



Flow Cytometry in PNH: Clinical and Laboratory Perspectives

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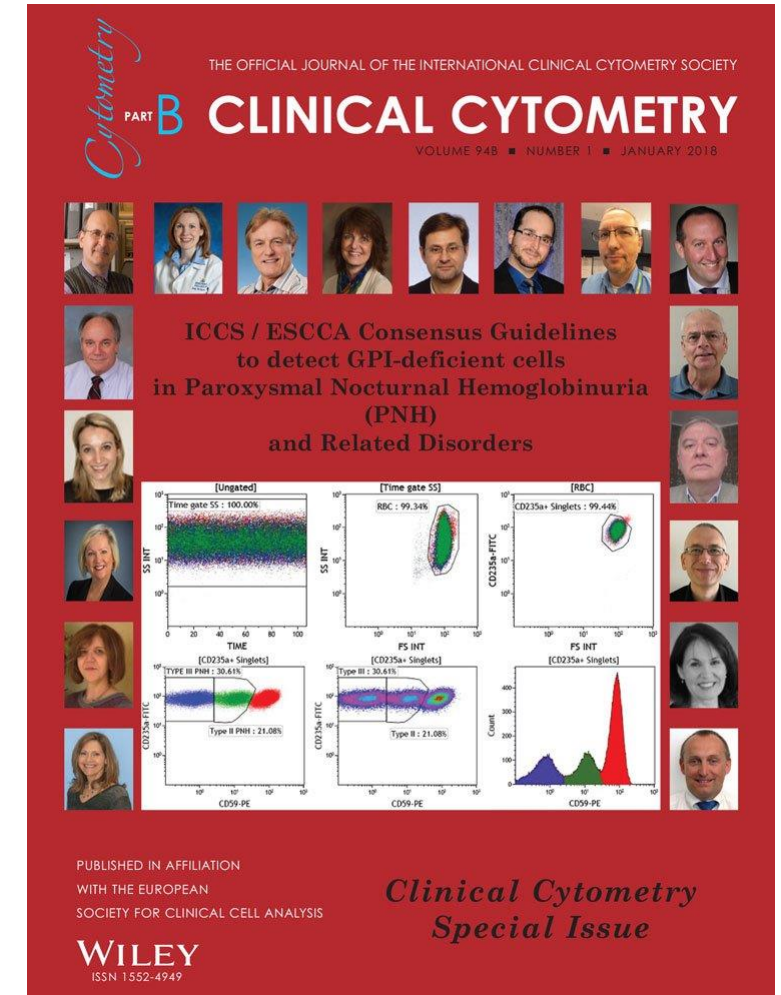
- Consultancy and honoraria Daniel Payne
 - Sysmex
 - Novartis
 - AstraZeneca
- Consultancy and honoraria Louise Arnold
 - Novartis
 - Sobi
 - AstraZeneca / Alexion

- Early recognition
- Improved management
- Aligned data
- Effective communication



PNH Flow Cytometry

- Set of standards
 1. Principles
 2. Reagent selection
 3. Sample preparation
 4. Flow cytometer setup
 5. Data analysis
 6. Reporting of results
 7. Ongoing verification
- Audit local practice for compliance



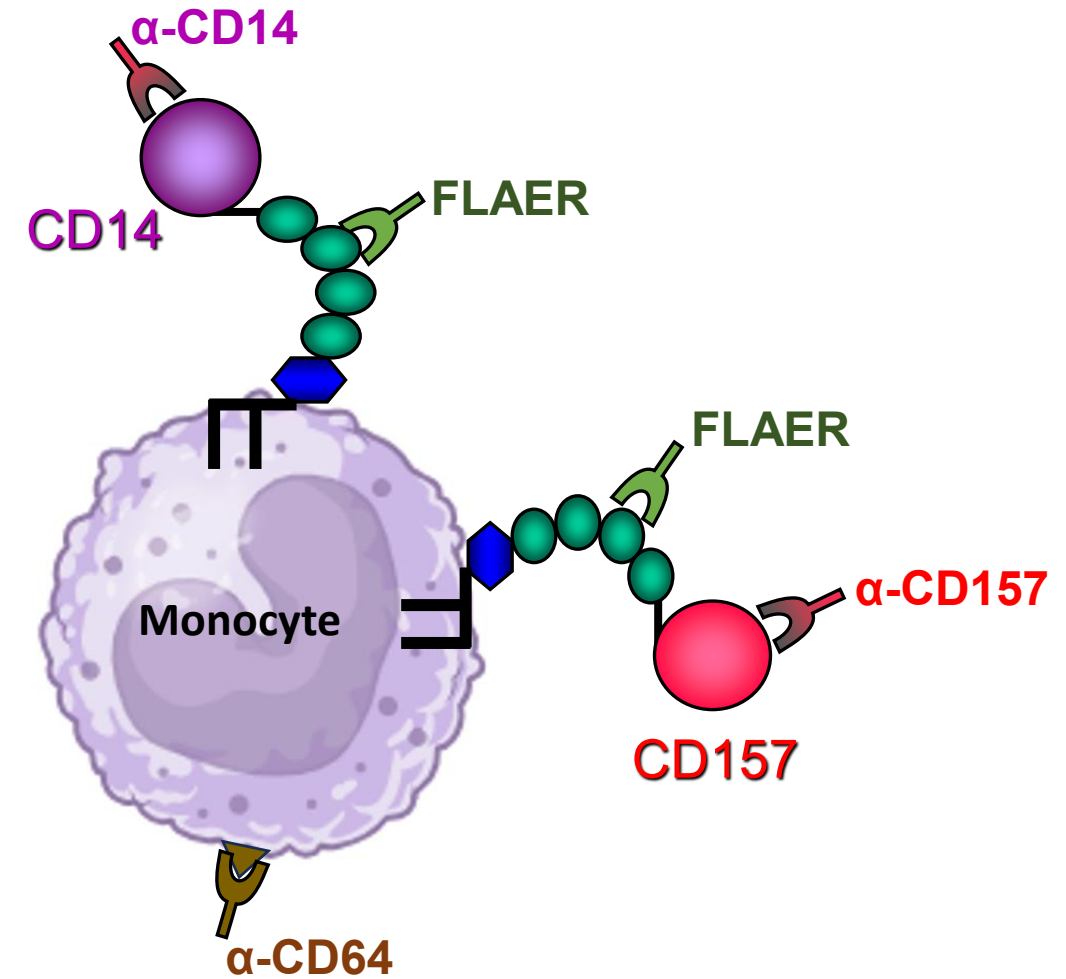
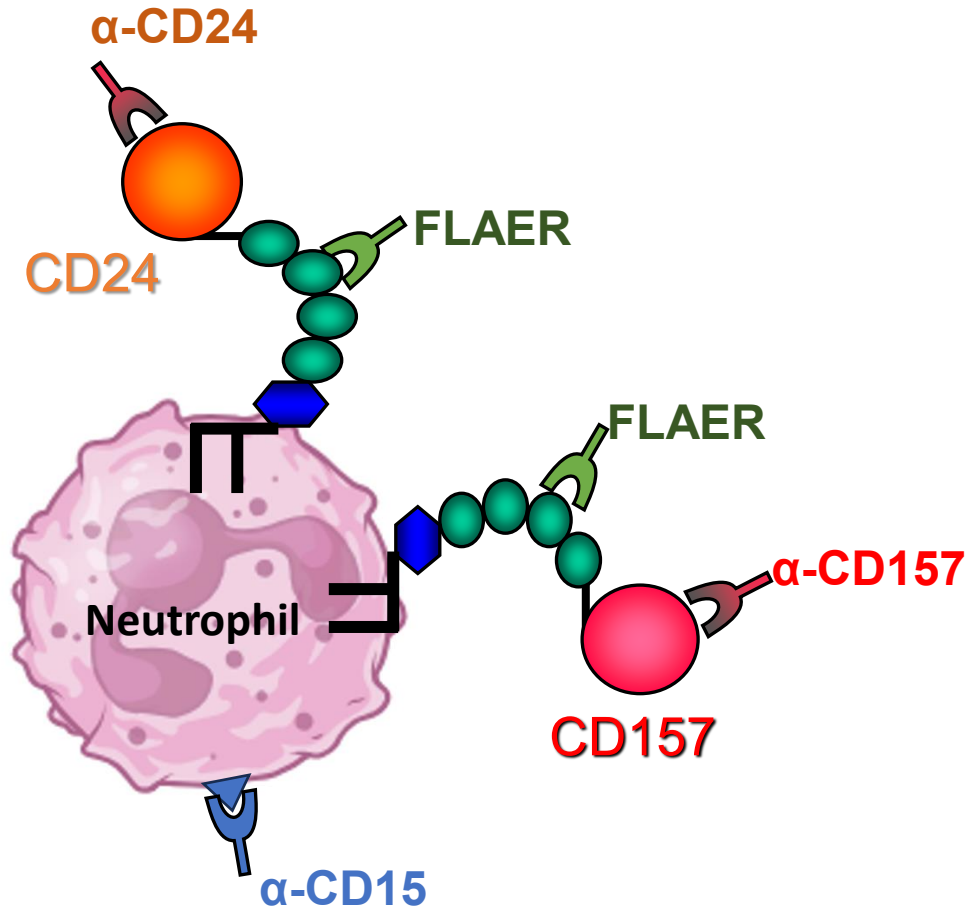
Sutherland *et al* 2018 Cytometry Part B: Clinical Cytometry

Flow Cytometry in Practice: Case 1

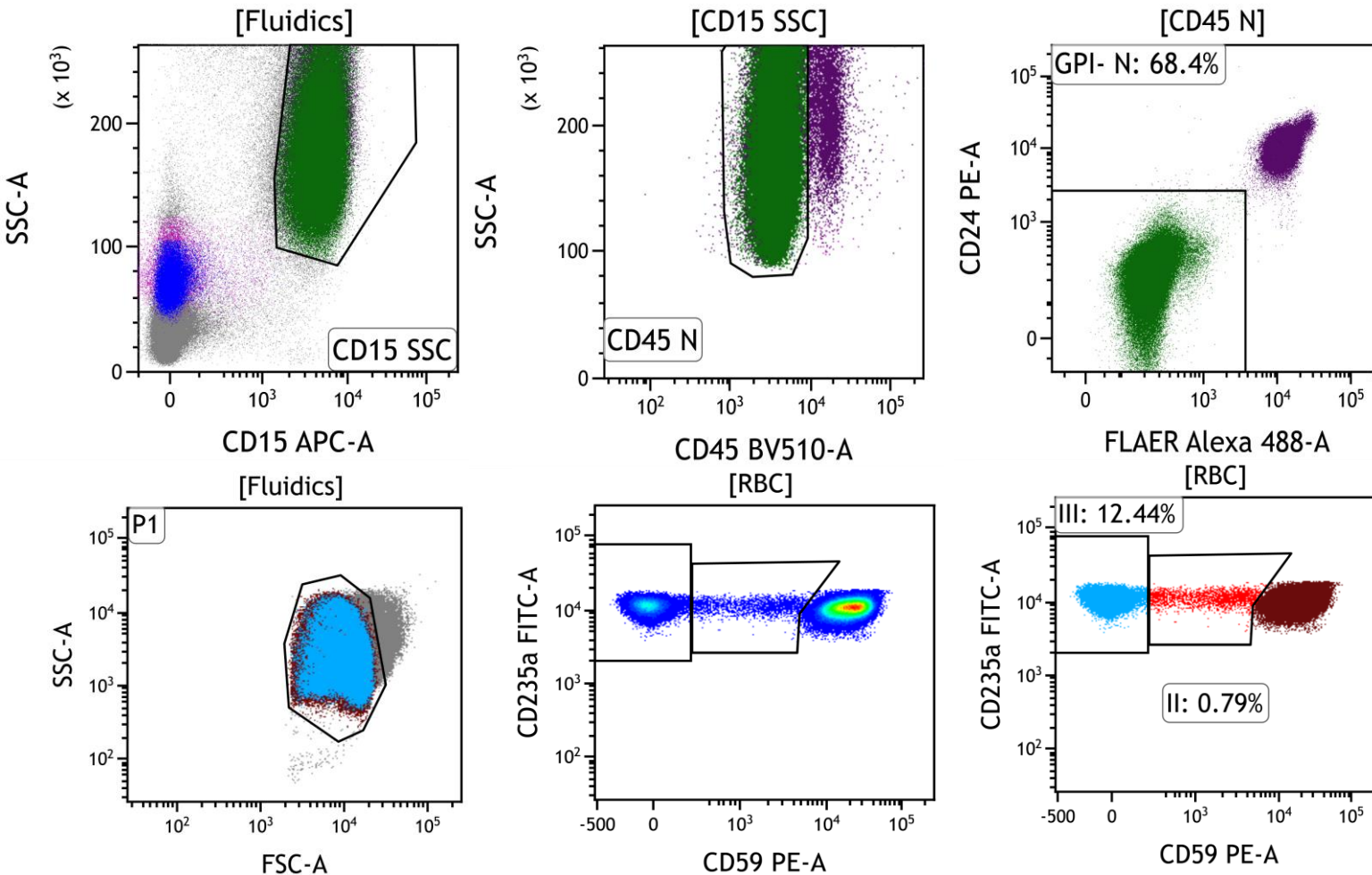
- May 2003
 - Initial symptoms occurred
 - 2 hospital admissions over the year
- August 2004
 - Black urine, Dysphagia, Jaundice
 - LDH 3263 IU/L (13xULN)
 - HB 70g/L, platelets $50 \times 10^9/L$
 - Erythrocyte transfusions
 - Folic acid & ferrous sulphate
- December 2004
 - Blood sample sent for PNH testing



Identification of PNH White Blood Cell



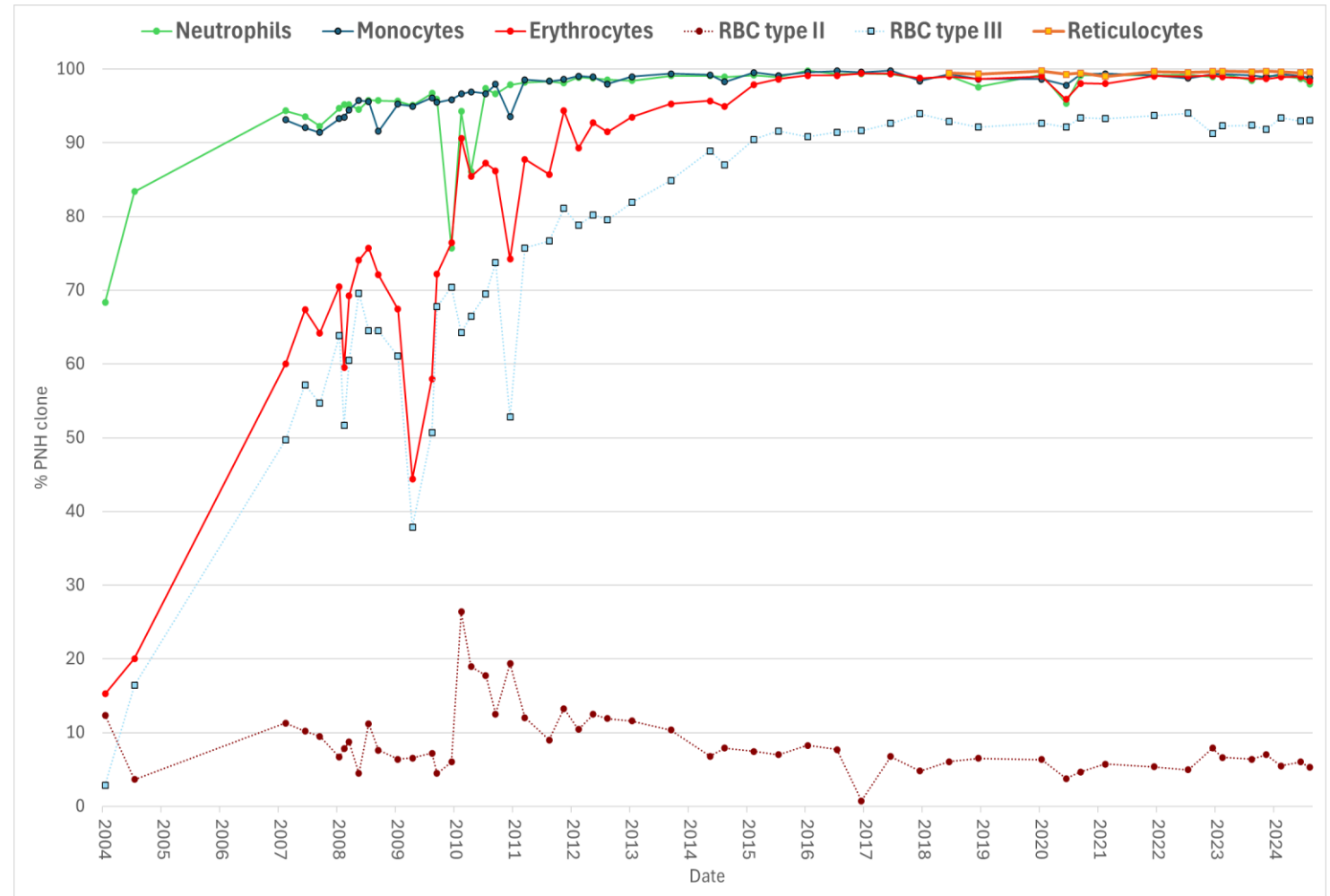
Flow Cytometry in Practice: Case 1



- Neutrophil PNH clone 68.4%
- Confirmed diagnosis of PNH
- Red cell PNH clone 13.23%
 - 12.44% type III cells & 0.79% type II cells

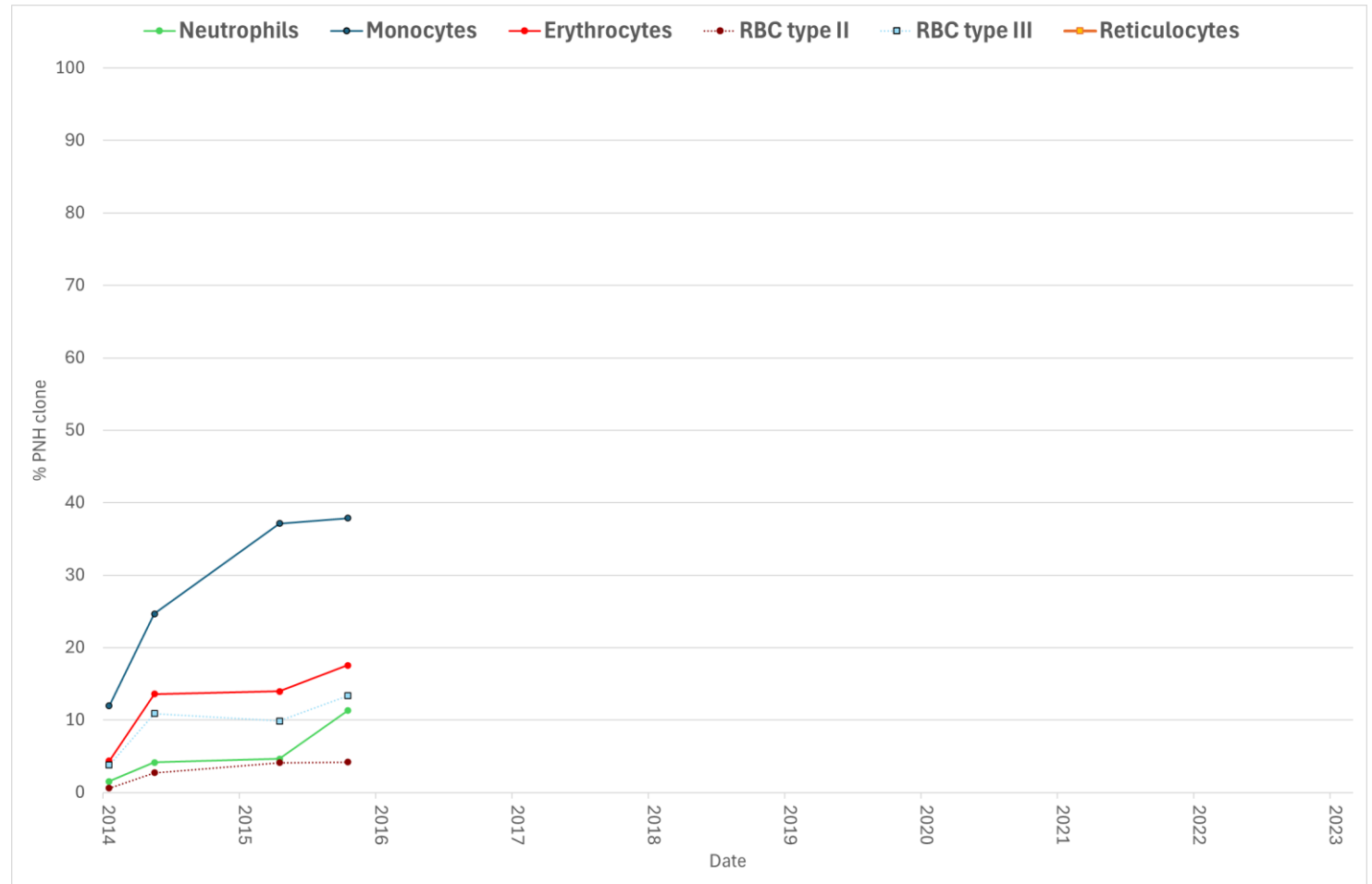
Flow Cytometry in Practice: Case 1

- September 2005 Eculizumab
- June 2021 Ravulizumab
 - LDH 224 IU/L
- September 2016 to 2025
 - Large PNH Clone 99%
 - Hb 132g/L
 - Platelets 51 x10⁹/L
 - LDH 350 IU/L



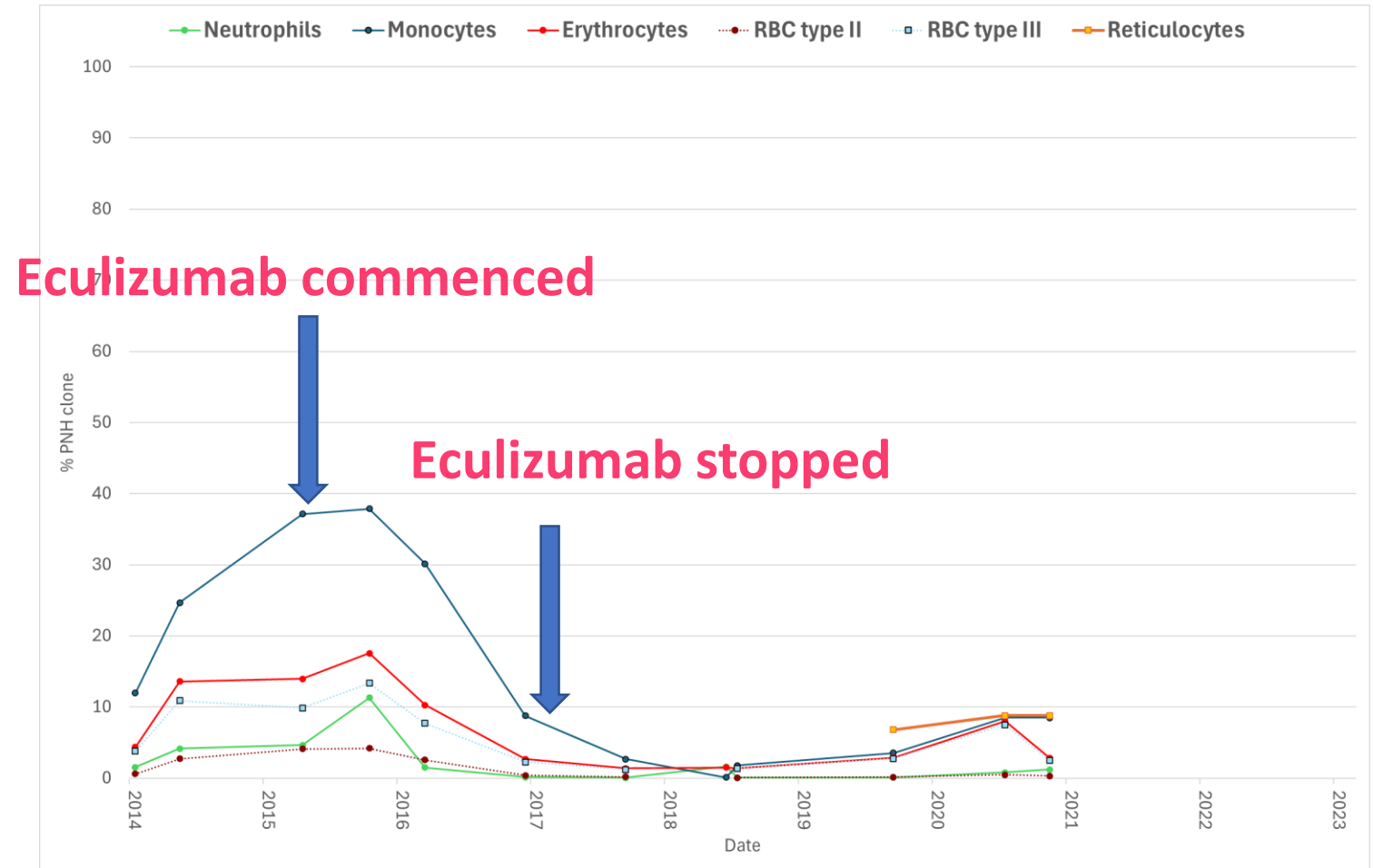
Flow Cytometry in Practice: Case 2

- 2006
 - Severe Aplastic Anemia
 - ATG + cyclosporine
 - Full recovery
- 2014-2015
 - Pregnant
 - Fall in counts
- Cyclosporine
- Eculizumab

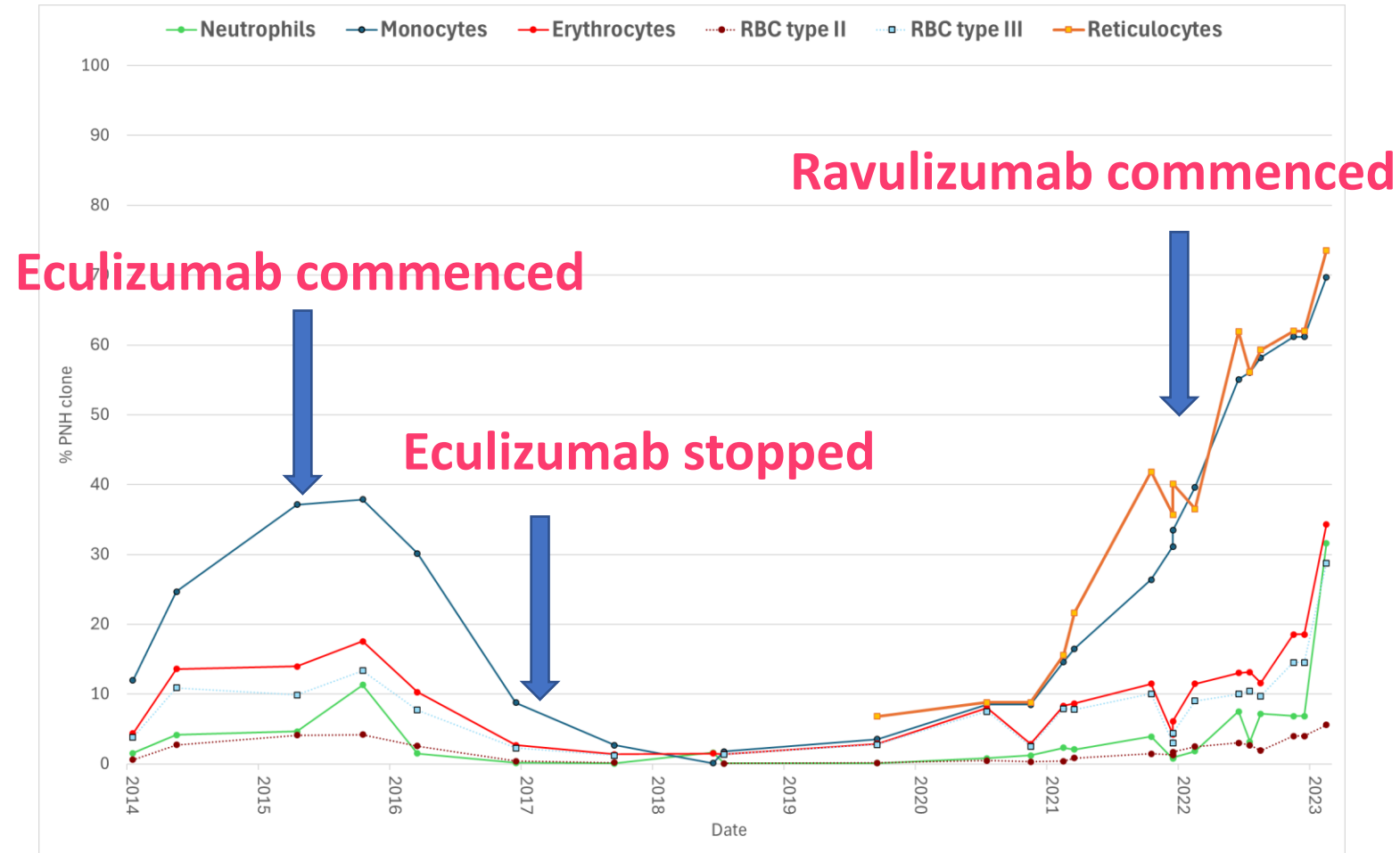
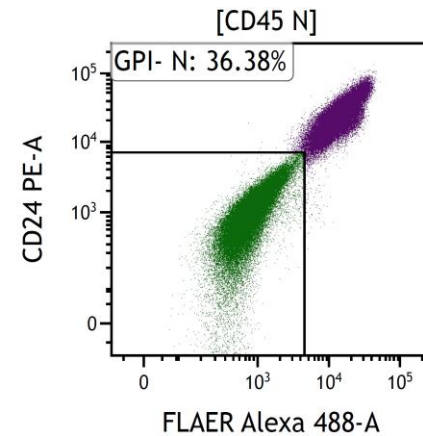
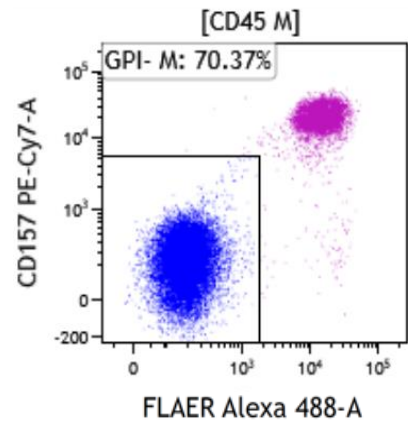
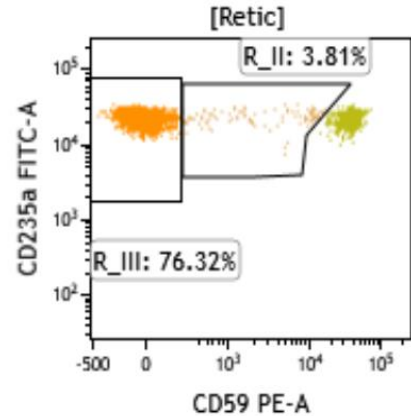
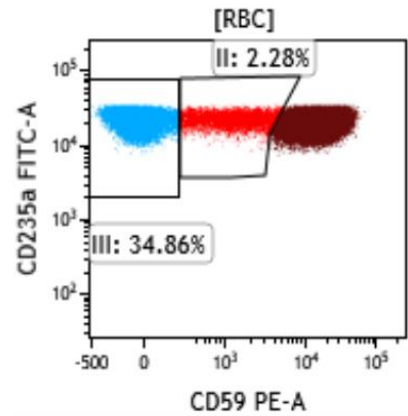


Flow Cytometry in Practice: Case 2

- 2017-2021
 - Patient's condition stable
 - Maintaining good quality of life
- What is important?



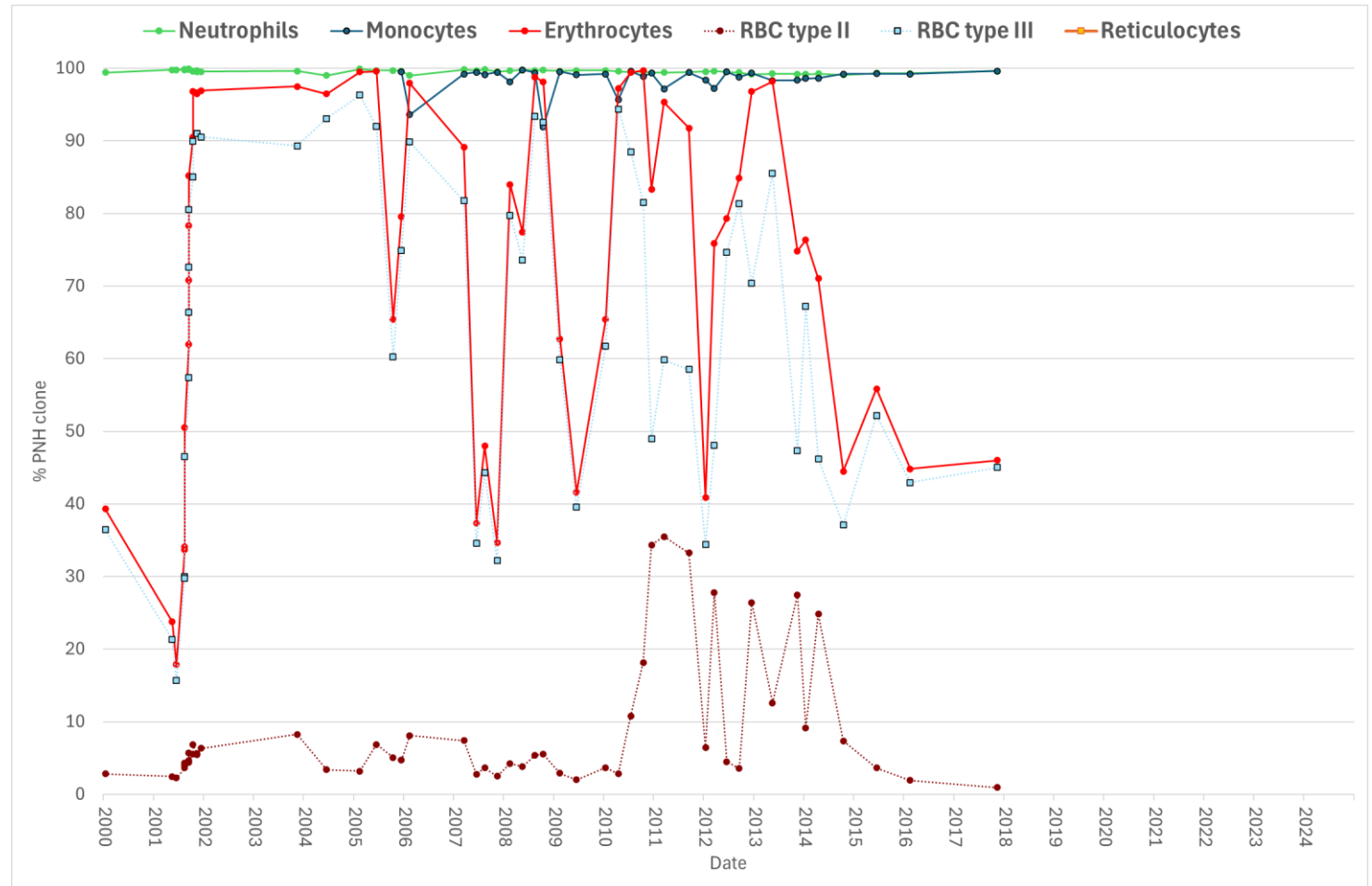
Flow Cytometry in Practice: Case 2



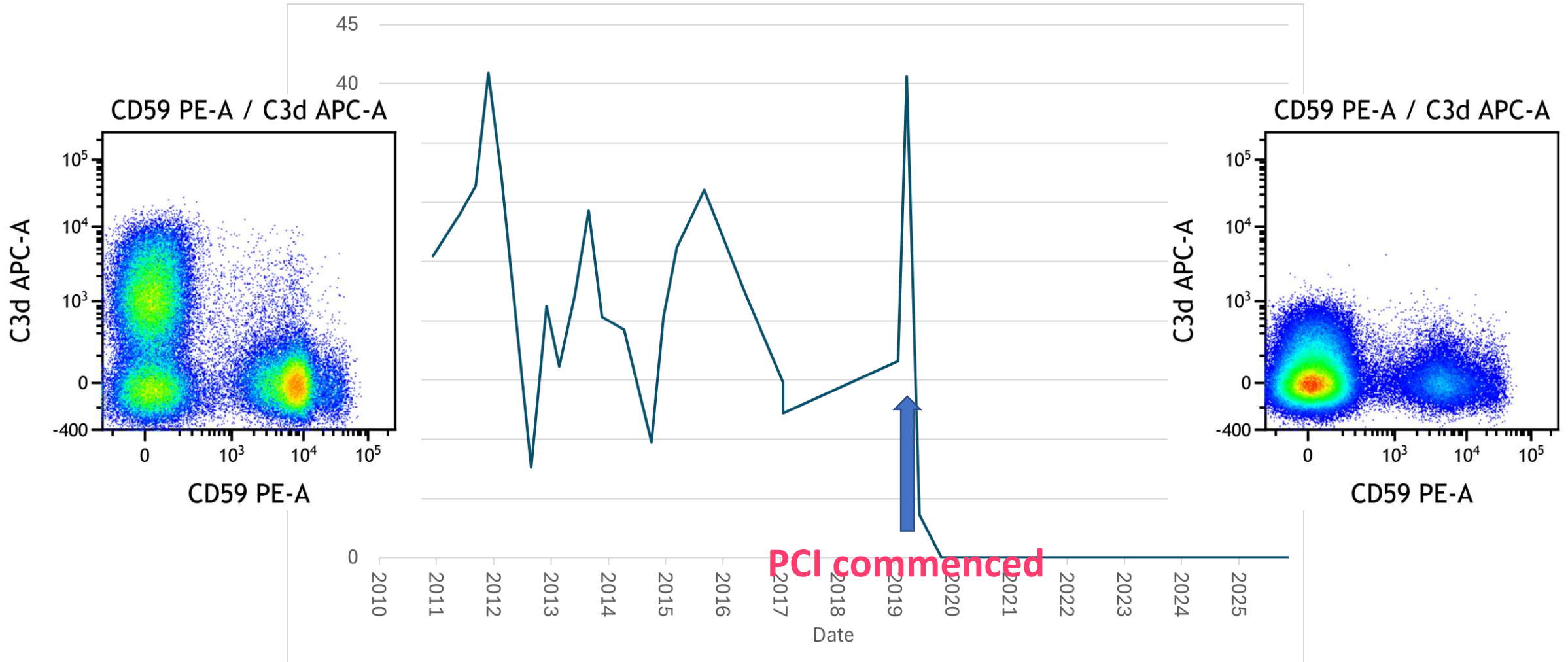
- Monitor the PNH clone at least annually
- If the clone is increasing or decreasing or other symptoms / change in results
 - More frequent reviews
- Consider investigations
- Adjust therapy depending on all results and symptoms
- Close monitoring and discussion

Flow Cytometry in Practice: Case 3

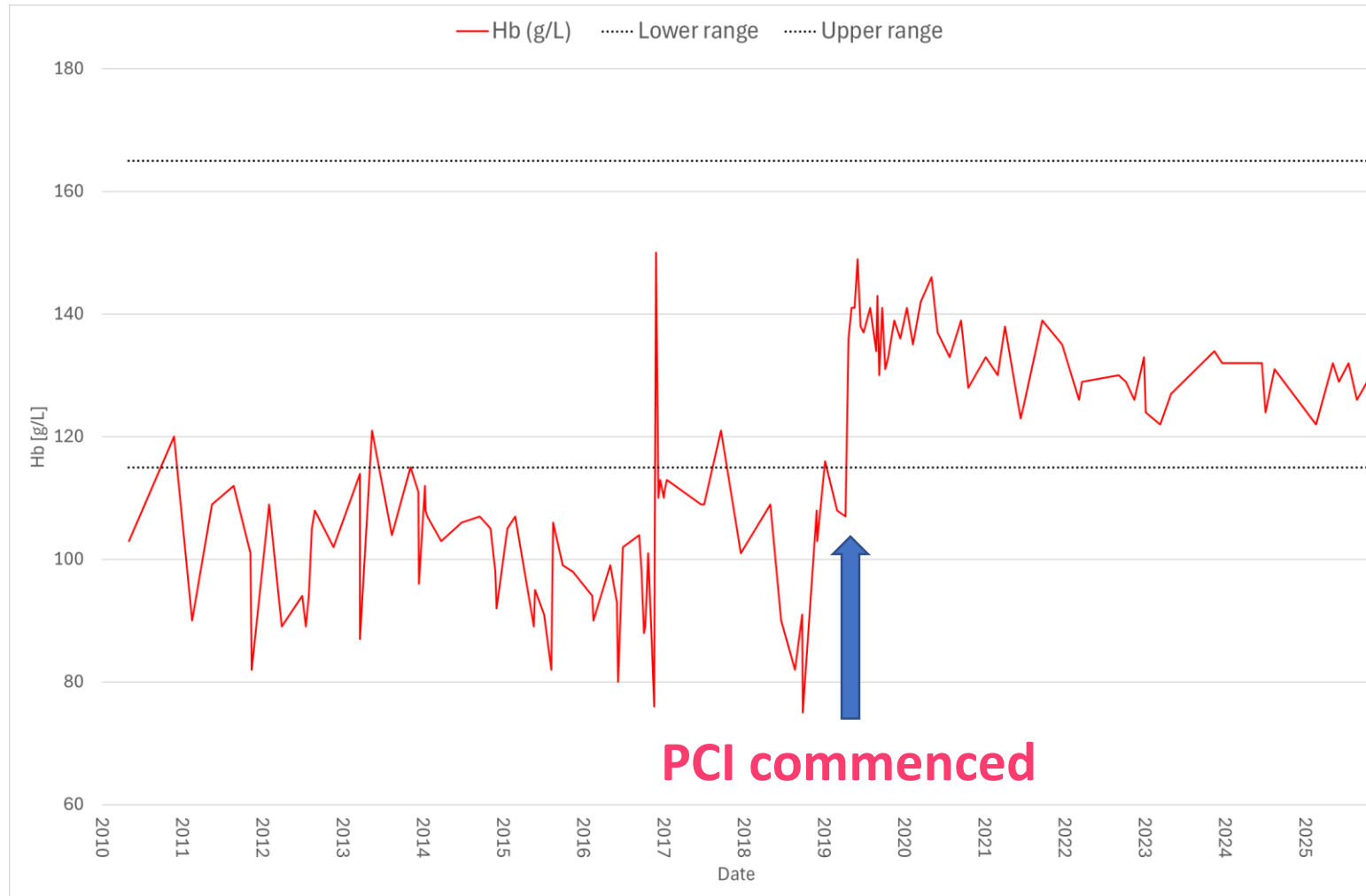
- 1997 Aplastic Anemia
- 2001 Large PNH clone
- 2002 Eculizumab
- 2006-2018
 - Erythrocyte transfusions
 - Why is the graph so busy?



Assessment of Extravascular Hemolysis (C3d on PNH RBC)

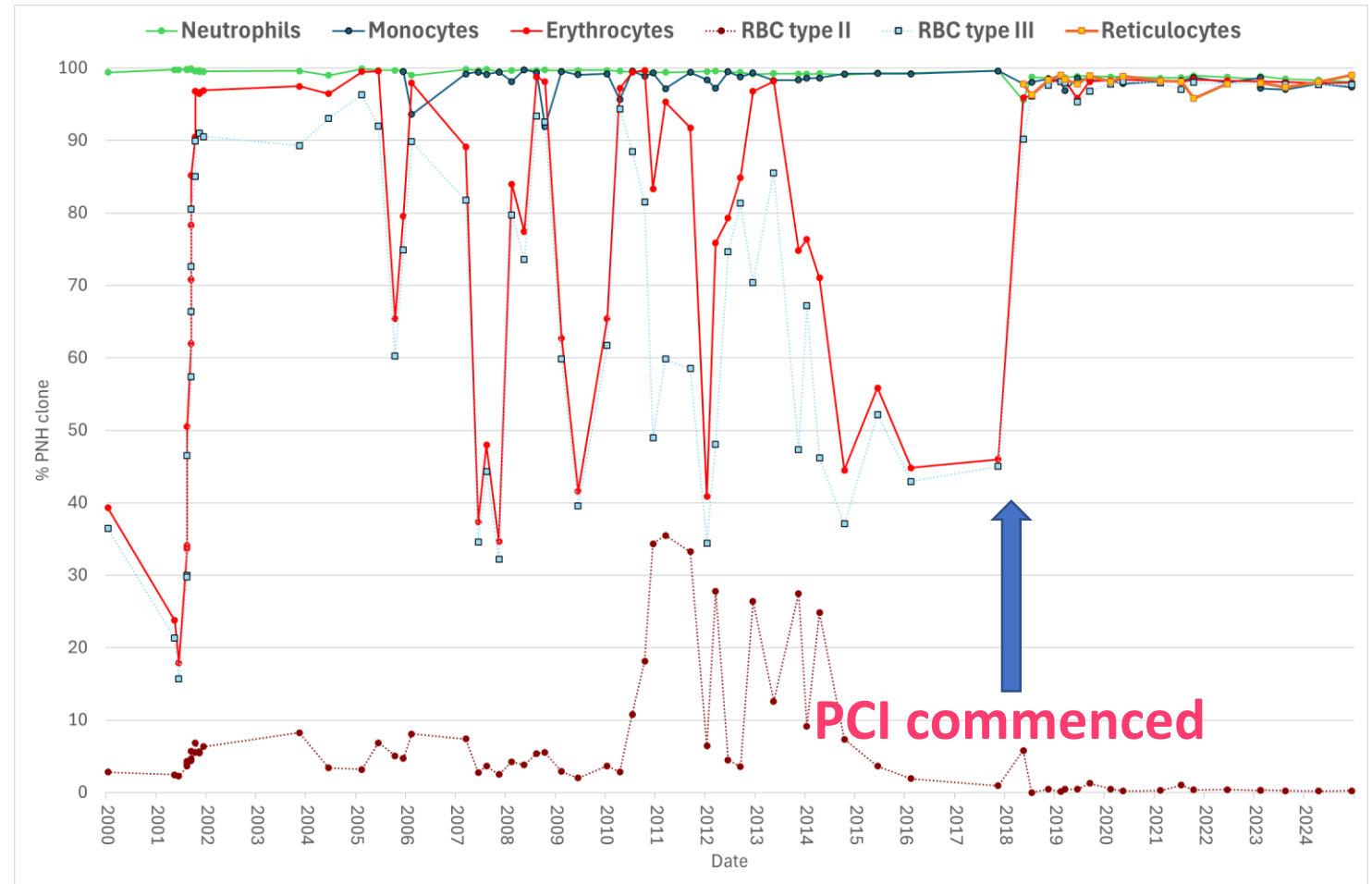


Assessment of Extravascular Hemolysis (hemoglobin)



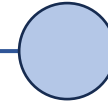
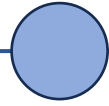
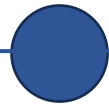
Flow Cytometry in Practice: Case 3

- 2019
 - Commenced Proximal Complement Inhibition (PCI)
- Received intermittent venesections

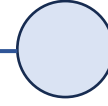


From Early Results to Modern Diagnostics

2002



2025



- PNH neutrophils & red cell clone
- Start of complement inhibition in PNH
- Impact on QoL

- Extravascular hemolysis
- Proximal complement inhibition
- PK/PD assays
- CH50 on eculizumab

- Additionally able to evaluate PNH reticulocytes cell clone & C3d loading
- Individualised patient care
- Focus on improving morbidity & mortality

- Check laboratory is performing external quality assurance / sample sharing / part of a network
- Discuss and question unusual / atypical results
- Ask for advice, speak to PNH experts
- Work together with your laboratory
 - Appropriate alerts and pathways in place for new patients
 - Useful tools – graphs, assessing immature red cells/reticulocytes



THANK YOU!