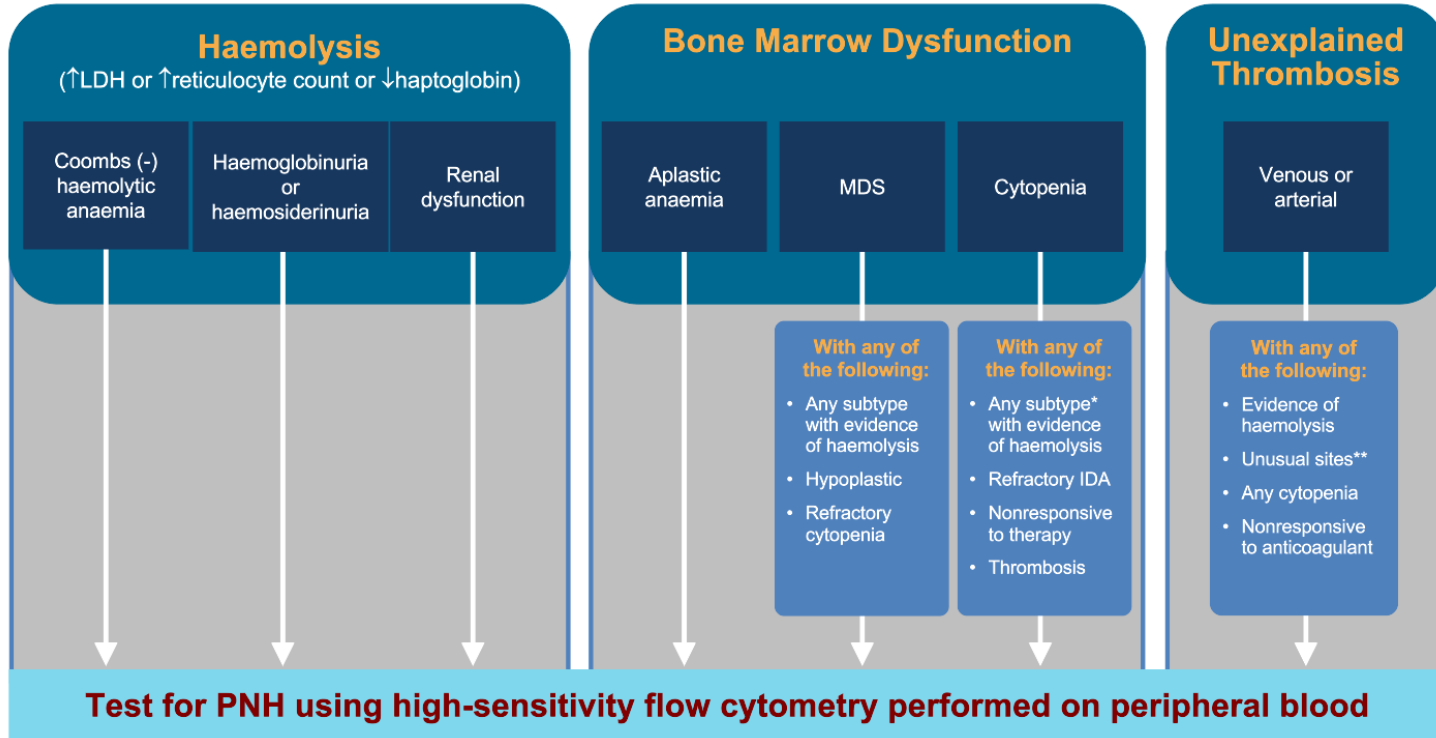
PNH behandeld met Eculizumab: C3 opsonisatie vindt nog steeds plaats (want geen rem via CD55) → extravasculaire hemolyse.

→ Rol voor proximale complementremmers.

PNH – intravasculaire hemolyse



PNH – indicatie flowcytometrie diagnostiek



PNH – flowcytometrie diagnostiek

Criteria voor PNH evaluatie:

- ≥ 2 verschillende markers tegen
- ≥ 2 verschillende GPI-verankerde eiwitten of GPI zelf op
- ≥ 2 verschillende celpopulaties (niet lymfocyten)

Immuunfenotypering:

- MoAb:
 - Erytrocyten: CD55, CD59
 - Monocyten: CD14, CD157
 - Neutrofiele granulocyten: CD24, CD157
- FLAER expressie: fluorescent pro-aerolysine (bacterieel toxine) dat GPI-ankers bindt.

PNH – flowcytometrie diagnostiek

Rapportage:

- Geen PNH: GPI-geankerd eiwit+ en FLAER+
- PNH kloon aantoonbaar met verlies GPI-geankerd eiwit en FLAER-
 - Geringe kloon: 0.1-1% (o.a. bij aplastische anemie)
 - PNH kloon: >1%

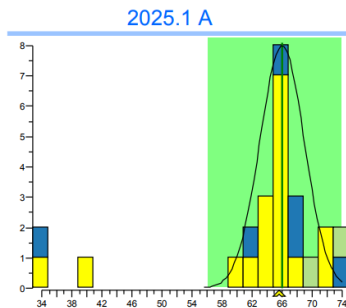
PNH rondzending 2025

PNH rondzending 2025

- De expertwaarde is gebaseerd op de resultaten van alle deelnemers met exclusie van uitbijters.
- Er wordt geen expertwaarde vastgesteld voor methoden met een te beperkt aantal deelnemers.

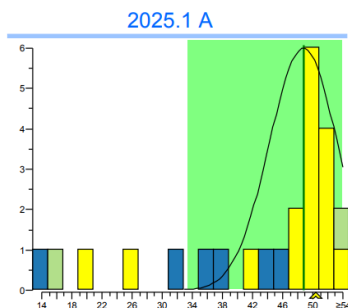
PNH 2025.1 - Erythrocyten

Ery CD59 totaal



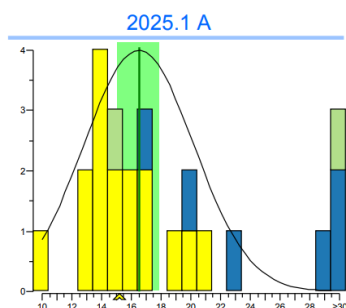
	cons.	meth.	exp.	lab
gem.	66.0	65.2	66	65.6
SD	3.0	1.8		
n	25	17		
nu	4	4		
rec.	99%	101%	99%	

Ery CD59 type II

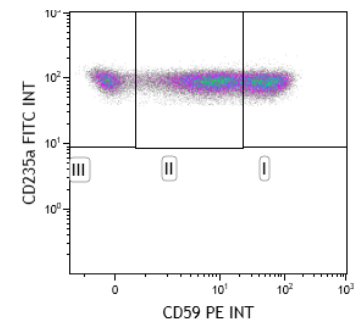
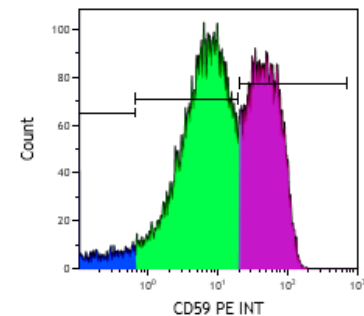


	cons.	meth.	exp.	lab
gem.	49	50	48.8	50.4
SD	5	2		
n	24	16		
nu	5	3		
rec.	103%	101%	103%	

Ery CD59 type III



	cons.	meth.	exp.	lab
gem.	16.50	15.25	16.5	15.2
SD	3.71	2.22		
n	24	16		
nu	4	0		
rec.	92%	100%	92%	



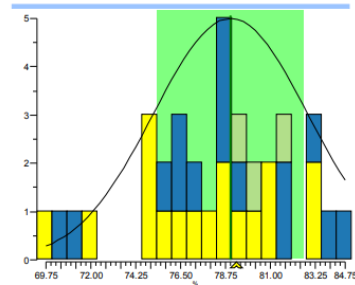
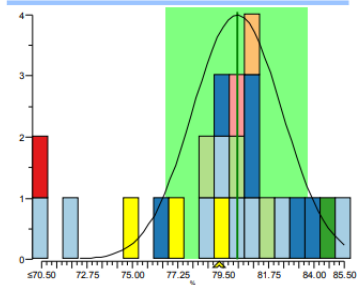
PNH 2025.1 - Monocyten

Mono CD14

Mono FLAER

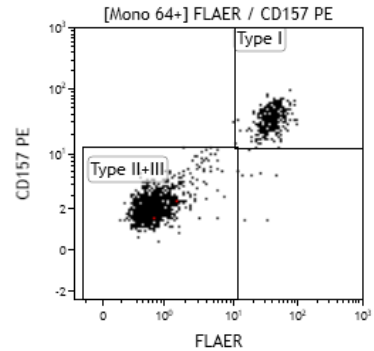
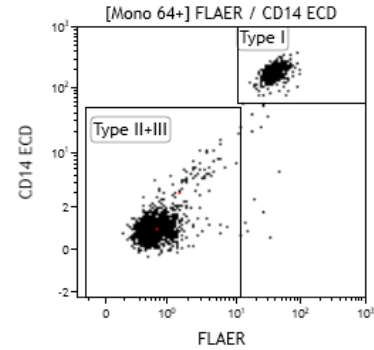
2025.1 A

2025.1 A



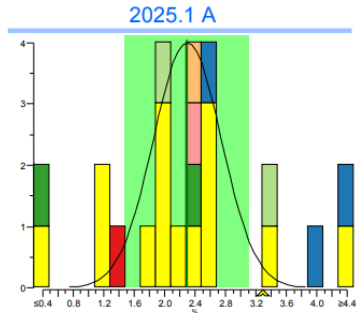
	cons.	meth.	exp.	lab
gem.	80.2	77.3	80.2	79.3
SD	2.2			
n	24	3		
nu	3	0		
rec.	99%	103%	99%	

	cons.	meth.	exp.	lab
gem.	79.0	78.0	79	79.3
SD	3.9	4.3		
n	35	17		
nu	0	0		
rec.	100%	102%	100%	



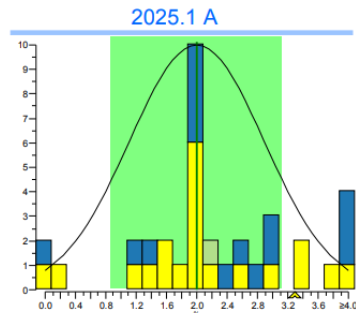
PNH 2025.1 - Granulocyten

Granulo CD24

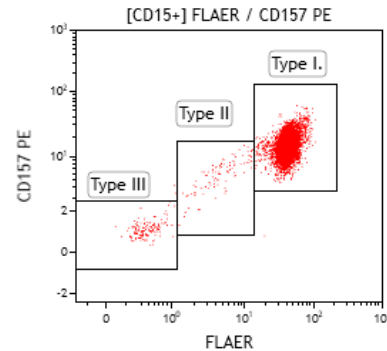
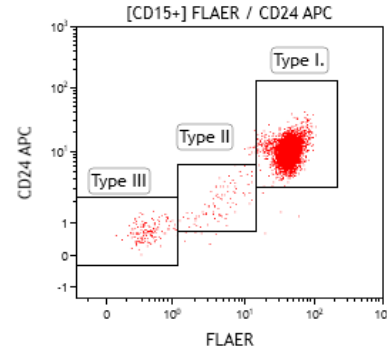


	cons.	meth.	exp.	lab
gem.	2.30	2.10	2.3	3.3
SD	0.44	0.59		
n	24	14		
nu	5	2		
rec.	143%	157%	143%	

Granulo FLAER

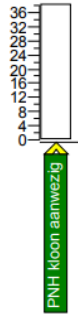


	cons.	meth.	exp.	lab
gem.	2.00	2.00	2	3.3
SD	0.89	0.82		
n	36	20		
nu	2	0		
rec.	165%	165%	165%	



PNH 2025.1 - Conclusie

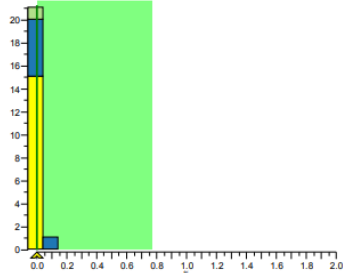
2025.1 A



PNH 2025.2 - Erythrocyten

Ery CD59 totaal

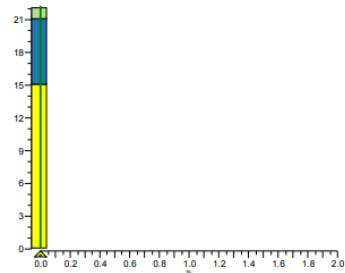
2025.2 A



	cons.	meth.	exp.	lab
gem.	0.00	0.00	0.00	0
SD	0.00	0.00		
n	22	15		
nu	1	0		
rec.				

Ery CD59 type II

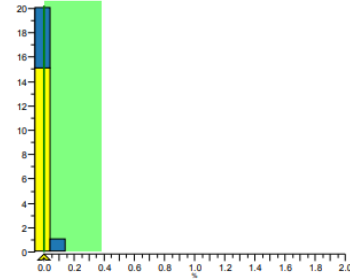
2025.2 A



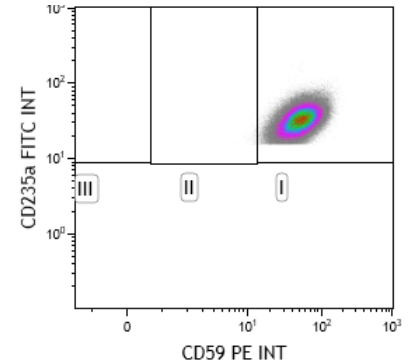
	cons.	meth.	exp.	lab
gem.	0.00	0.00	0.00	0
SD	0.00	0.00		
n	22	15		
nu	0	0		
rec.				

Ery CD59 type III

2025.2 A



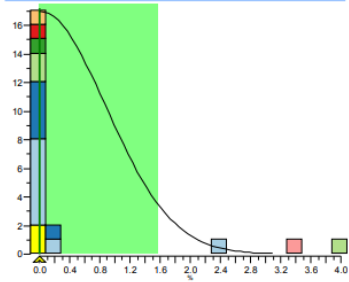
	cons.	meth.	exp.	lab
gem.	0.00	0.00	0.00	0
SD	0.00	0.00		
n	21	15		
nu	1	0		
rec.				



PNH 2025.2 - Monocyten

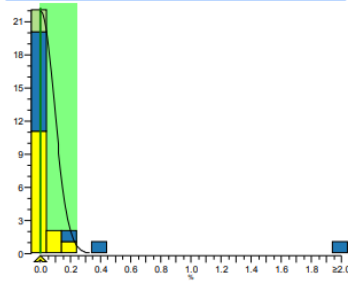
Mono CD14

2025.2 A



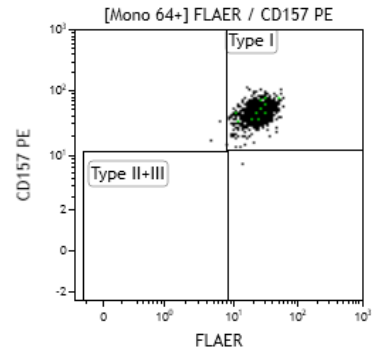
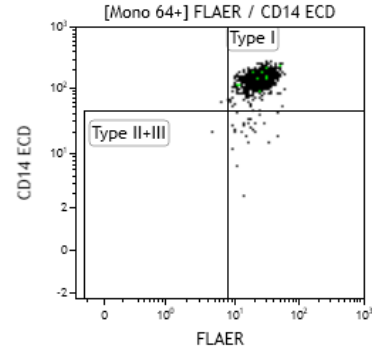
Mono FLAER

2025.2 A



	cons.	meth.	exp.	lab
gem.	0.0	0.0	0.00	0
SD	0.9			
n	22	2		
nu	1	0		
rec.				

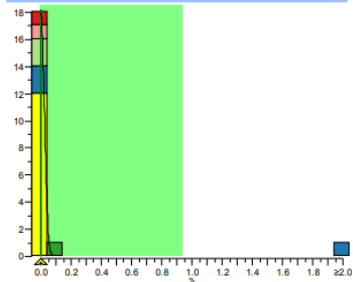
	cons.	meth.	exp.	lab
gem.	0.00	0.00	0.00	0
SD	0.09	0.04		
n	28	14		
nu	1	1		
rec.				



PNH 2025.2 - Granulocyten

Granulo CD24

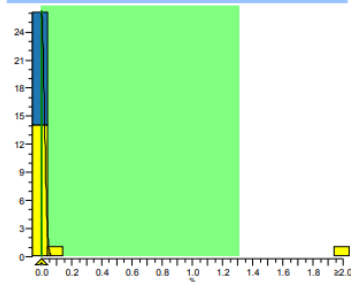
2025.2 A



	cons.	meth.	exp.	lab
gem.	0.00	0.00	0.00	0
SD	0.02	0.00		
n	20	12		
nu	1	0		
rec.				

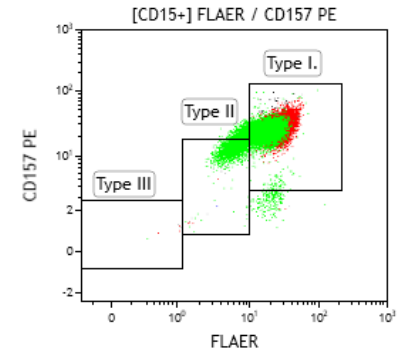
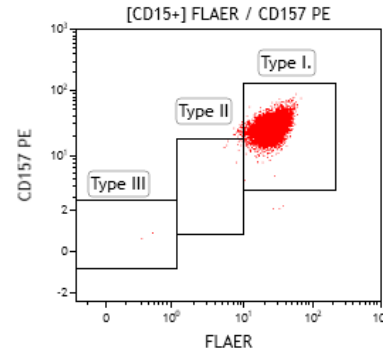
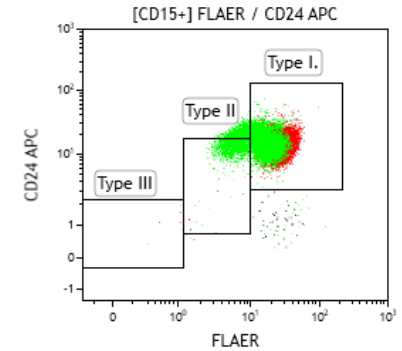
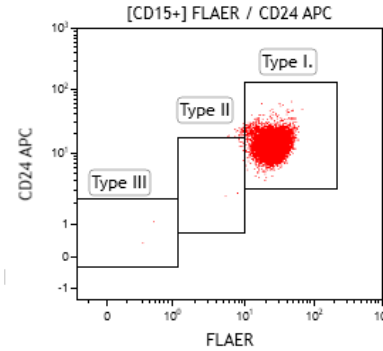
Granulo FLAER

2025.2 A



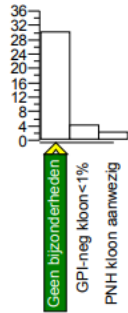
	cons.	meth.	exp.	lab
gem.	0.00	0.00	0.00	0
SD	0.02	0.03		
n	28	16		
nu	1	1		
rec.				

Let op CD15dim eo exclusie!



PNH 2025.2 - Conclusie

2025.2 A

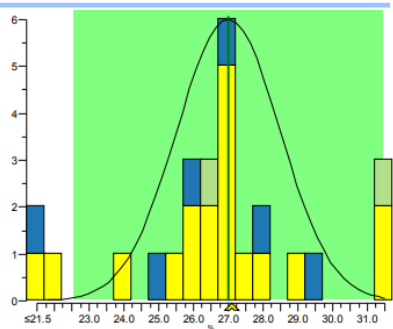


- Alleen numerieke getallen kunnen worden meegenomen in analyse. Indien $<0,1$ ingevoerd dan $0,1$ verwerkt in analyse.
- Eindconclusie “Geen bijzonderheden” is leidend.

PNH 2025.3 - Erythrocyten

Ery CD59 totaal

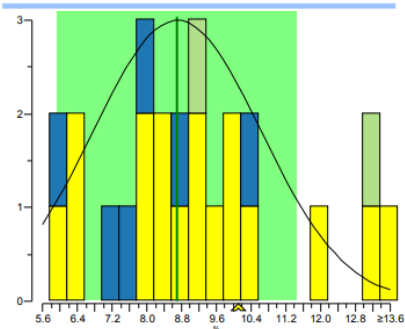
2025.3 A



	cons.	meth.	exp.	lab
gem.	27.0	27.0	27.0	27.1
SD	1.5	1.5		
n	26	18		
nu	6	4		
rec.	100%	100%	100%	

Ery CD59 type II

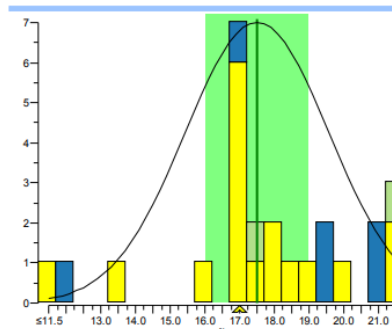
2025.3 A



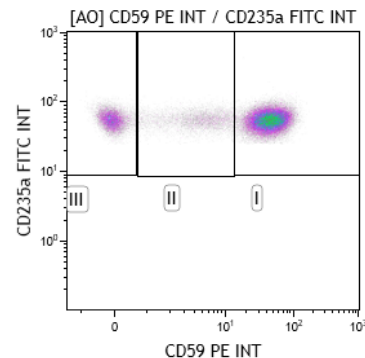
	cons.	meth.	exp.	lab
gem.	8.7	9.0	8.7	10.1
SD	1.9	1.5		
n	25	17		
nu	1	1		
rec.	116%	112%	116%	

Ery CD59 type III

2025.3 A



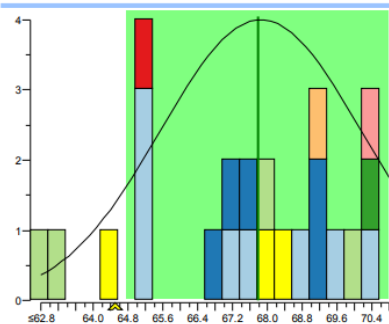
	cons.	meth.	exp.	lab
gem.	17.5	17.0	17.5	17.0
SD	2.1	1.5		
n	25	17		
nu	4	3		
rec.	97%	100%	97%	



PNH 2025.3 - Monocyten

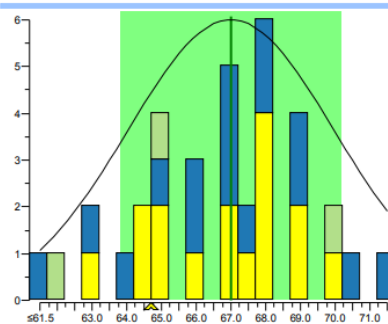
Mono CD14

2025.3 A



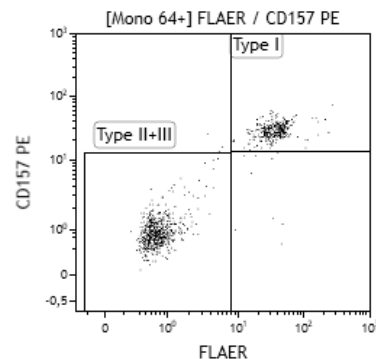
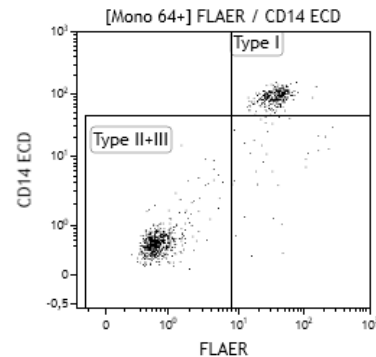
Mono FLAER

2025.3 A



	cons.	meth.	exp.	lab
gem.	67.8	68.0	67.8	64.5
SD	2.3			
n	24	3		
nu	1	1		
rec.	95%	95%	95%	

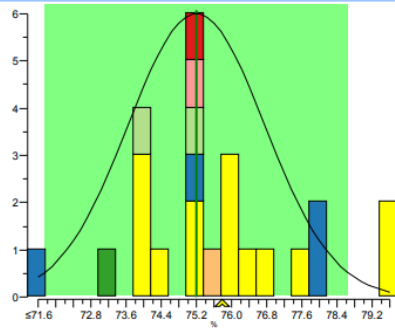
	cons.	meth.	exp.	lab
gem.	67.0	67.2	67.0	64.7
SD	3.0	2.2		
n	35	16		
nu	1	0		
rec.	97%	96%	97%	



PNH 2025.3 - Granulocyten

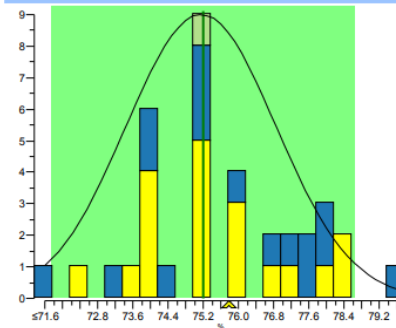
Granulo CD24

2025.3 A



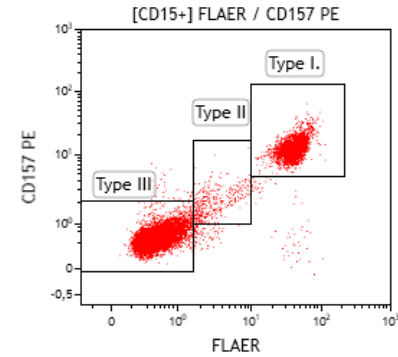
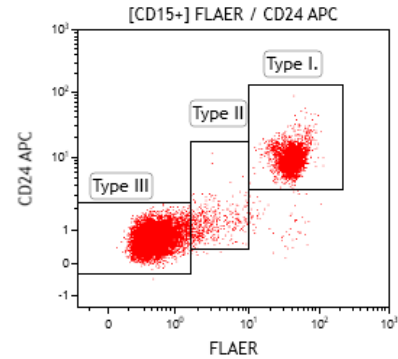
Granulo FLAER

2025.3 A



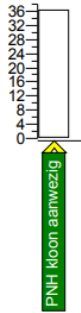
	cons.	meth.	exp.	lab
gem.	75.2	75.9	75.2	75.8
SD	1.6	1.9		
n	24	14		
nu	1	0		
rec.	101%	100%	101%	

	cons.	meth.	exp.	lab
gem.	75.2	75.2	75.2	75.8
SD	1.7	1.8		
n	36	19		
nu	1	0		
rec.	101%	101%	101%	



PNH 2025.3 - Conclusie

2025.3 A



PNH kloon in erythrocyten

Evaluatie type I vs. II vs. III ery's

- Veelal minder representatief voor totale kloongrootte dan de WBC klonen door:
 - Hemolyse.
 - Kloonderdunning met normale ery's als gevolg van recente transfusies.
- Inzicht in PNH kloon type III binnen ery's is relevant voor beoordeling van de effectiviteit van complementremming.

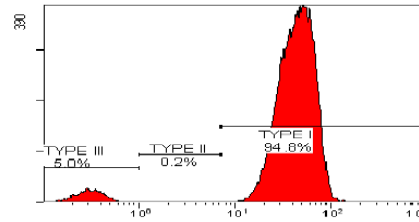
Effect Eculizumab behandeling op kloongrootte

Bescherming van PNH erythrocyten tegen MAC-vorming → toename Type III kloon.

Patiënt:

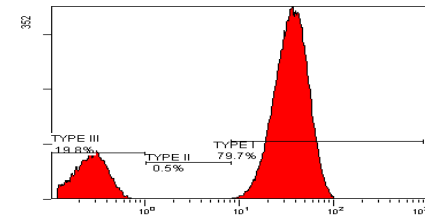
- 95% PNH kloon in WBCs
- Beloop over ca 26 weken

Initial visit: 5% PNH cells



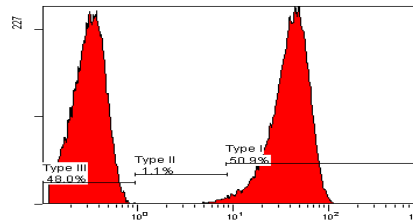
CD59-PE

Visit 2: 19% PNH cells



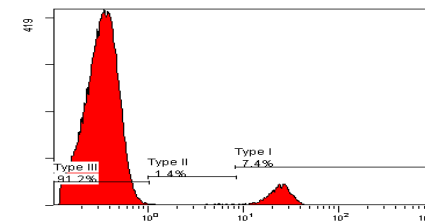
CD59-PE

Visit 3: 48% PNH cells



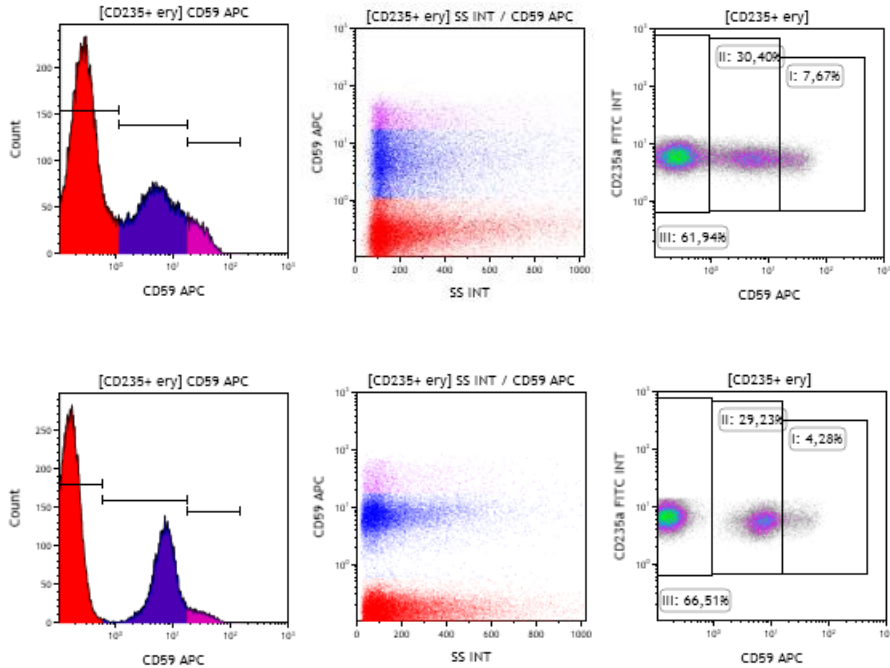
CD59-FITC

Visit 4: 91% PNH cells



CD59-FITC

Lastige beoordeling type I vs. II ery's: wat is echt?



Lastige beoordeling type I vs. II ery's: wat is echt?

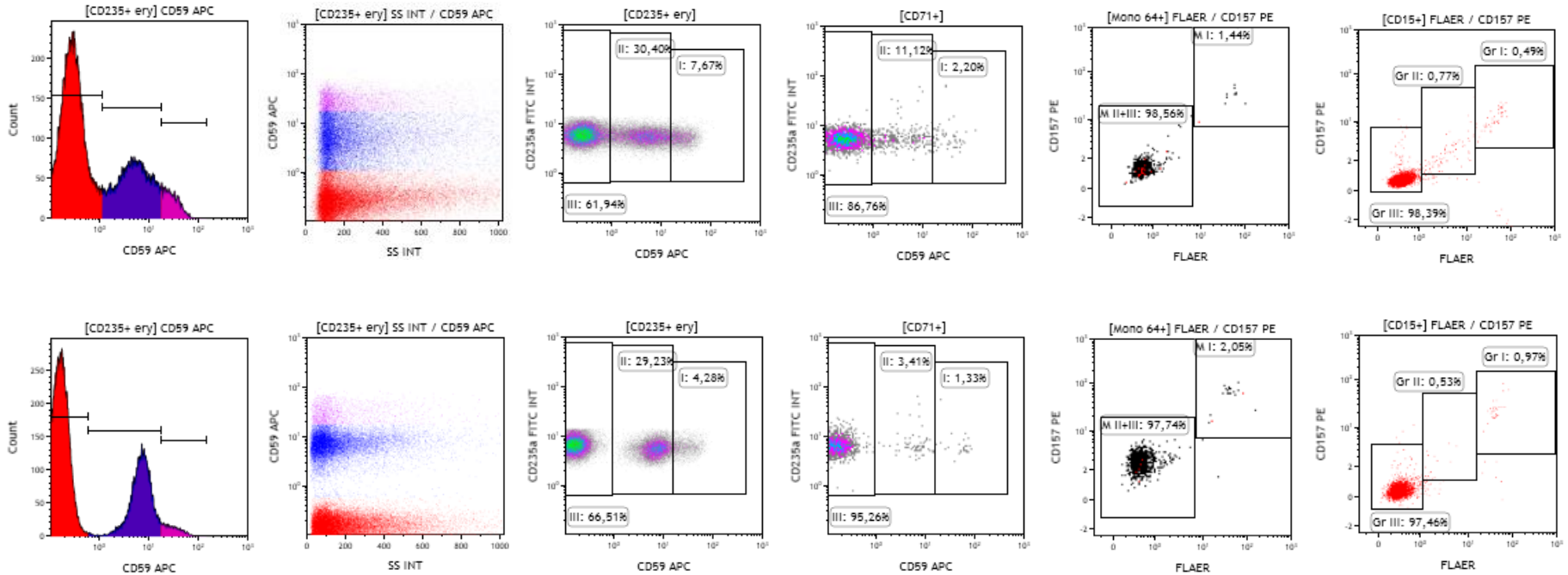
CD59 interpretatie wordt bemoeilijkt door:

- Recente/frequente erythrocyten transfusies.
- Hemolyse.
- Erythrocyt leeftijd: oudere mature ery's hebben lagere CD59 expressie.
- Weinig erythropoïese in AA of andere BMF syndromen.
- Een grote PNH type II kloon die overlapt met normale erythrocyten.

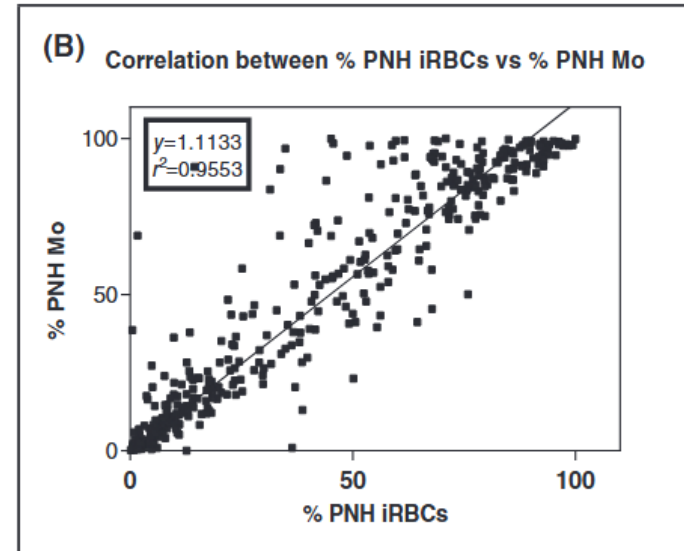
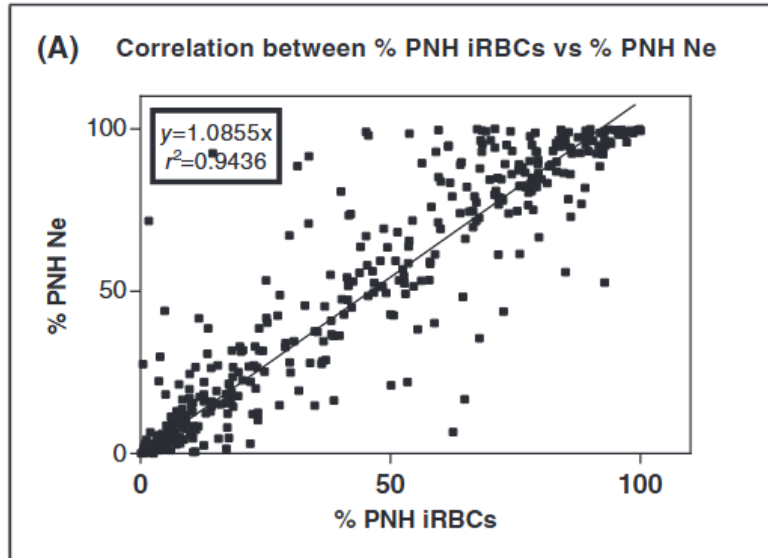
Alternatief:

- Analyse van immature erythrocyten (kernhoudend en/of reticulocyten) middels CD71:
 - Niet gevoelig voor hemolyse
 - Geen significante verdunning na transfusie van mature erythrocyten.

PNH kloon in erythrocyten: CD235⁺ vs. CD71⁺



PNH kloon in iRBC vs. PNH kloon in WBC populaties



Vragen?