Role of circulating tumor DNA in response prediction and assessment of clonal evolution

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Conflict of interest

Research Support:	Gilead, Abbvie, Janssen, Cellestia
Employee	No
Consultant	No
Major Stockholder	No
Speakers Bureau	No
Honoraria	Gilead, Abbvie, Janssen, Roche, AstraZeneca
Scientific Advisory Board	Gilead, Abbvie, Janssen, AstraZeneca, MSD

Agenda

- General notions and practicalities
- ctDNA in DLBCL
- ctDNA in cHL

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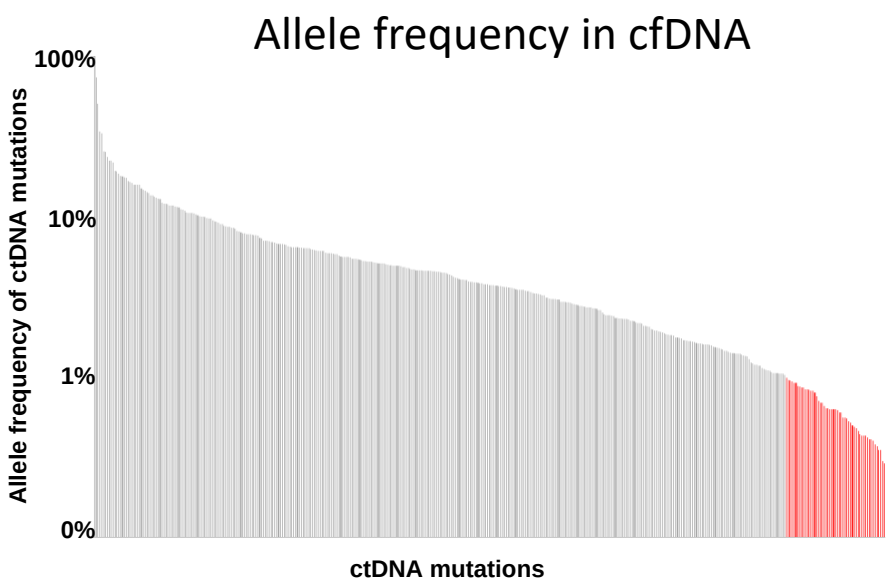
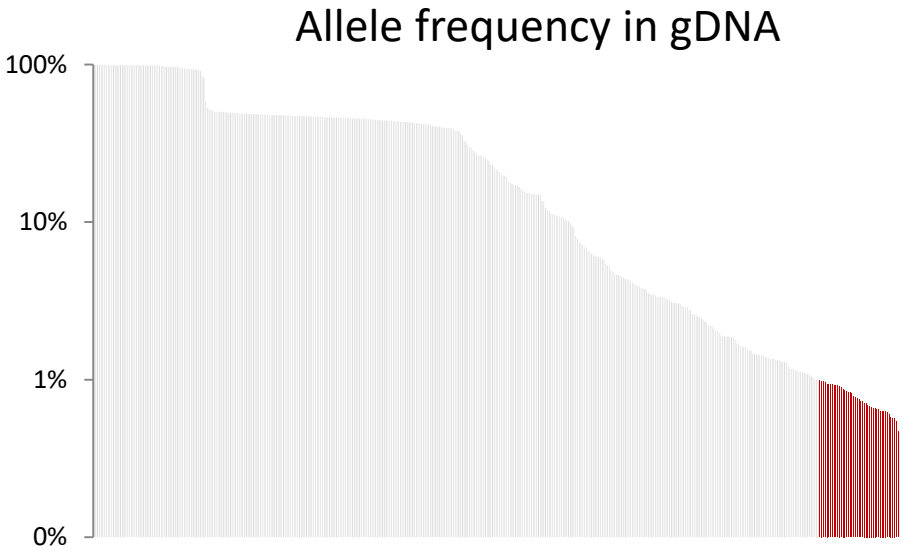
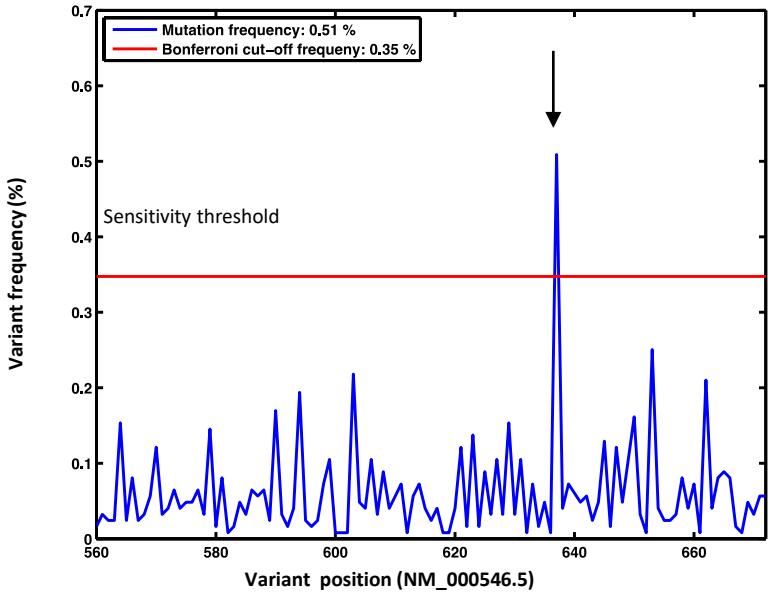
ctDNA is of low abundance:
Optimization of sensitivity and specificity of NGS is mandatory







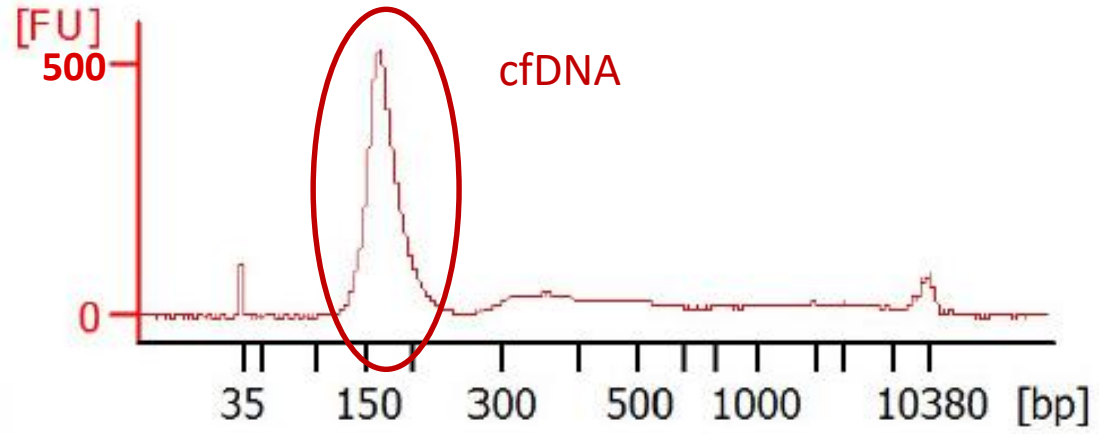
True mutations

Background noise of NGS

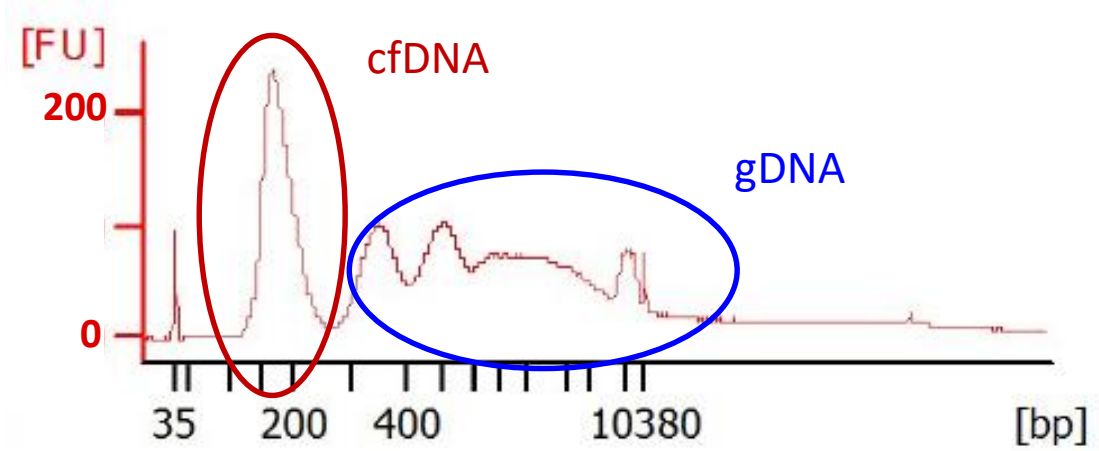
Spina V, et al. Blood 2018

Pre-analytics is critical

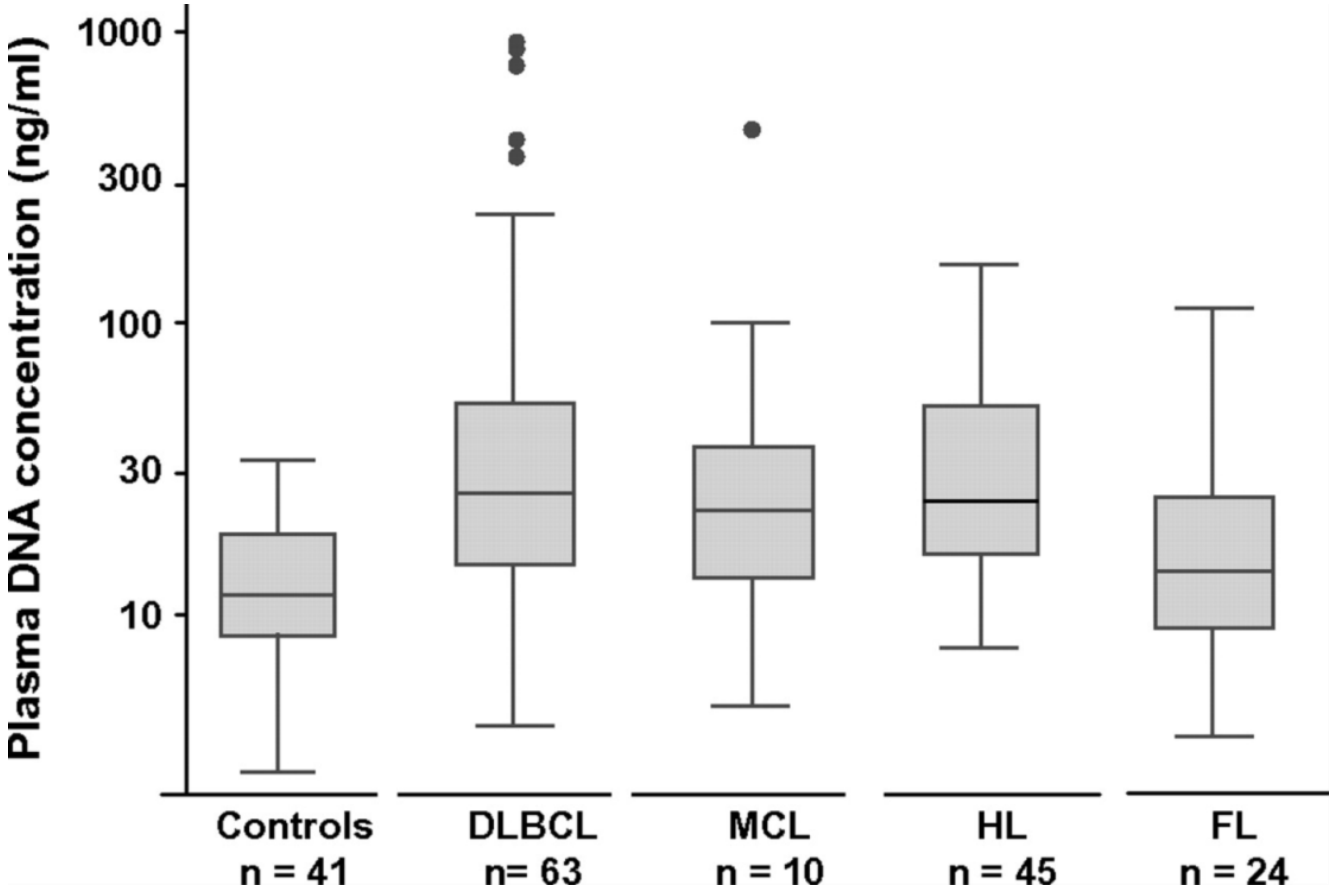
cfDNA sample of good quality: peak sized between 100 and 200 bp



cfDNA of poor quality: gDNA contamination



cfDNA circulates in small amounts

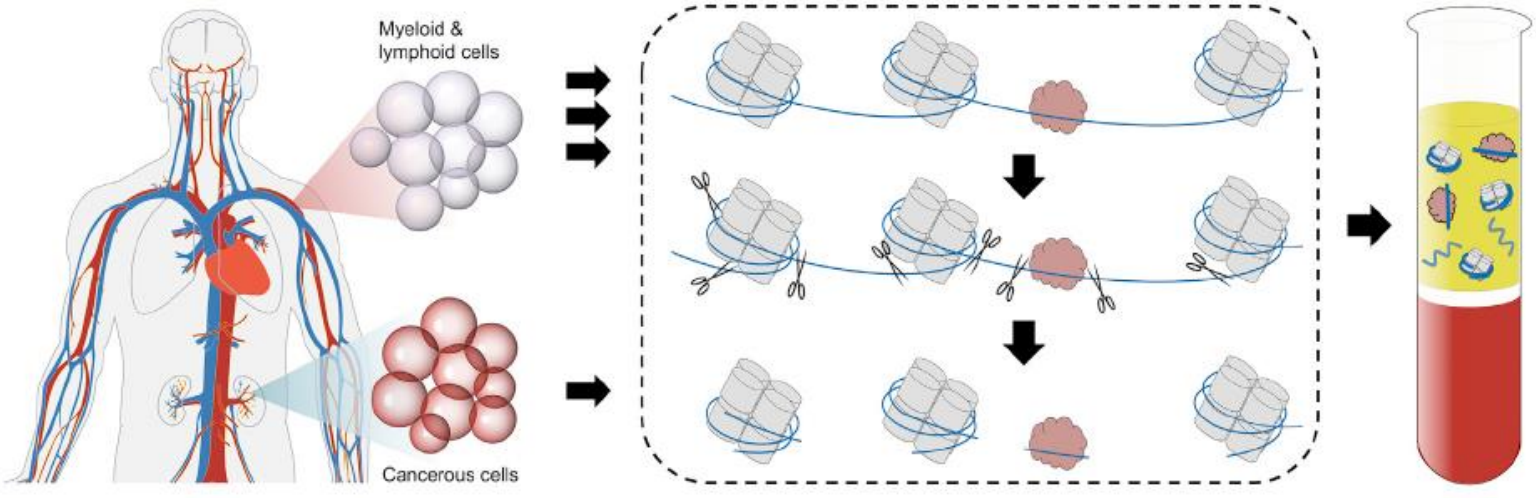


Hohaus S et al. Ann Oncol. 2009;20(8):1408-1413

Challenges in the identification of small abundant ctDNA variants by NGS

- Input DNA (at least 32 ng)
- Library preparation chemistry (capture based, molecular barcoding)
- Coverage (>2000X >80% target region)
- Bioinformatic pipeline for variant calling (catalogue of systematic errors)

The origin of cell free DNA in healthy subjects and cancer patients



- In healthy individuals cfDNA derives from apoptosis of normal hematopoietic cells
- In tumor patients cfDNA is released by tumor apoptotic cells
- ctDNA is distinguished from other cfDNA by the presence of somatic mutations representative of tumor biology absent in normal cells

Snyder, Cell 2016

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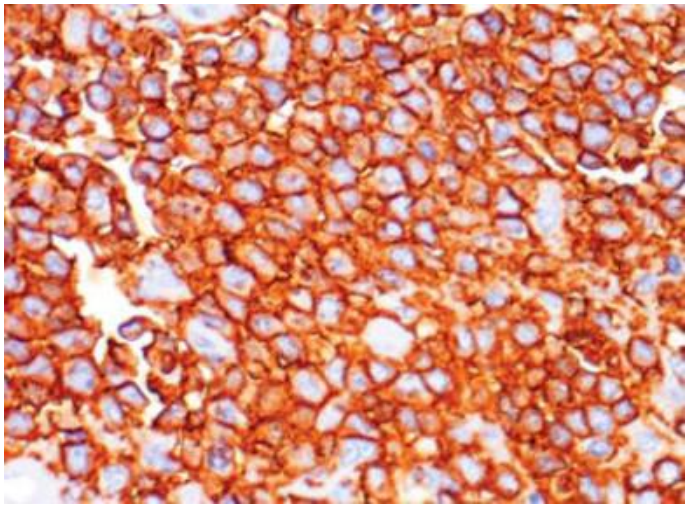
- ctDNA in cHL

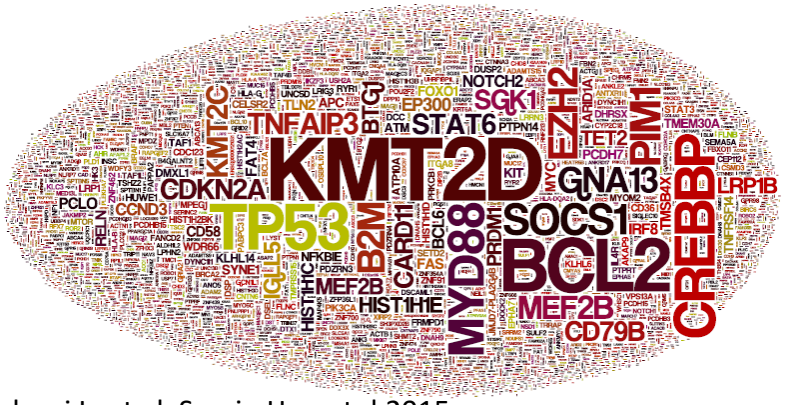
Diffuse large B-cell lymphoma vs classical Hodgkin lymphoma

DLBCL

Tumor cells are enriched in the mass



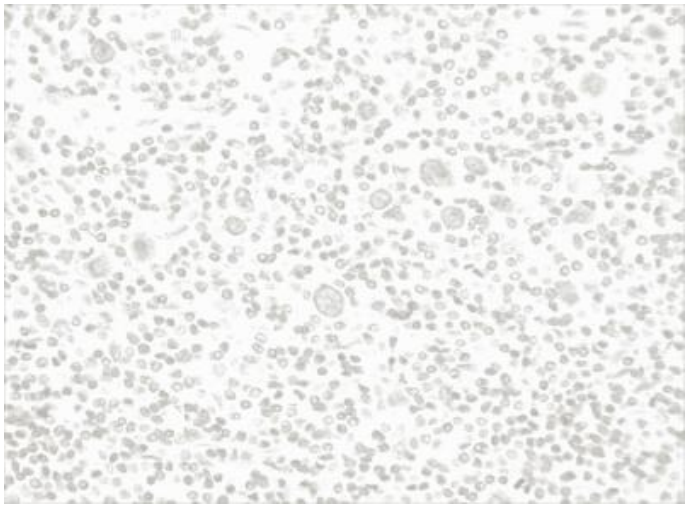
Exome sequencing data from >1000 cases



Pasqualucci L, et al. Semin Hematol 2015

cHL

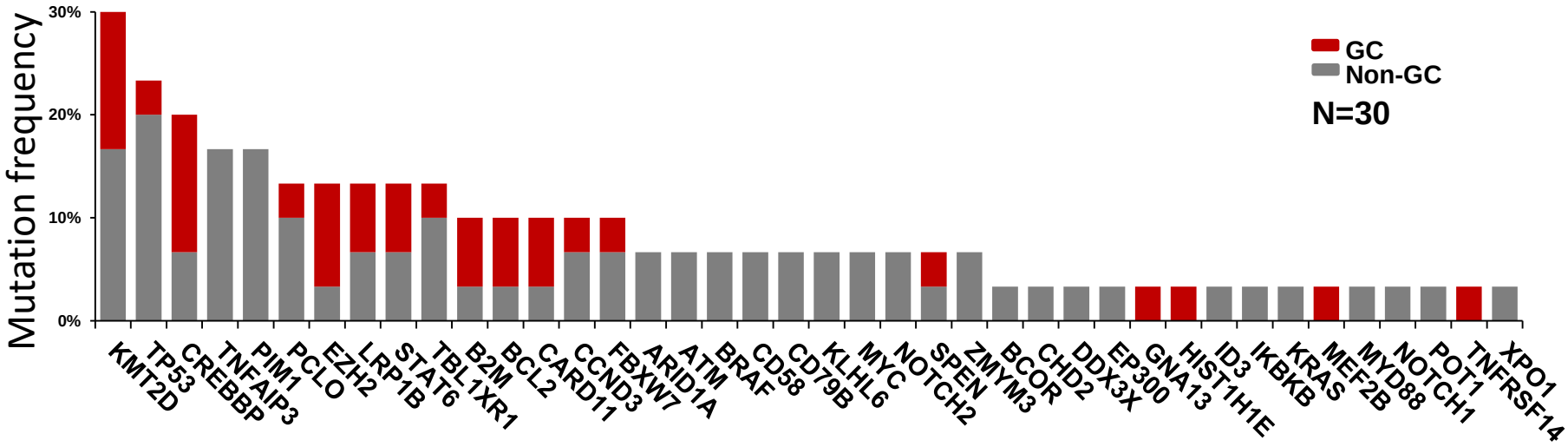
Tumor cells are rare in the mass

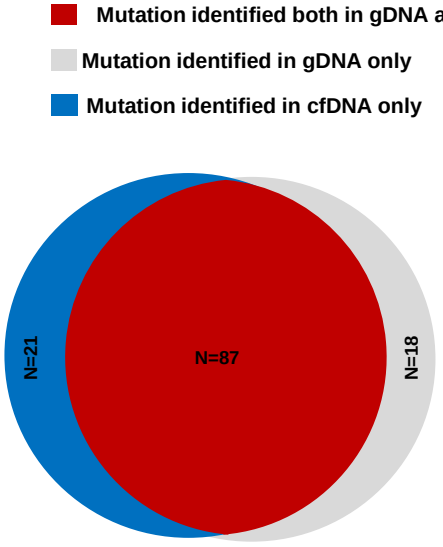


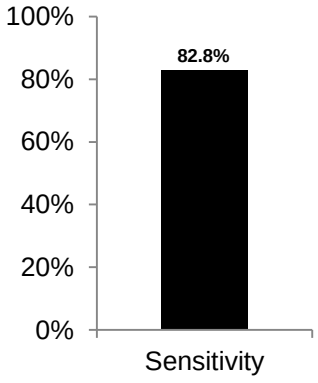
Exome sequencing data from only 10 cases

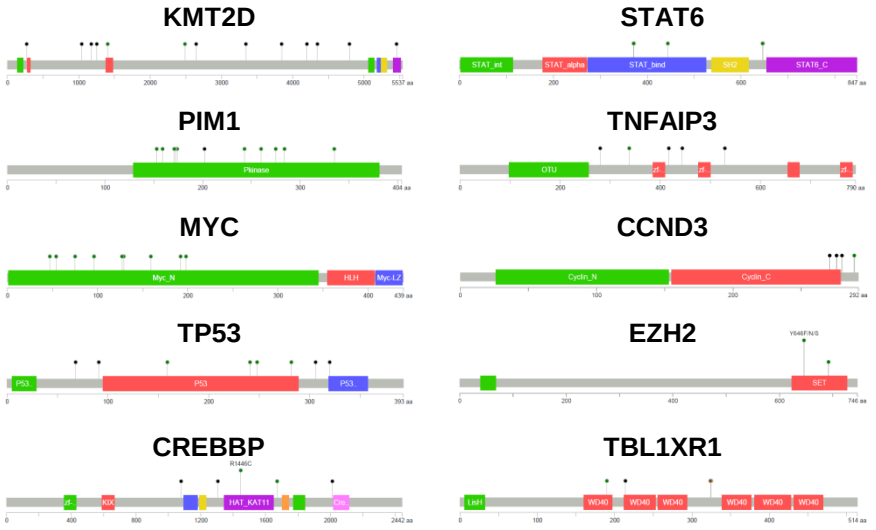
Reichel J, et al. Blood 2015

ctDNA mirrors the genetics of DLBCL cells



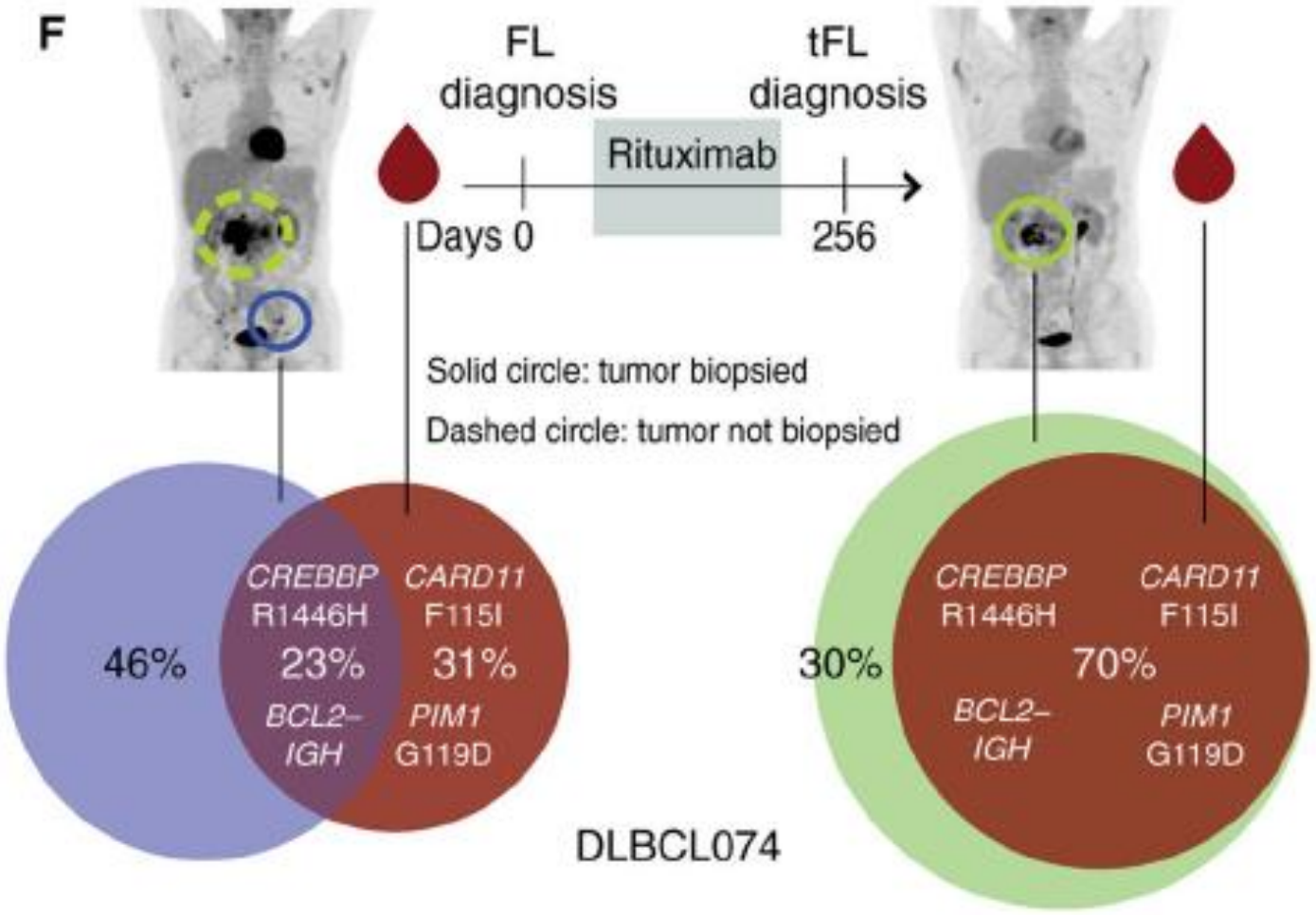






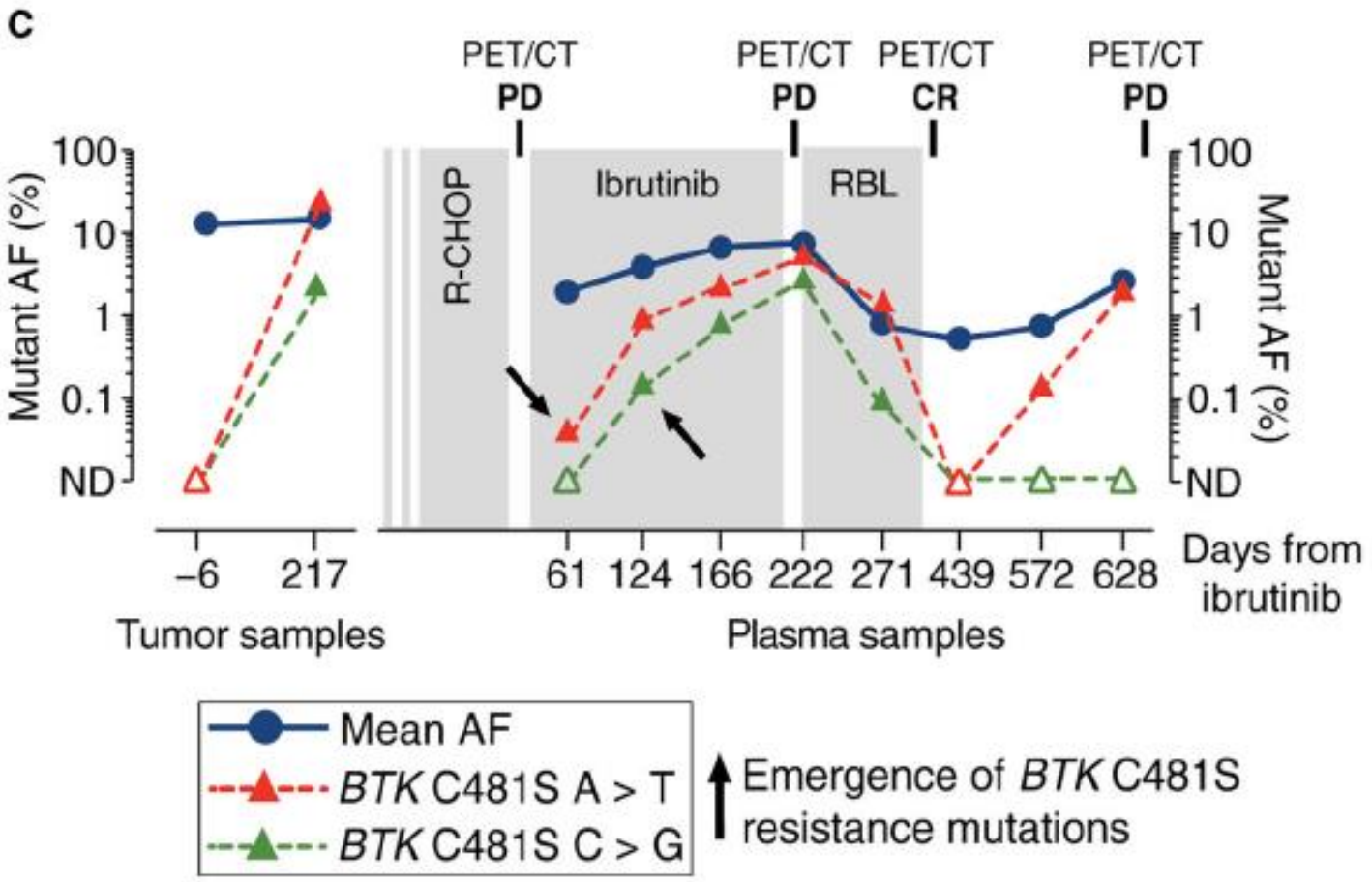
Rossi D, et al. Blood 2017

Circulating tumor DNA resolves the spatial heterogeneity of lymphomas



Scherer F, et al. Sci Transl Med 2016

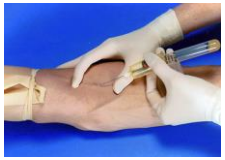
Longitudinal cfDNA genotyping allows Non invasive detection of ibrutinib resistance mutations

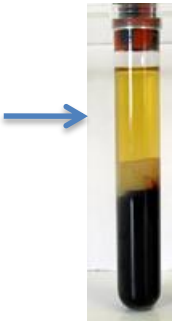


Scherer F, et al. Sci Transl Med 2016

LymphoSIGHT™ platform

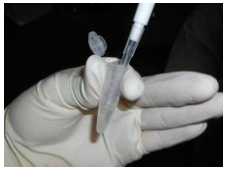
1) Collect 10cc peripheral blood

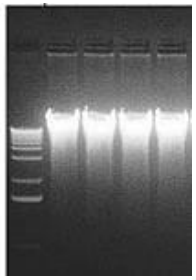




Serum

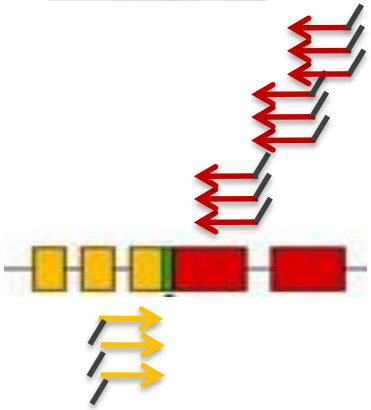
2) Extract DNA





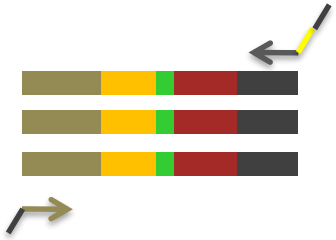
Genomic DNA

3) Amplify VDJ with multiplex PCR



PCR amplicons

4) Prepare for sequencing with common PCR



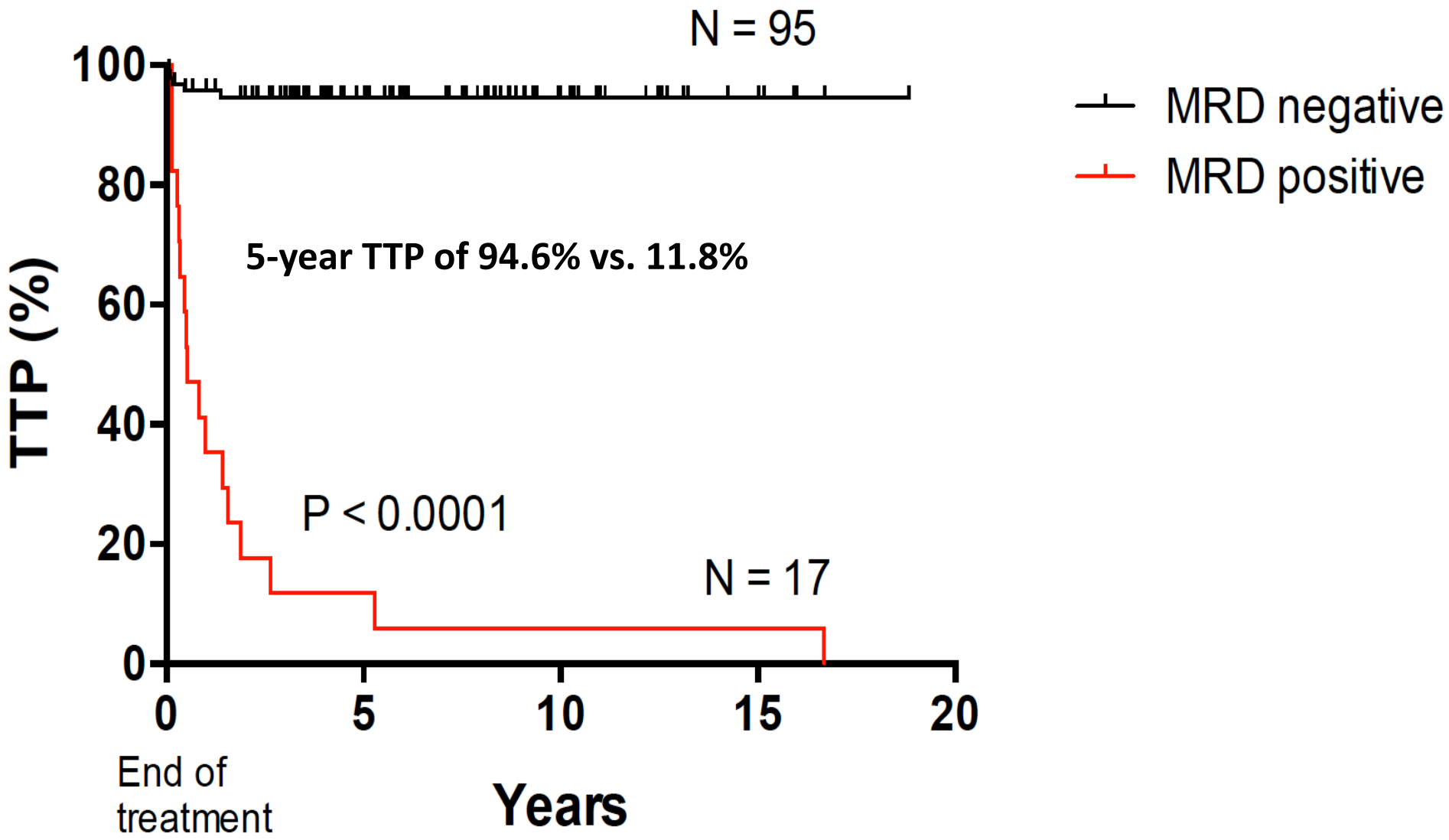
Sequencing library

5) Sequence ~1M 100bp reads

```
CTGGCCCCAGTAGTCATACCAACTAGCG
TTGGCCCCAGAAATCAAGACCATCTAAA
ACGGCCCCAGAGATCGAAGTACCAGTGT
TTGGCCCCAGACGTCCATATTGTAGTAG
CTGGCCCCAGAAGTCAGACCGGCTAACA
```

Sequence data

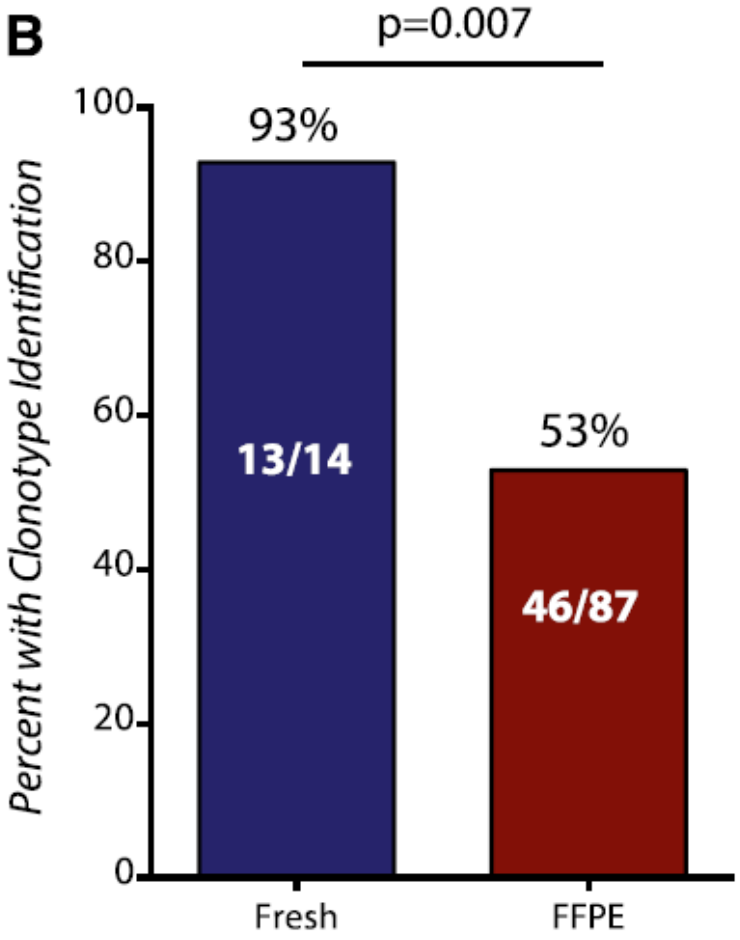
MRD at the end of treatment predicts progression



Roschewski M et al. *Lancet Oncol*, 2015

Limitations of Ig-HTS as tumor fingerprint in the Liquid biopsy

Recovery rate of the tumor IG rearrangement from DLBCL tissue biopsies



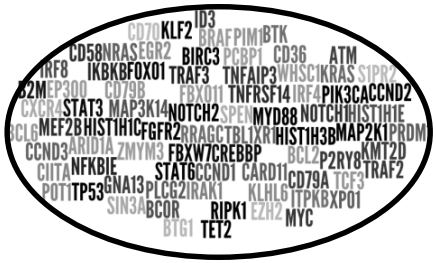
Kurtz DM et al. *Blood*, 2015.

Mutational profile as tumor fingerprint

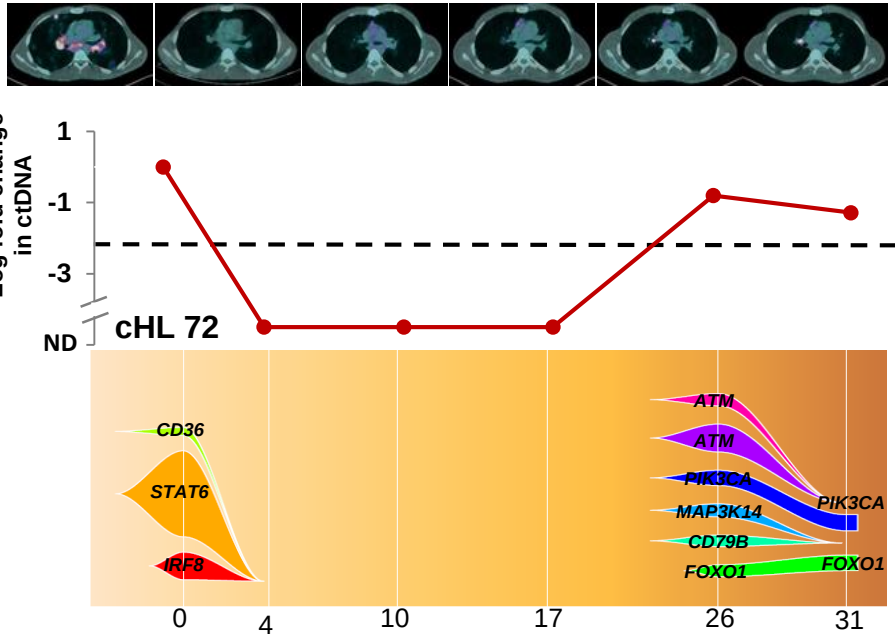
PB granulocytes

Plasma

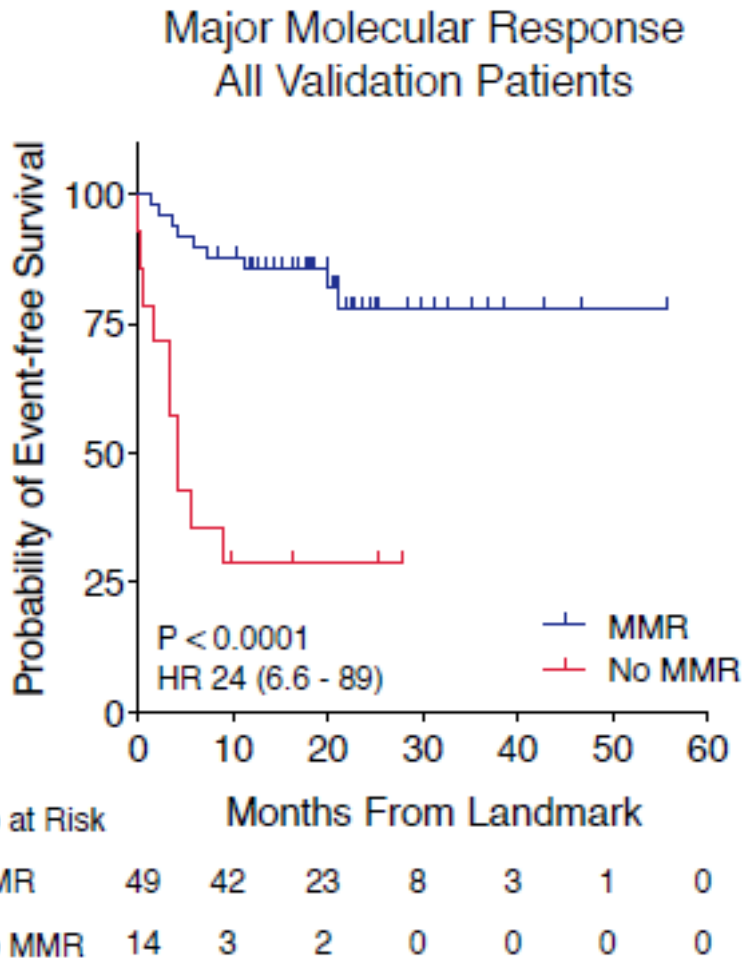
Ultra deep sequencing

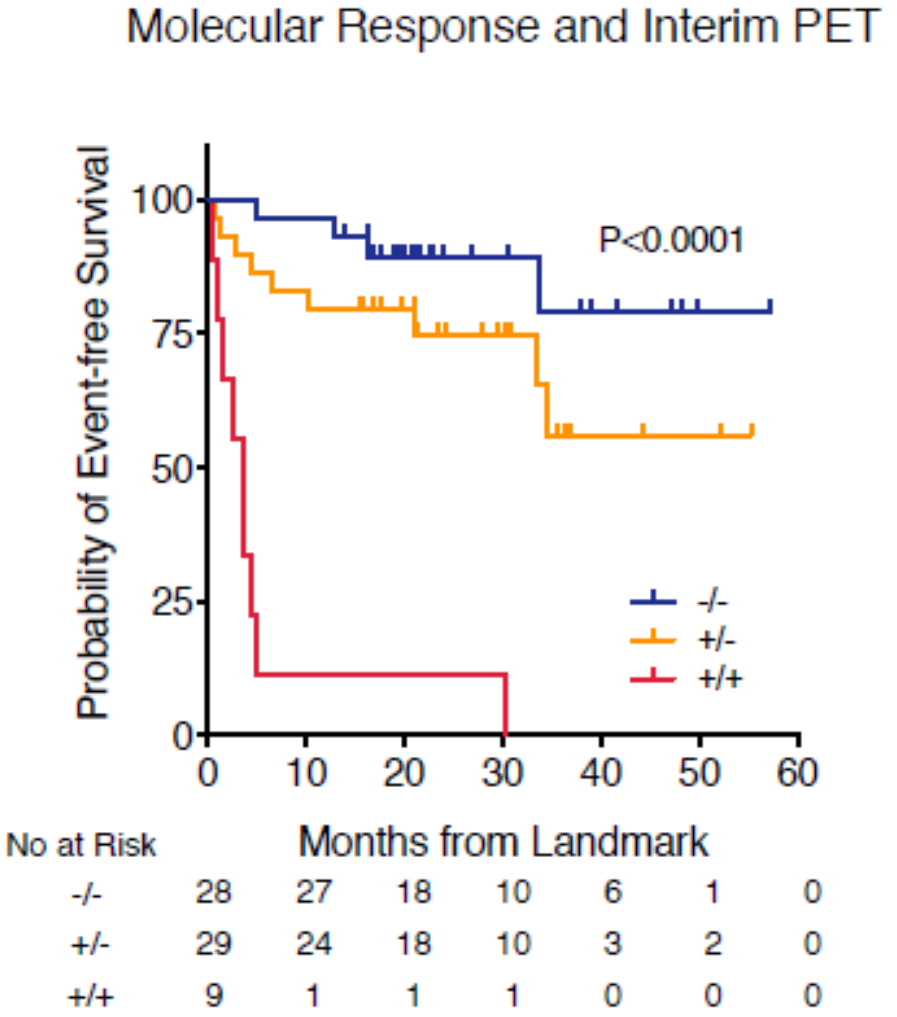


Resolution of the tumor mutation profile



The prognostic value of molecular response is independent of interim imaging





Kurtz, J Clin Oncol 2018

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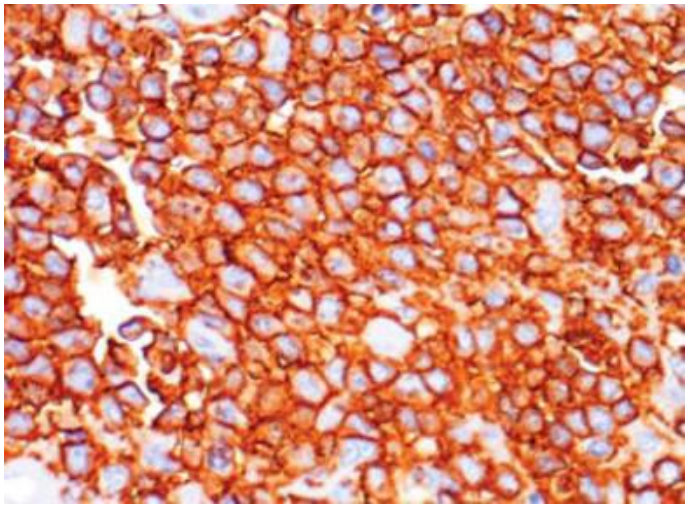
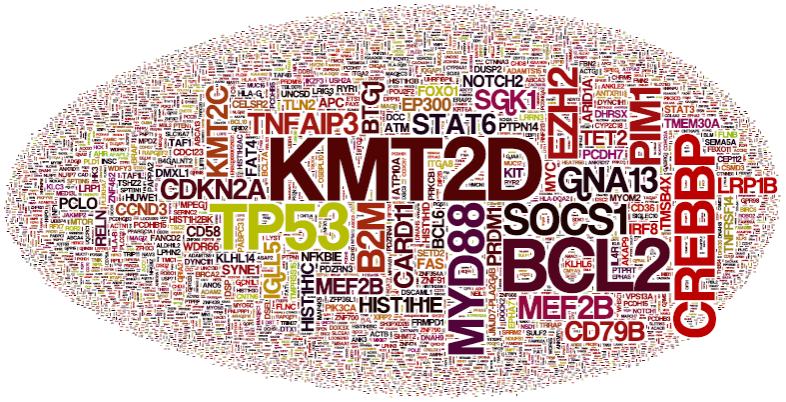
Understanding of the genetics of DLBCL vs cHL

DLBCL

Tumor cells are enriched in the mass

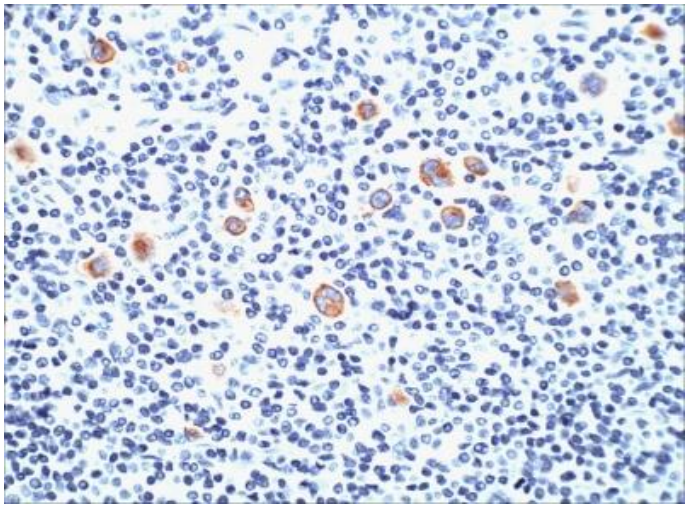


Exome sequencing data from >1000 cases



cHL

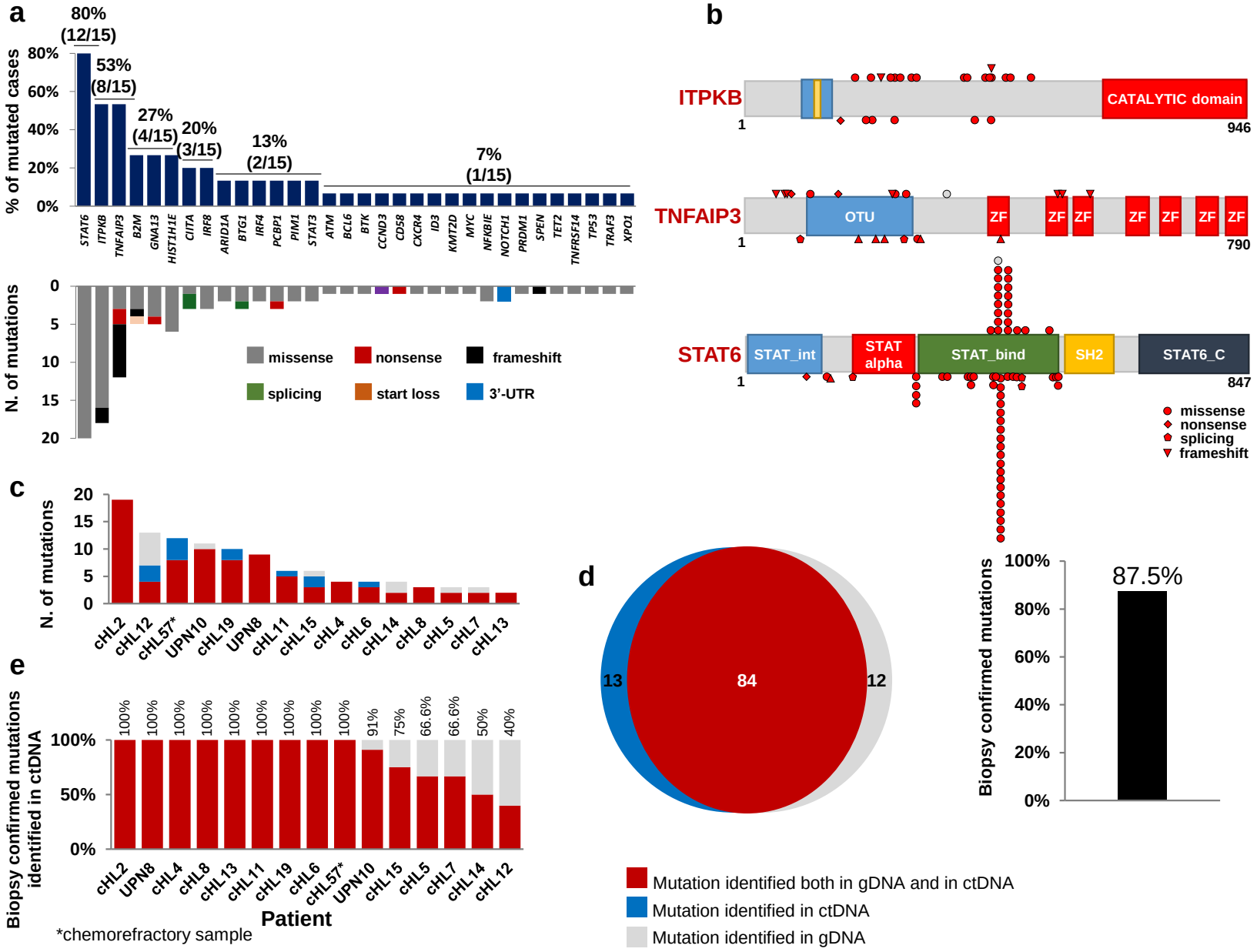
Tumor cells are rare in the mass



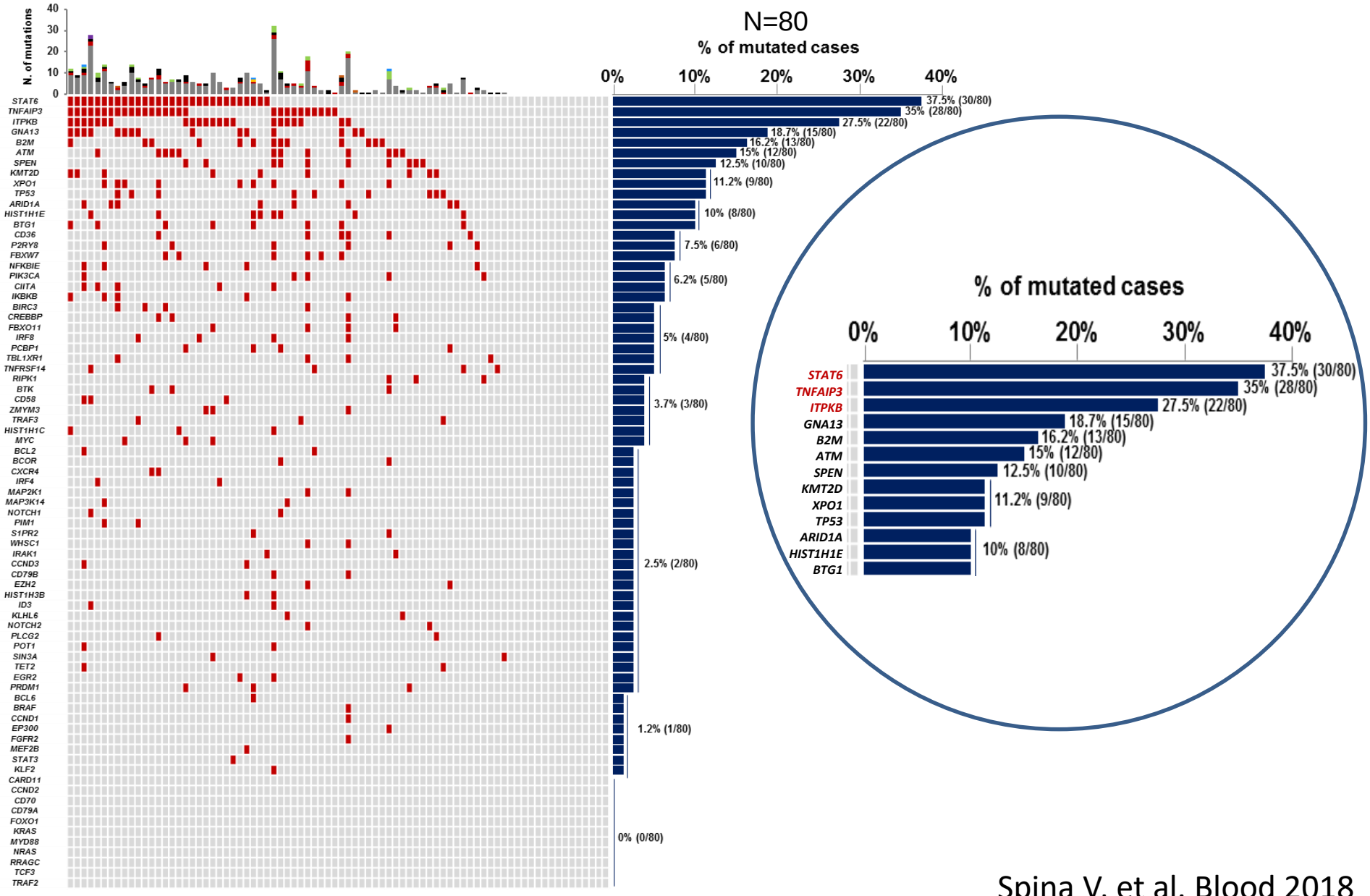
Exome sequencing data from only 10 cases

Reichel J, et al. Blood 2015

ctDNA mirrors the genetics of HRS cells



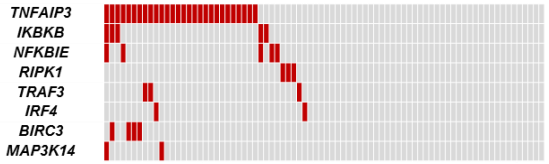
Mutational landscape of newly diagnosed cHL



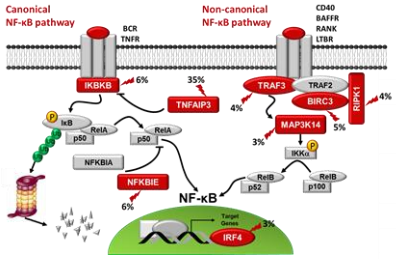
Spina V, et al. Blood 2018

Mutated pathways in newly diagnosed cHL

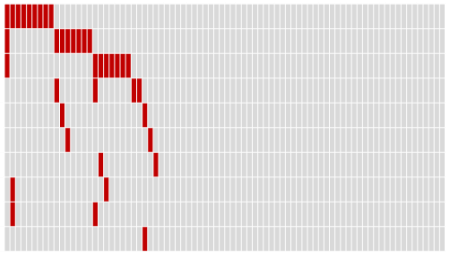
NF-κB



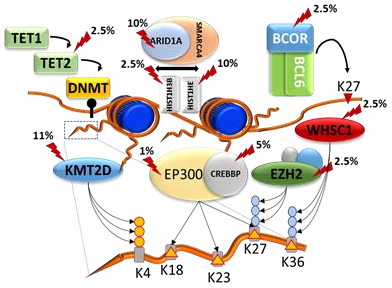
46.2% (37/80)



Epigenetic genes

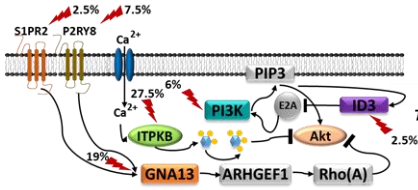


35% (28/80)

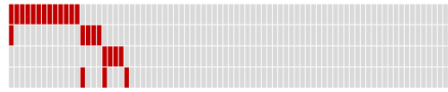


PI3K-AKT

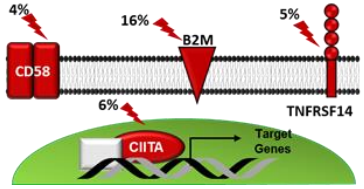
46.2% (37/80)



immune surveillance genes



27.5% (22/80)

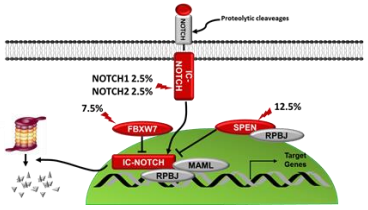


Cytokine signaling

37.5% (30/80)

NOTCH pathway

20% (16/80)



Spina V, et al. Blood 2018

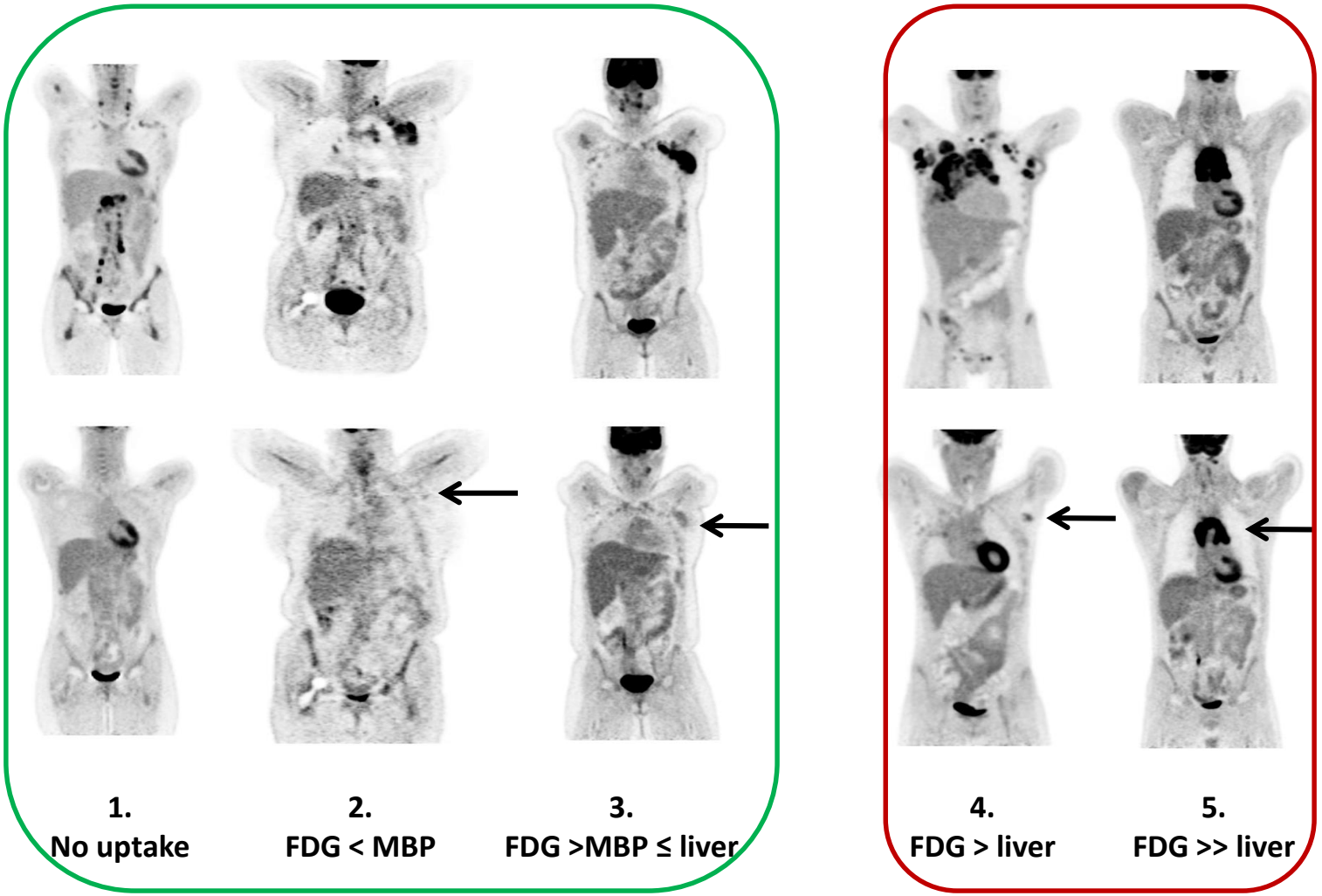
Interim PET/CT accuracy in cHL

False negative rate = 3%

False positive rate = 19%

Baseline PET

Interim PET



1. No uptake

2. FDG < MBP

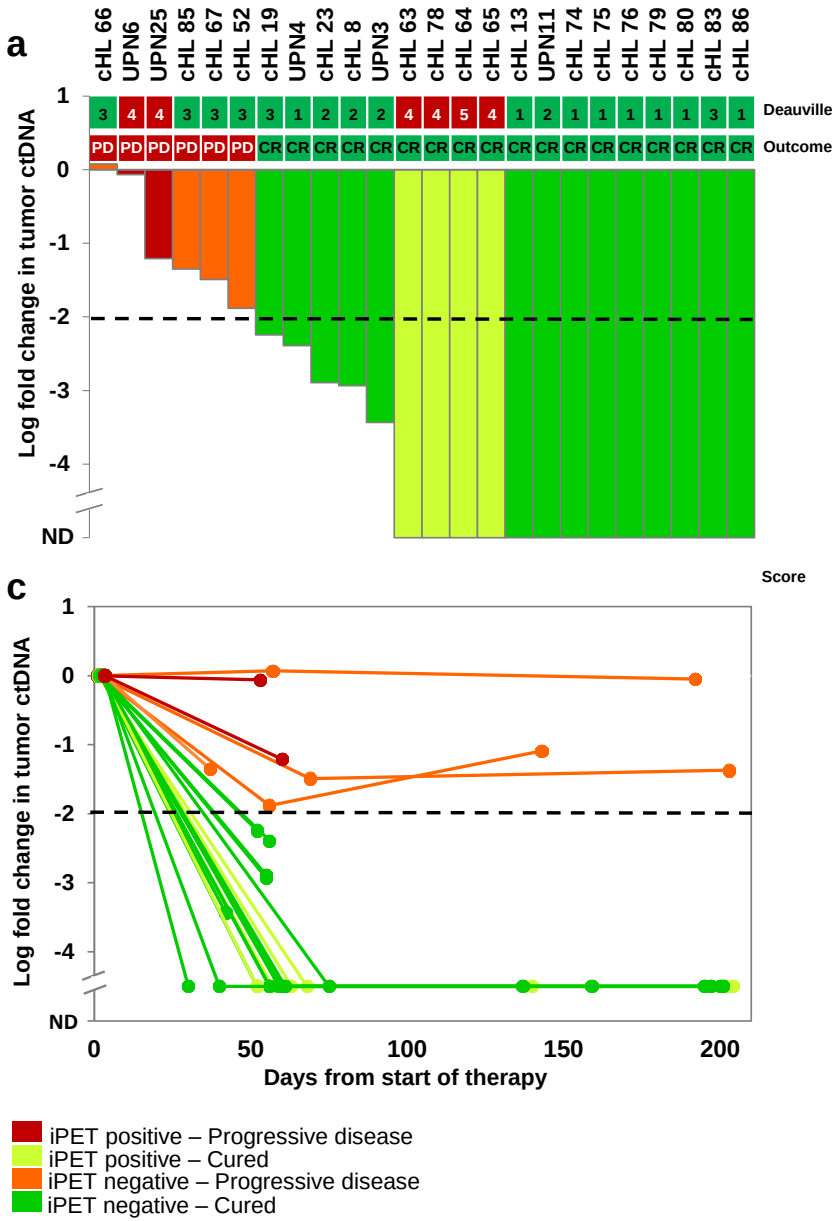
3. FDG >MBP ≤ liver

4. FDG > liver

5. FDG >> liver

Terasawa, et al J Clin Oncol 2009 27:1906-1914

Changes in tumor cfDNA complement iPET

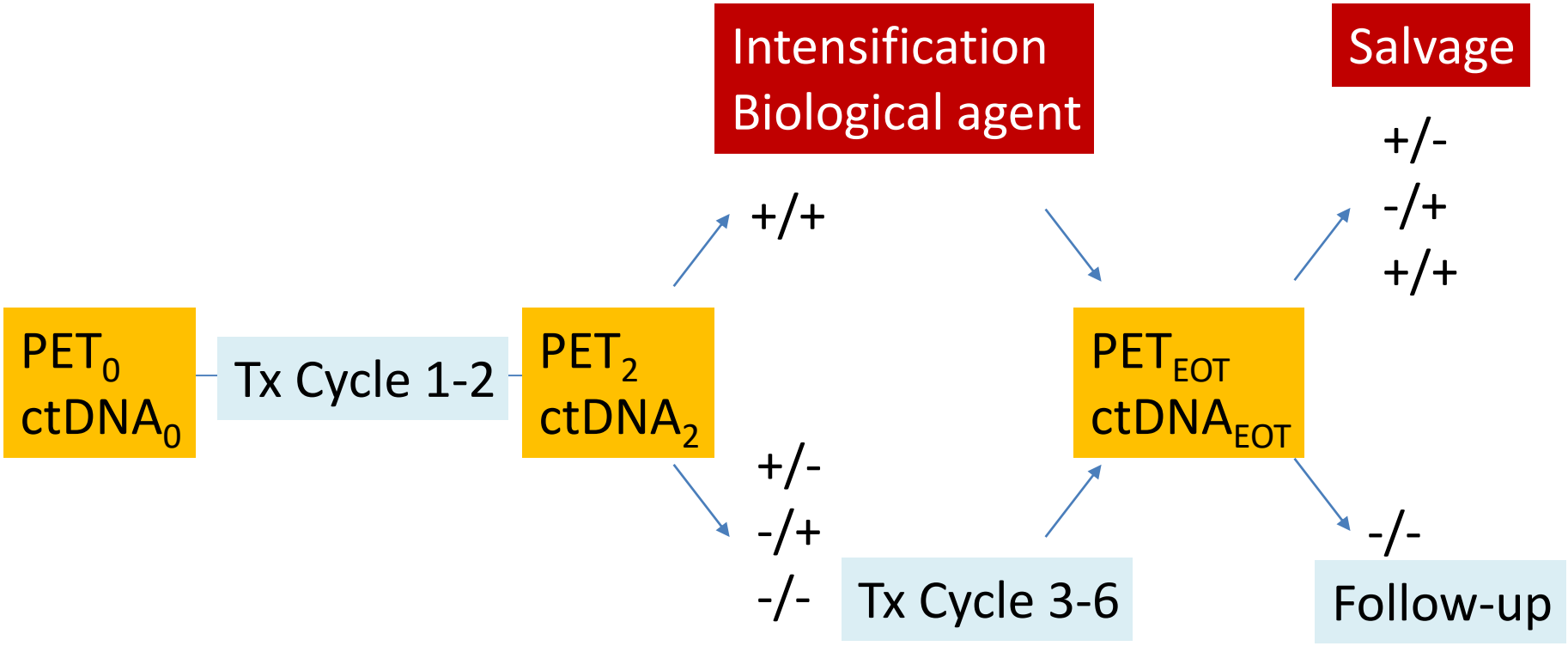


Spina V, et al. Blood 2018

Conclusions

- Molecular markers informing treatment: BCR signaling mutations, EZH2 mutations
- Clonal evolution: mutation resistance monitoring
- Molecular markers informing response to therapy: minimal residual disease monitoring

Clinical trial design incorporating ctDNA assessment



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Gianluca Gaidano

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Fondation Nelia et Amadeo Barletta
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erc European Research Council Scientific Council

Unrestricted research grant from Gilead and Abbvie

SAVE THE DATE: June 18-22, 2019