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Detection of recurrent somatic variants in cell-free DNA as a tool for disease monitoring in Hodgkin lymphoma

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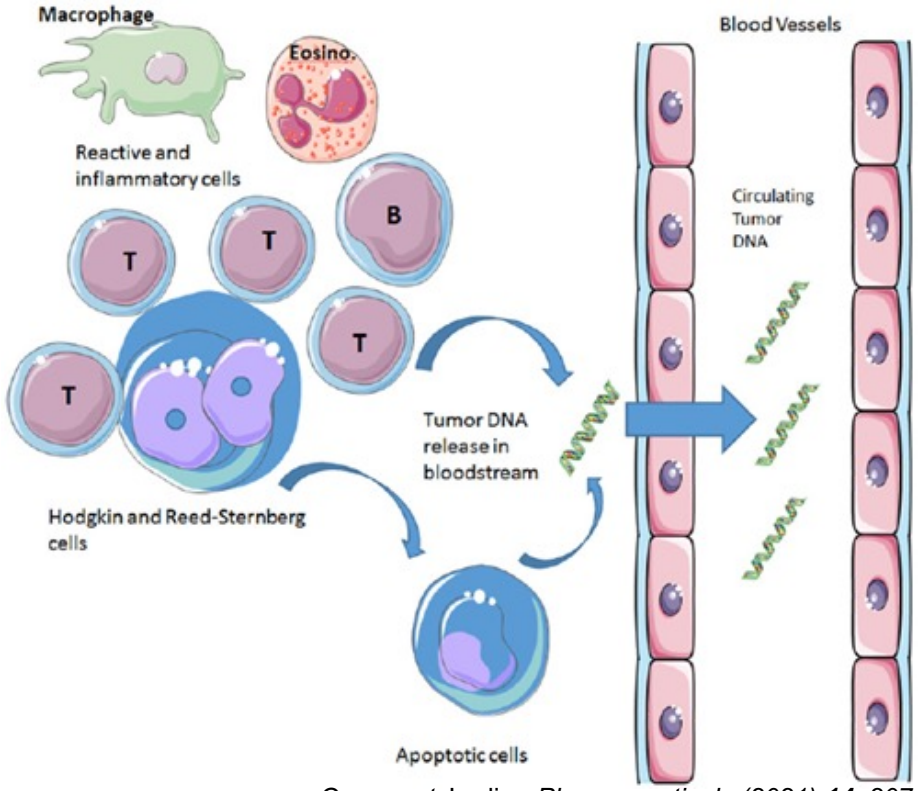
Current state of HL

- Up to 80 % pts can be cured; 20 – 30 % progression or relapsed
- PET/CT scan – evaluation for treatment response
  - False positive/false negative results → looking for new approaches



Camus *et* Jardin., *Pharmaceuticals (2021)* ***14****, 207.*

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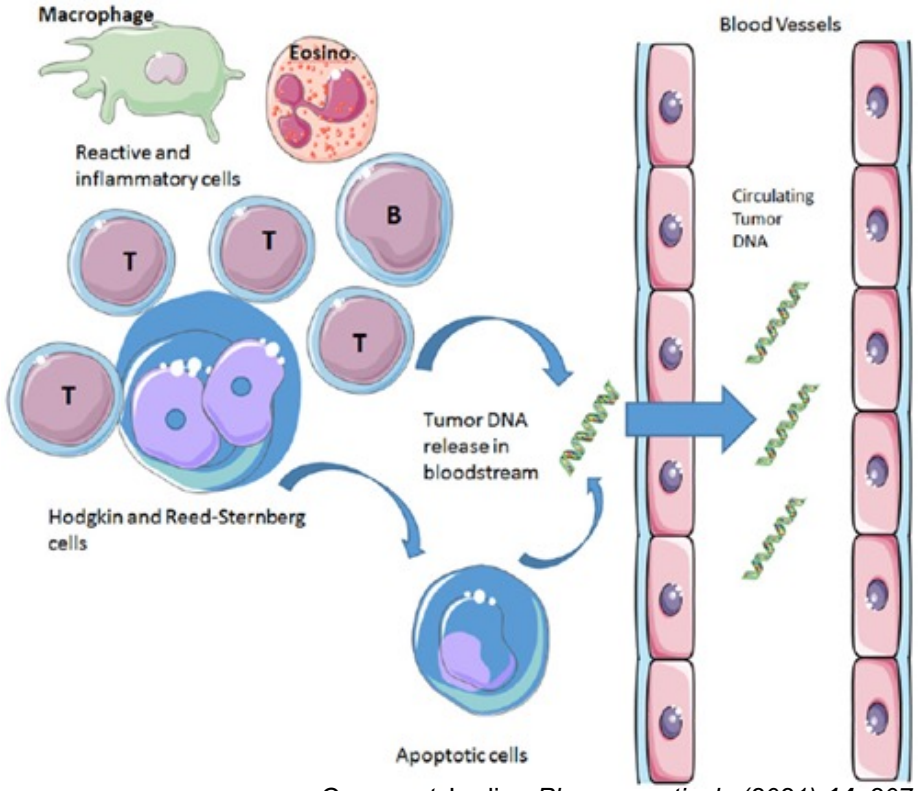


Camus *et* Jardin., *Pharmaceuticals (2021)* ***14****, 207.*

Liquid biopsy

- Hodgkin genotyping
- Minimal residual disease monitoring (MRD)

NGS approach

- *Target enrichment panel*
  - *B2M, CD36, CIITA, GNA13, HIST1H1E, ITPKB, NFKBIE, PTPN1, SOCS1, SPEN, STAT6, TNFAIP3* and *XPO1*
    - Based on Spina *et al.*, 2018 and Camus *et al.*, 2021

- Library
  - *SureSelect XT HS2 technology (Agilent Technologies) – target enrichment with molecular barcodes*
  - Input to library preparation - min. 40 – 50 ng of cfDNA

- Sequencing
  - *NovaSeq6000 (Illumina) - requirement for high coverage – 5000x*
  - *SureCall software (Agilent Technologies) –* VAF*= *0.5 %*

*VAF - Variant allele frequency

NGS approach
101 pts (94 dg., 7 relapse)

58 pts (54 dg., 4 relapse)
Sufficient cfDNA
More than 40 ng
68 % pts Ann arbor stage III – IV

↓

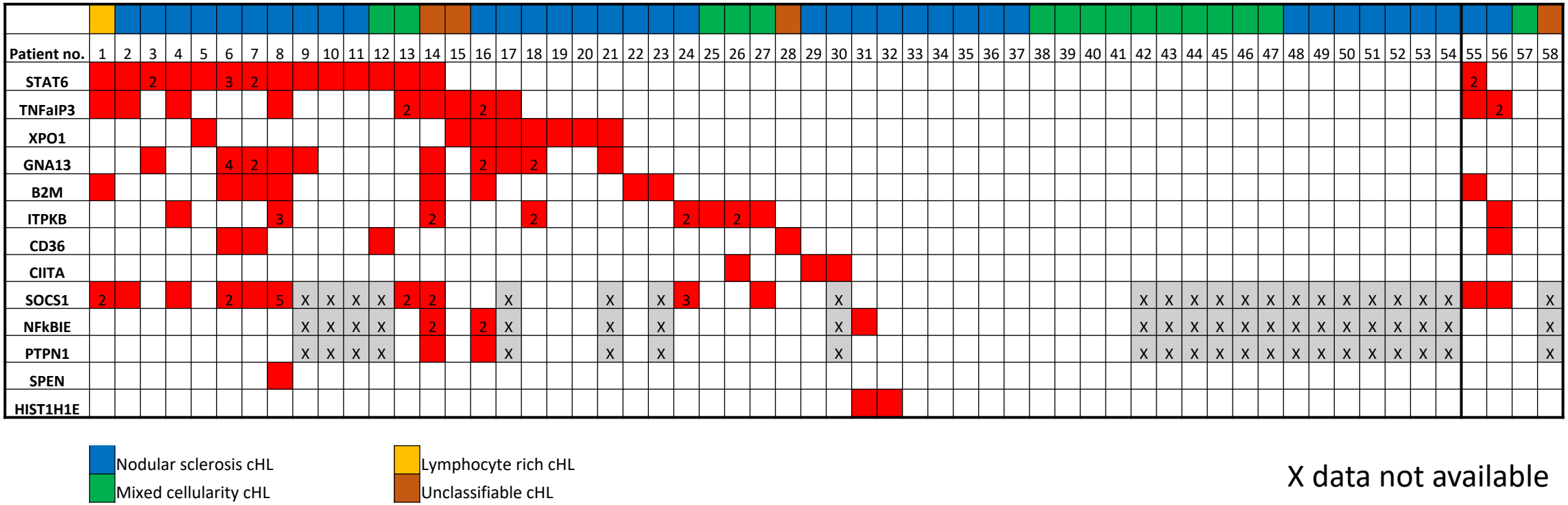
NGS

39 pts (36 dg., 3 relapse)
Insufficient cfDNA
Less than 40 ng
13 % pts Ann arbor stage III – IV
or
Failed library prep. (4 pts/dg.)

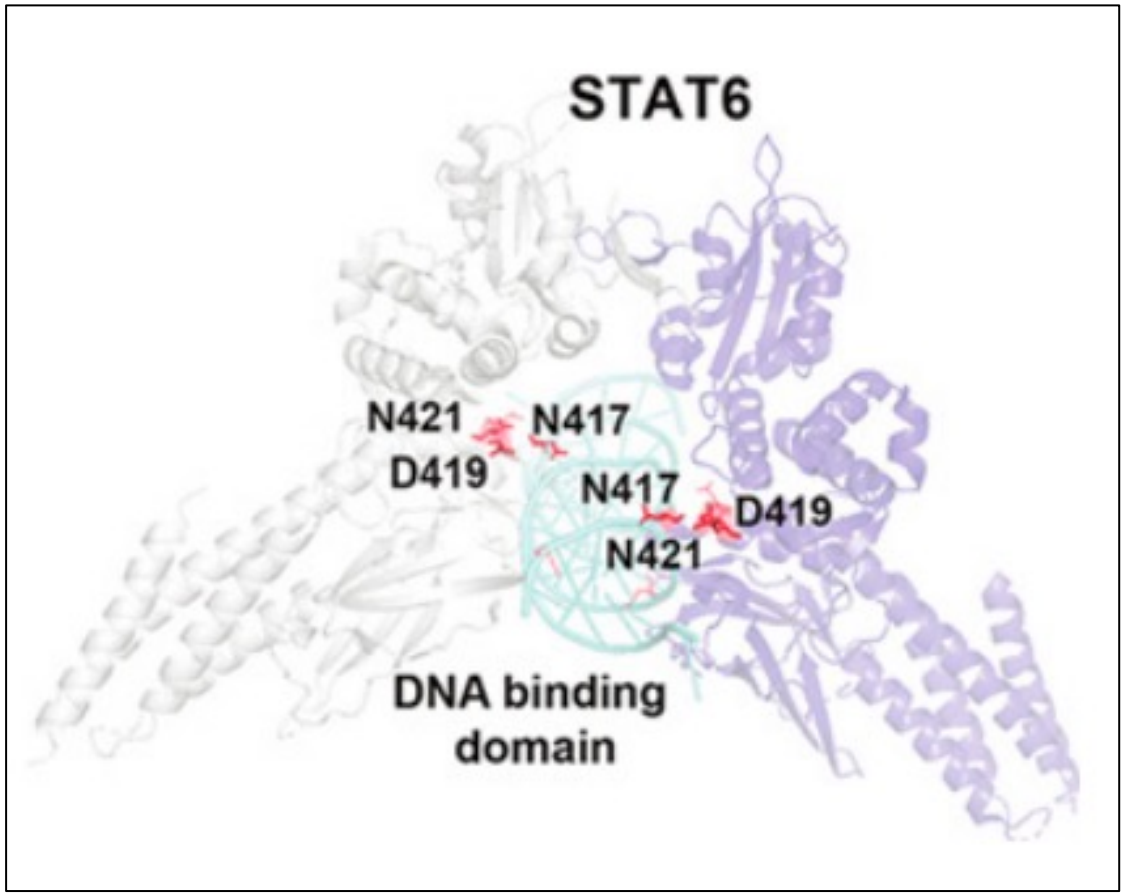
NGS results

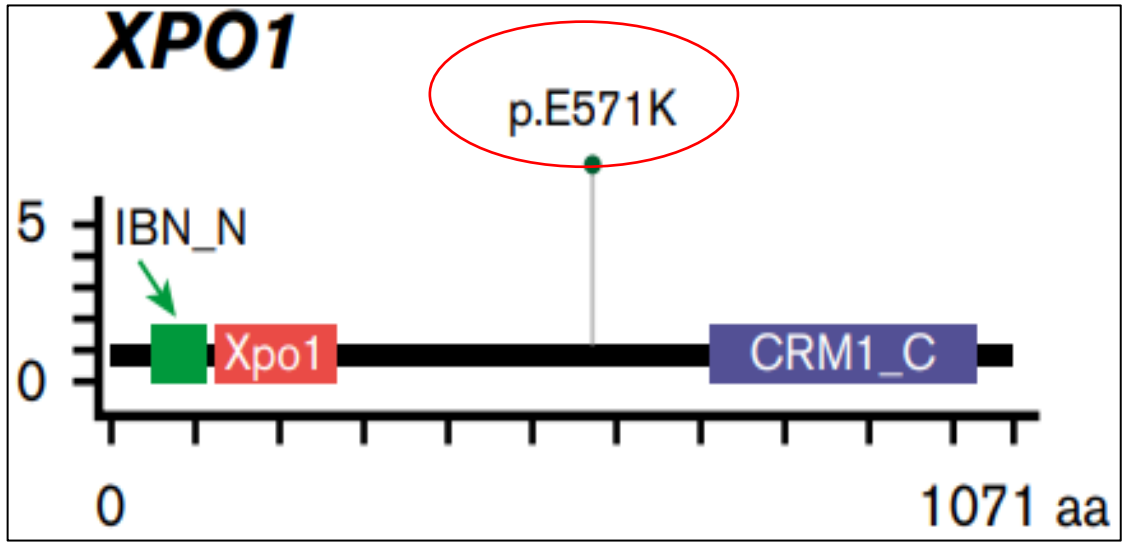
- 58 pts (54 dg., 4 relapse) - 33/58 (56.89 %), VAF (8.1 %; 1.5-36 %)

- *SOCS1* (12/36 pts; 33.33 %)
- *STAT6* (15/54 pts; 25.86 %)
- *TNFAIP3* (12/58 pts; 20.68 %)
- *GNA13 (10/58 pts; 17.24 %)*
- *ITPKB (9/58 pts; 15.51 %)*
- *XPO1* (8/58 pts; 13.79 %)



Markers for MRD testing revealed by NGS





Up to 40 % of all cHL (27.7 %)

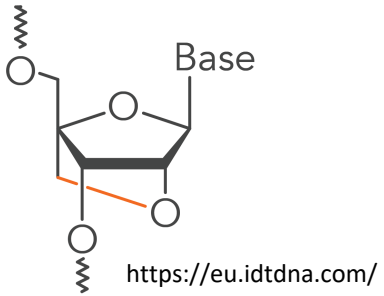
10 - 20 % of all cHL (13.8 %)

Spina *et al*., *Blood* (2018); **131** (22): 2413–2425;
Camus *et al*., Haematologica. (2021);**106** (1):154–162

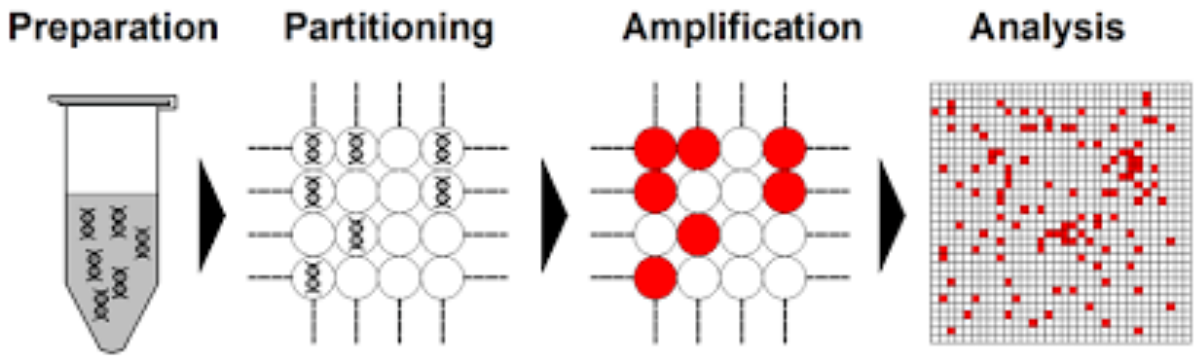
Camus *et al*., Heamatologica (2016); **101:** *1094-1101*.;
Spina *et al*., *Blood* (2018); **131** (22): 2413–2425

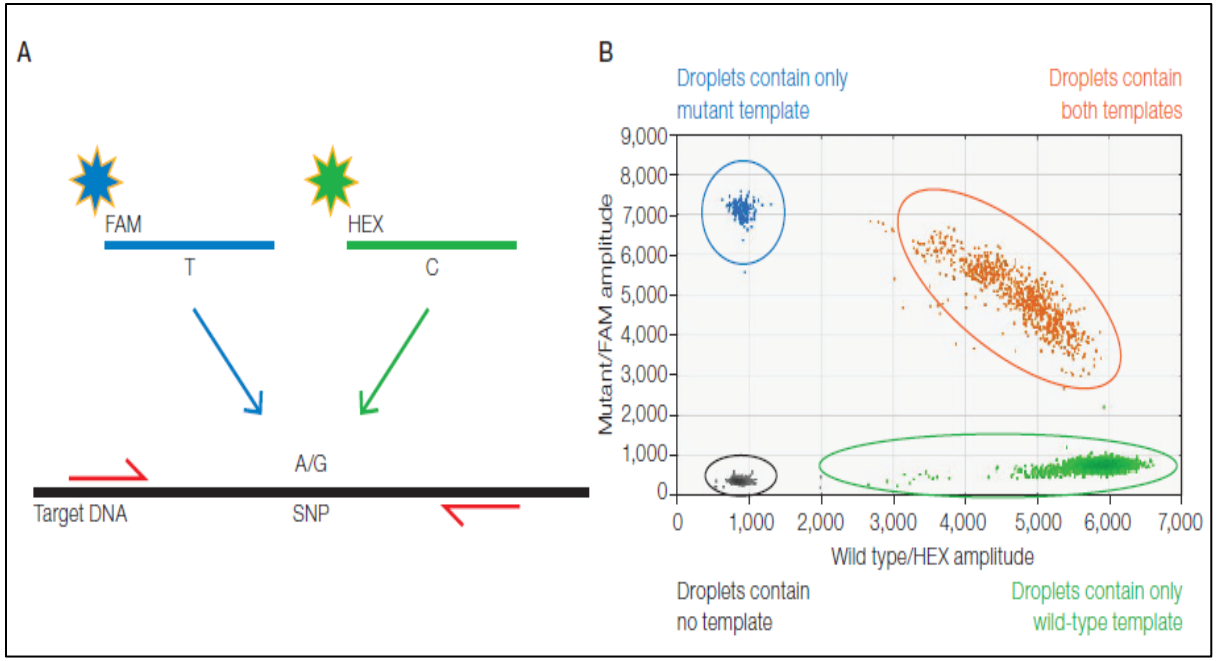
dPCR approach

- *QIAcuity™ probe PCR kit + nanoplate QIAcuity Nanoplate 26K 24-well (Qiagen)*
- Locked nucleid acid probes (LNA probes)
  - reduction of false positivity rates → better sensitivity



https://eu.idtdna.com/

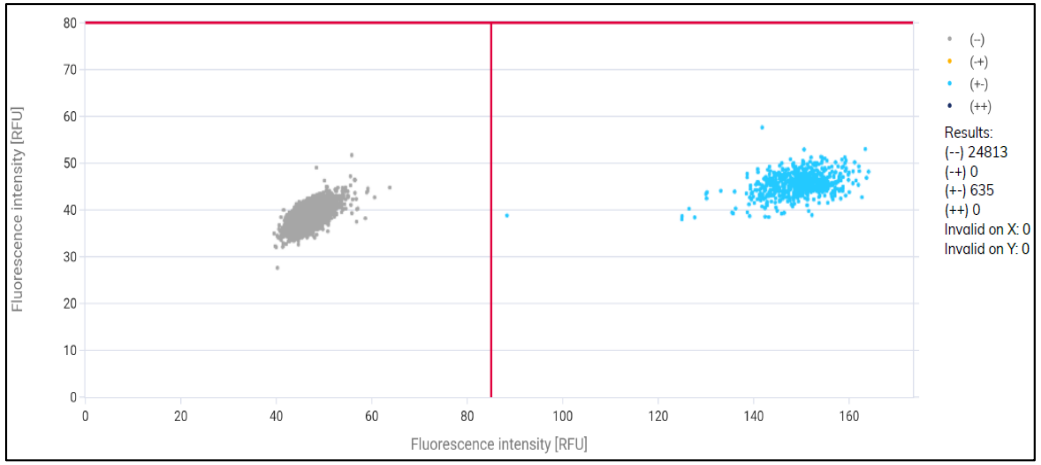




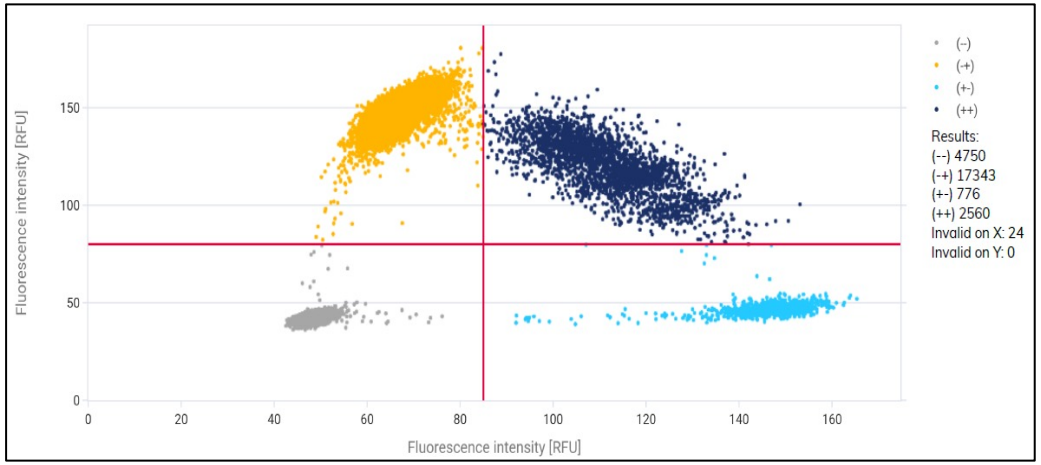
https://www.bio-rad.com/

MRD monitoring

- *STAT6, XPO1 – hotspots*
  - Sensitivity up to – *0.1 %*
- 6 pts with *XPO1* mutation p.E571K
- 12 pts with *STAT6* mutations
  - 8 pts p.N417Y
  - 1 pt p.G416R
  - 1 pt p.D419N
  - 1 pt p.N421K
  - 1 pt double mutant p.N417D + p.D419A



Negative control *STAT6* N417Y– only WT Donor cfDNA



Positivite control *STAT6* N417Y
0.1 picogram of gBlock with donor cfDNA

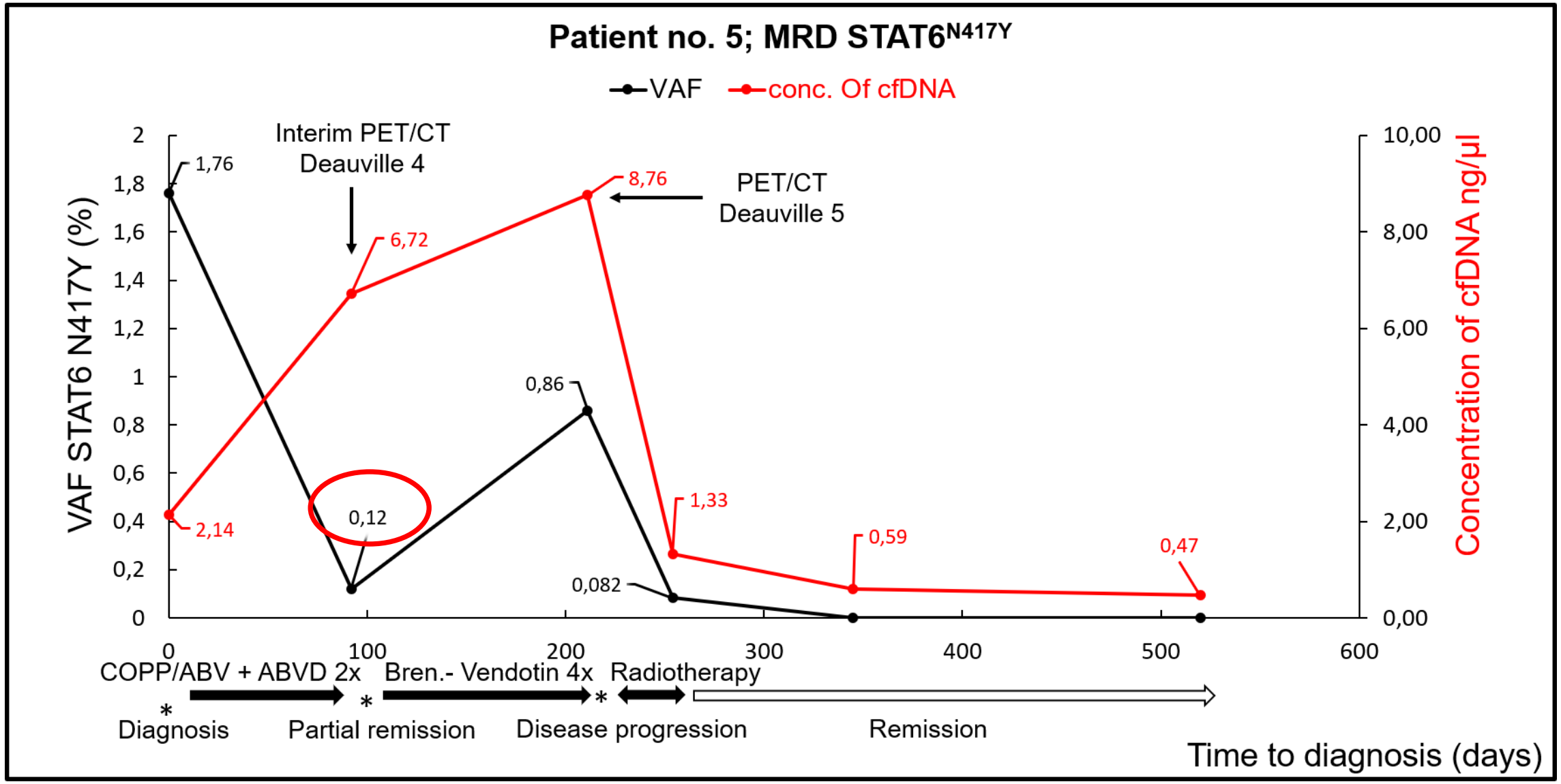
MRD monitoring

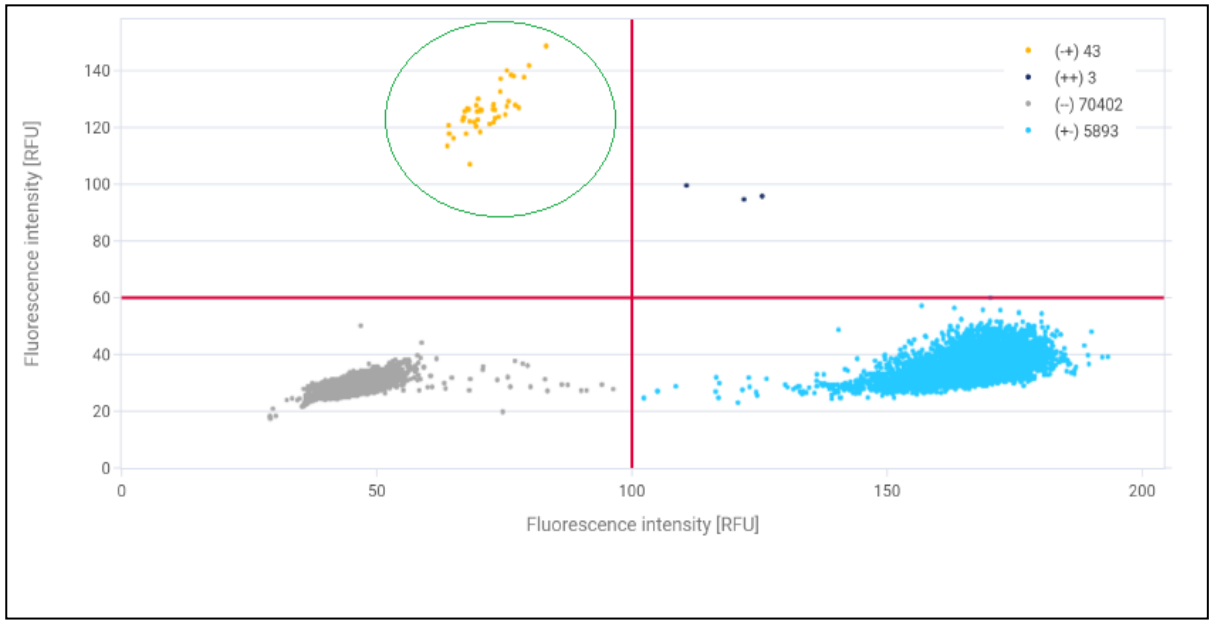
- *STAT6, XPO1 – hot spots* – Sensitivity up to – *0,1 %*

- 6 pts with *XPO1* mutation *p.*E571K ⟶ 1 pt early relapse

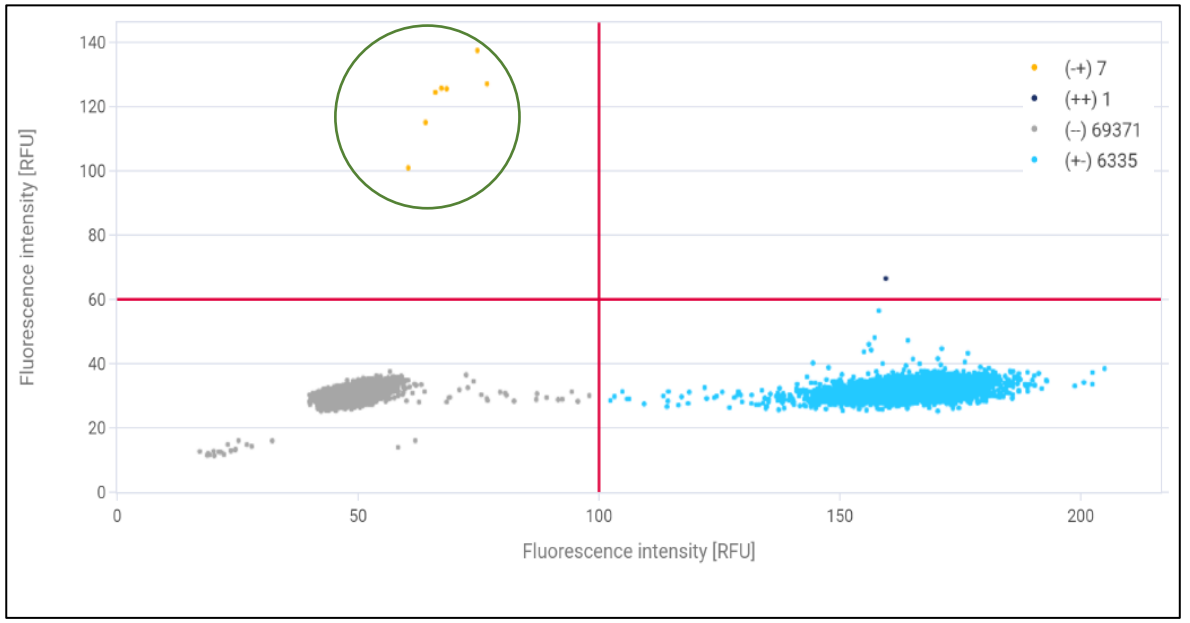
- 12 pts with *STAT6* mutations
  - 8 pts p.N417Y ⟶ 2 pts early relapse
  - 1 pt p.G416R 1 pt primary refractory
  - 1 pt p.D419N
  - 1 pt p.N421K
  - 1 pt double mutant p.N417D + p.D419A

MRD monitoring in patient with *STAT6* p.N417Y mutation using dPCR



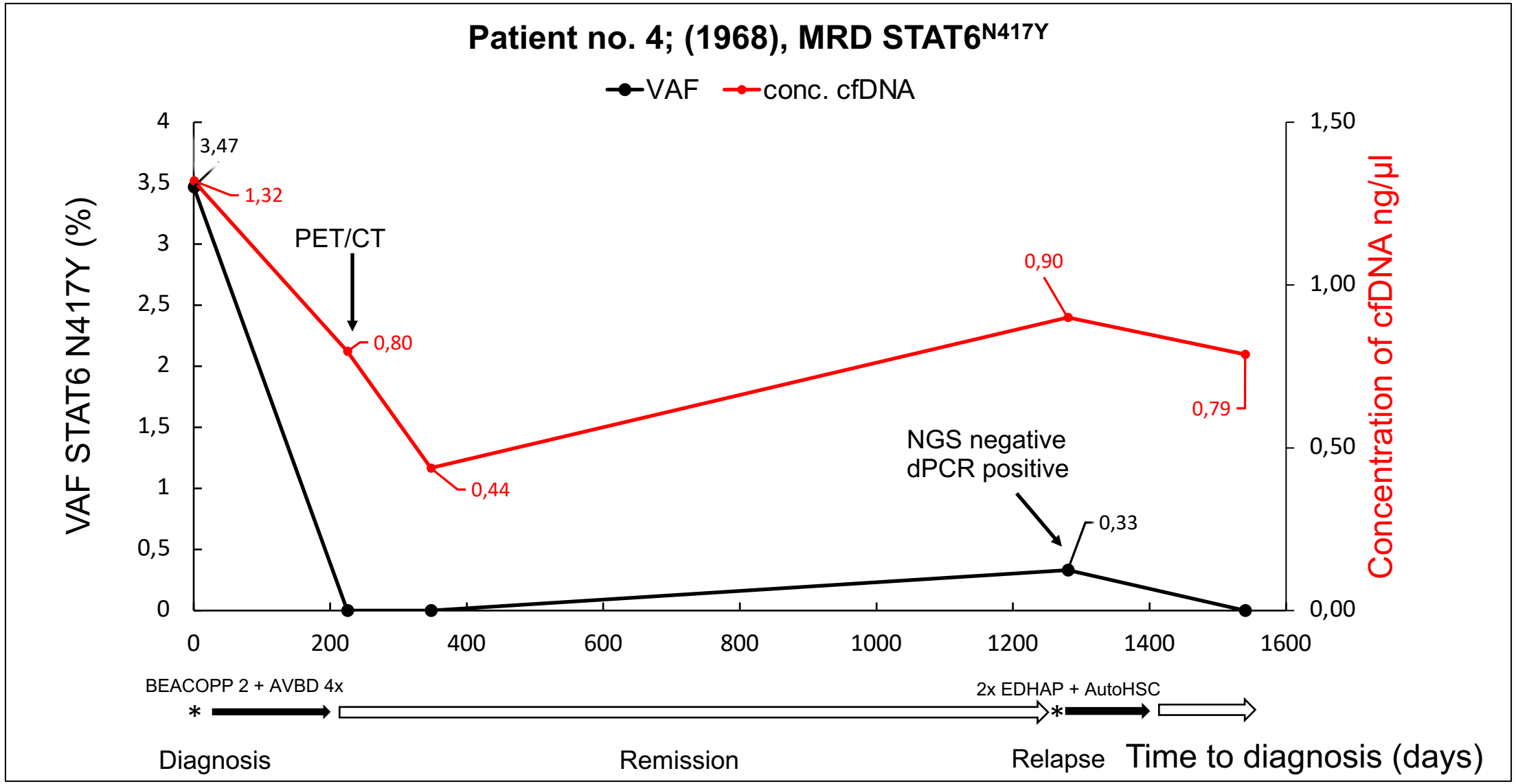


STAT6 p.N417Y
at the time of diagnosis, VAF = 0,76 % (NGS = 1,76 %)

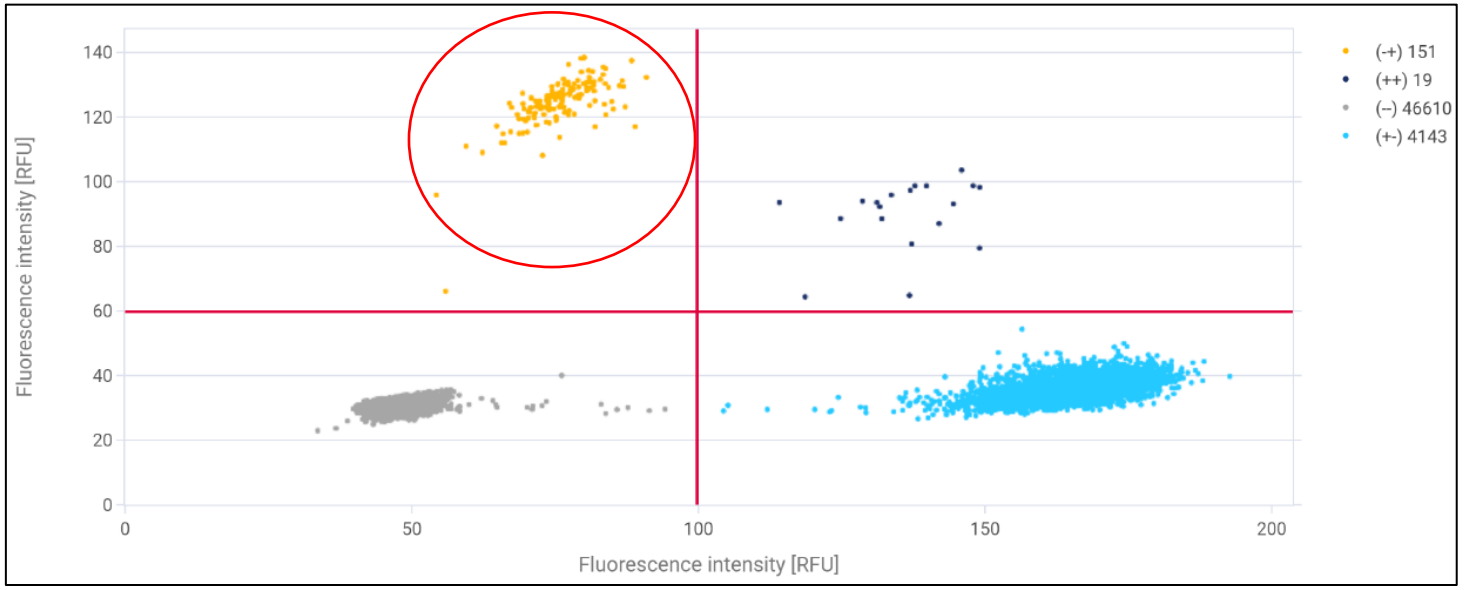


STAT6 p.N417Y
After first line of chemotherapy, VAF = 0,12 %

NGS/dPCR comparison?



NGS/dPCR comparison?



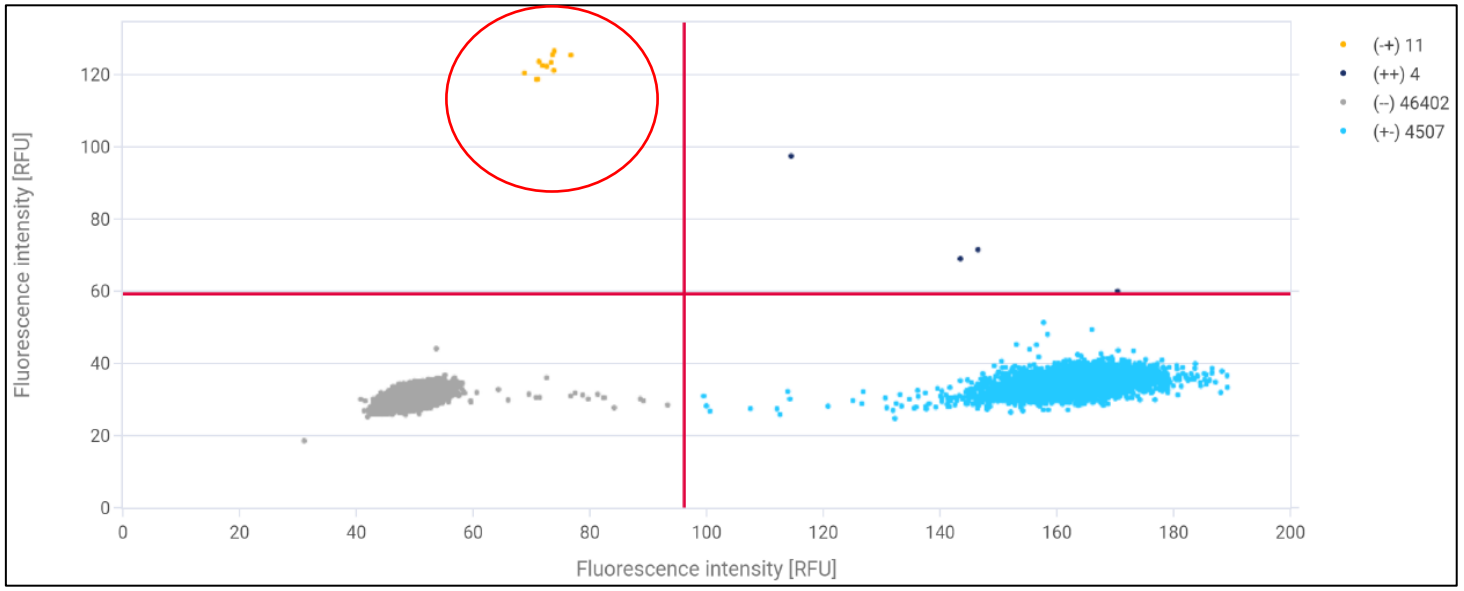
Dg. *STAT6* N417Y

NGS: 3,47 % VAF

Input NGS: 59,4 cfDNA

dPCR: 3,70 % VAF

Input dPCR: 33,0 ng cfDNA



Relapse *STAT6* N417Y

NGS: negative

Input NGS: 41,5 ng cfDNA

dPCR: 0,33 % VAF

Input dPCR: 27,0 ng cfDNA

Summary

- NGS – screening at the time of diagnosis
- Digital PCR – MRD monitoring in case of presence of molecular marker

Plasma Cell-Free DNA		
Characteristics	**Advantages**	**Disadvantages**
• Droplet digital PCR	• Short turnaround tim • Low cost • Detection of "hotspot" targetable activating mutations • Easy serial testing • 10^{-5} detection limit	• Not commercially available • Insufficient data to verify the reproducibility • False-positive and detection limit concerns
• Panel-directed Next-Generation Sequencing (CAPP-seq and other targeted panels)	• Measure disease burden, detect early relapse before radiological progression • Monitor variants' clearance in chemo sensitive patients versus non-responders patients who display persistent genetic alterations in plasma after treatment	• No standardized technique • Not commercially available • high cost due to elevated number of genes included in the panel • Need trained research teams with experienced bioinformaticians able to combine barcoding and unique molecular identifiers (UMIs) with integrated digital error suppression

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Thank you for your attention

Future goals

- 39 pts with insufficient amount of cfDNA → multiplex dPCR

- redesign of the NGS panel (new NGS/dPCR targets?)