BIOPSIA LIQUIDA

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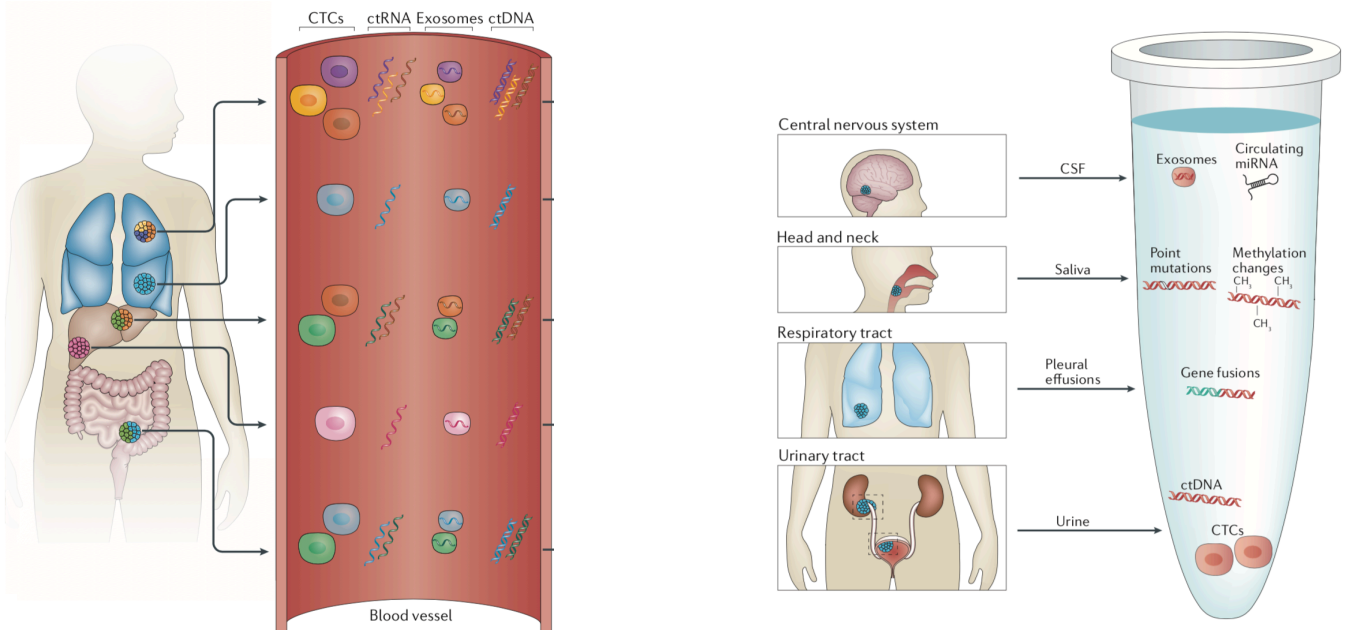
DISCLOSURES: GIANLUCA GAIDANO

- **Advisory boards, Speakers' bureau:** Janssen, Gilead, Abbvie, Roche, Morphosys, Amgen

- Non-financial interests: Chair of EHA Global Outreach Program, Board Member of Società Italiana di Ematologia, Fondazione Italiana Linfomi

The liquid biopsy

- A broad category of minimally invasive tests done on a sample of blood or other biological fluids
- Liquid biopsy can be used to analyze cell-free DNA (cfDNA), cells and vescicles such as exosomes that can originate from different healty tissues and also from cancers



Merker JD, *et al., JCO.* 2018; Siravegna G, *et al., Nat Rev Clin Oncol.* 2017

Liquid Biopsy vs Tissue Biopsy in hematology

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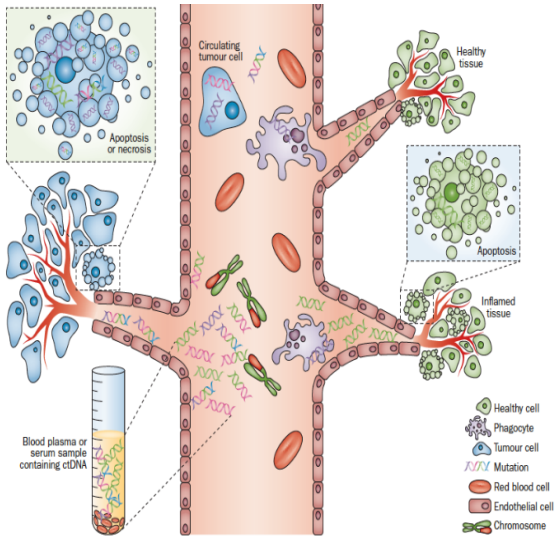
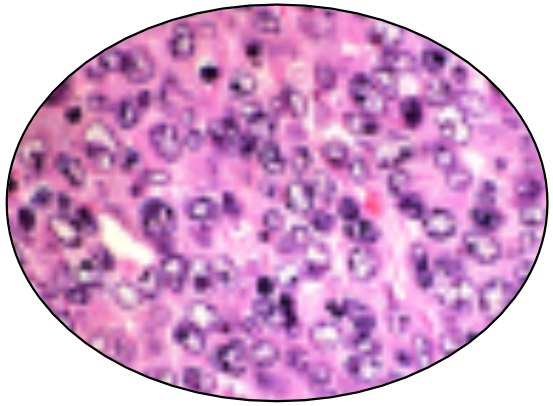
LIQUID biopsy during the clinical course:

- enables assessment of tumor heterogeneity and evaluation of all/most tumor localizations in real-time ("global view")
- allows monitoring of tumor dynamics and of treatment response



TISSUE biopsy during the clinical course:

- may not reflect the whole genetic picture of the disease
- may not be feasible based on patient conditions or tumor accessibility
- is unethical/impractical for *periodic* monitoring of disease progression/recurrence

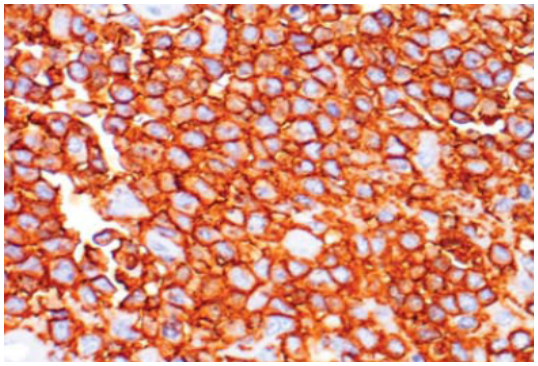


Crowley E, et al., *Nat Rev Clin Oncol*. 2013

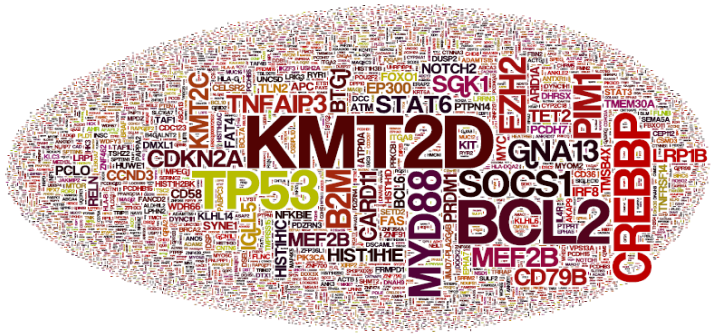
Diffuse large B-cell lymphoma vs Hodgkin lymphoma

DLBCL

Tumor cells are enriched in the mass

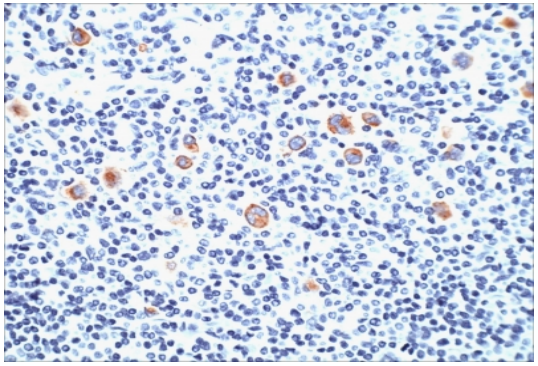


Exome sequencing data from >1000 cases



cHL

Tumor cells are rare in the mass



Exome sequencing data from only 10 cases

Pasqualucci L, et al., *Semin Hematol* 2015; Reichel J, et al., *Blood* 2015

Applications of ctDNA in DLBCL

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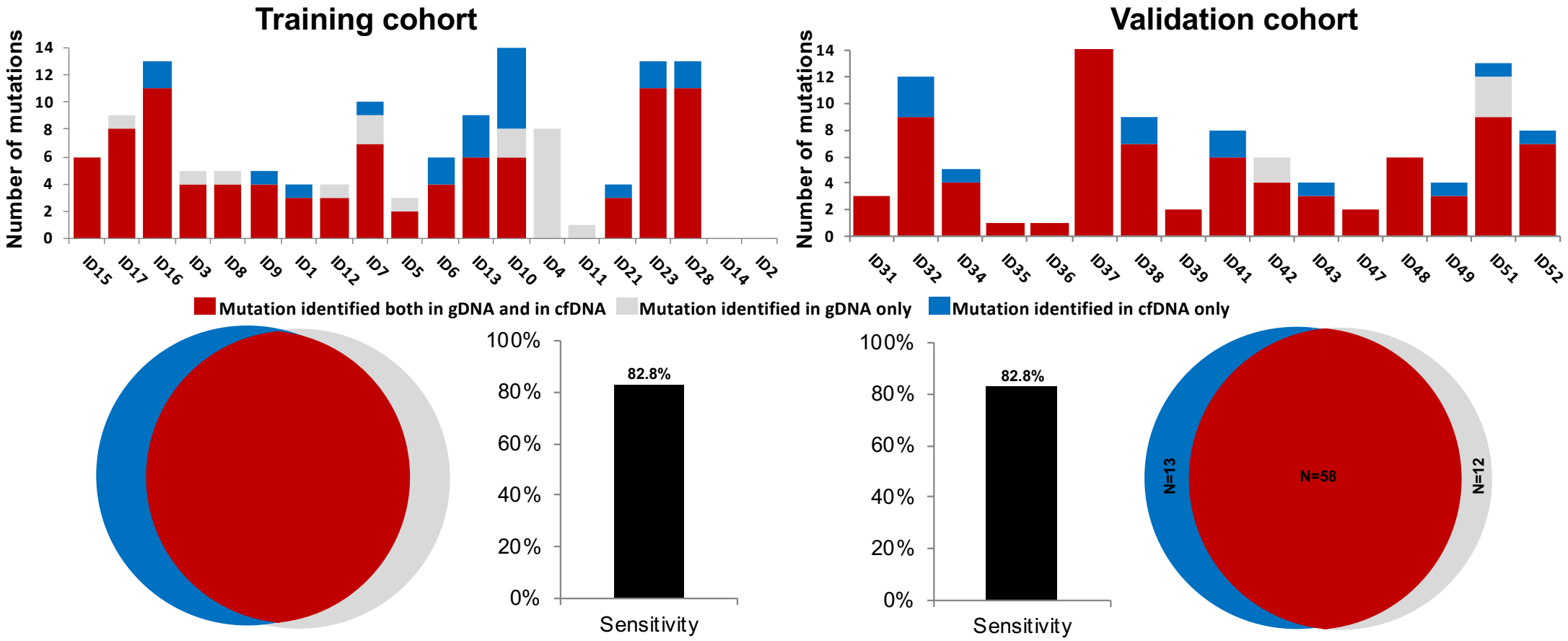
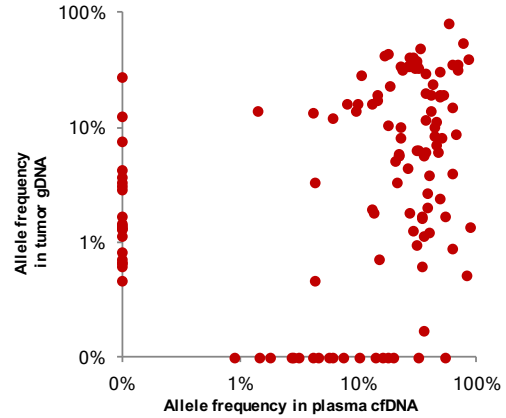
- **Genotyping**
- Clonal evolution
- Outcome prediction and MRD monitoring

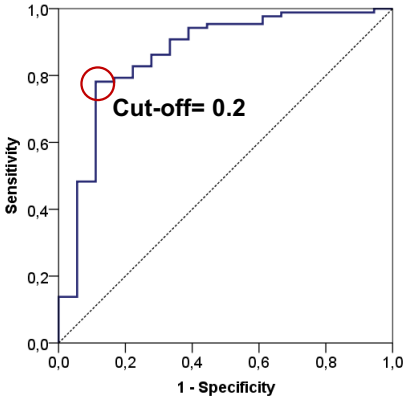
Plasma cfDNA genotyping vs tumor gDNA genotyping

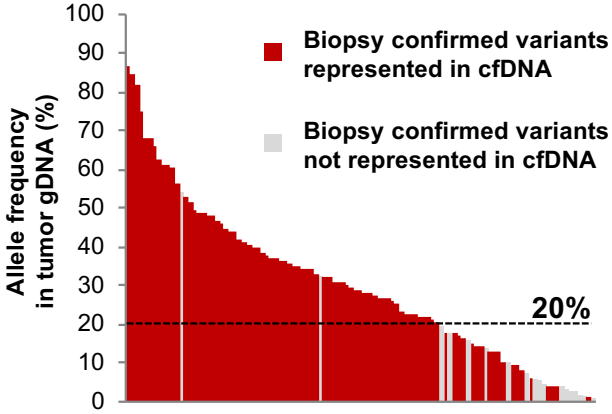


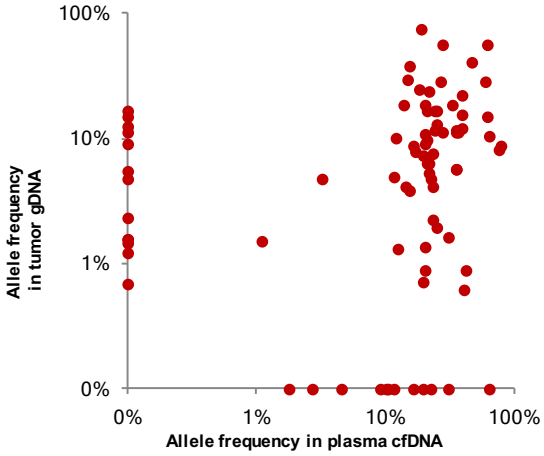
Rossi D, et al., *Blood*, 2017

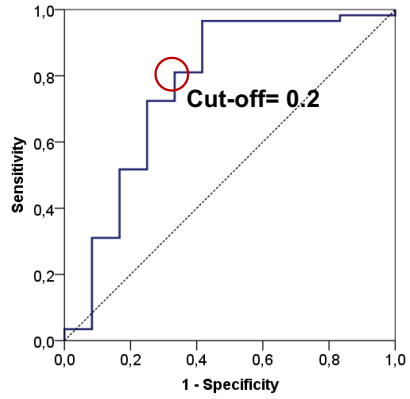
cfDNA genotyping has an optimal sensitivity for mutations represented in >20% of the tumor alleles

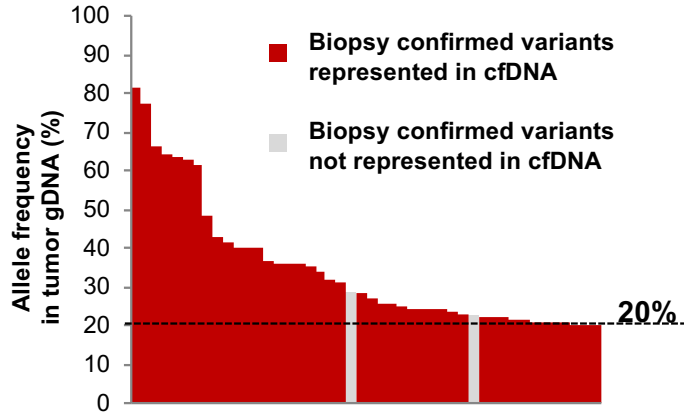






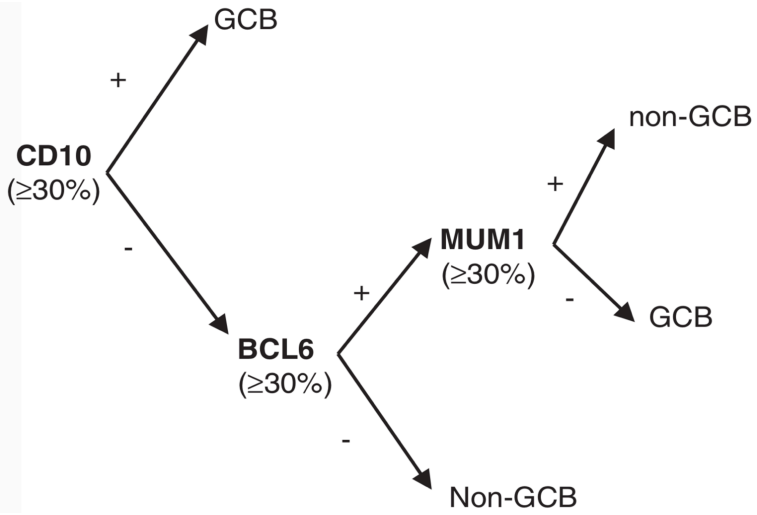


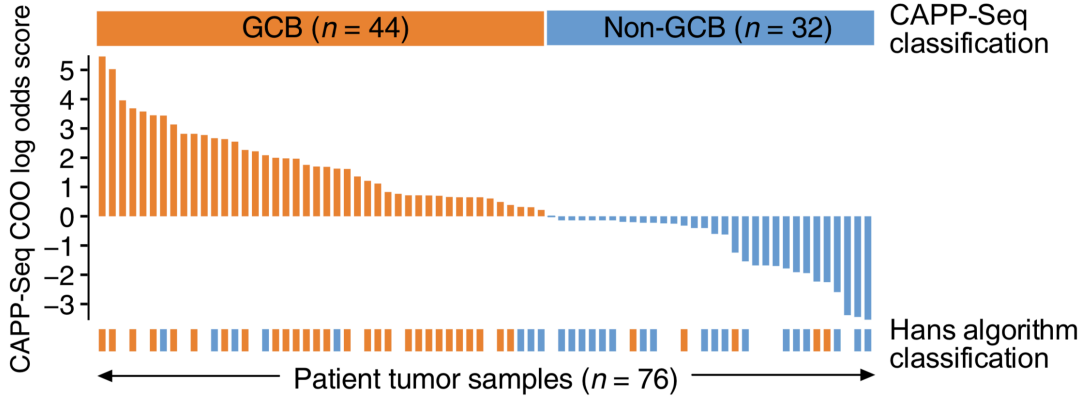


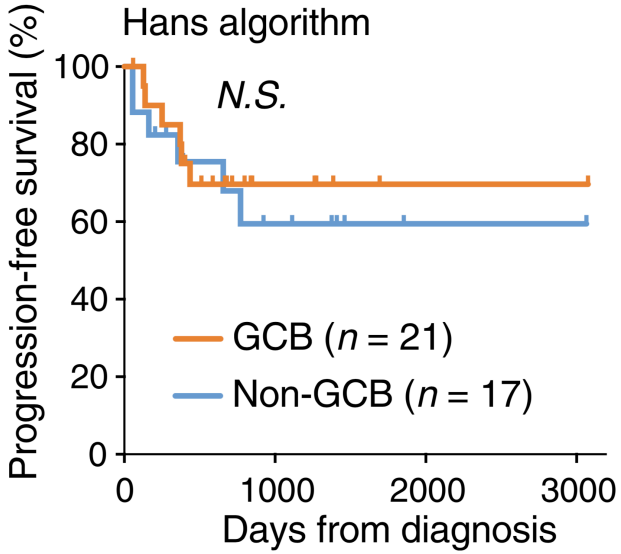


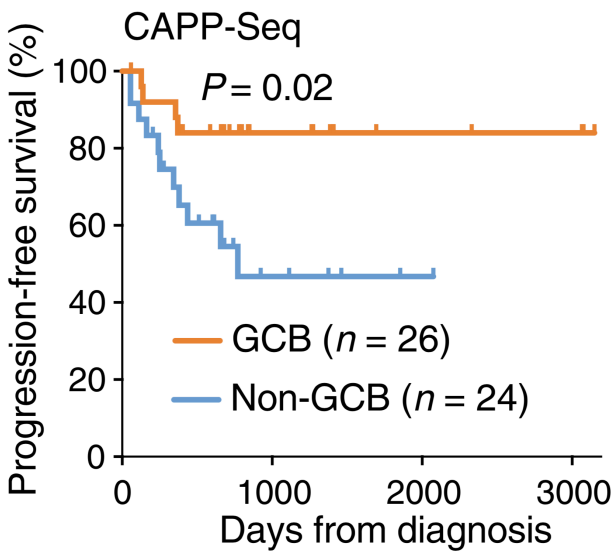
Rossi D et al., *Blood*, 2017

COO classification with cfDNA









Hans C et al., *Blood*, 2004; Scherer F et al., *Sci. Transl. Med* 2016

Applications of ctDNA in DLBCL

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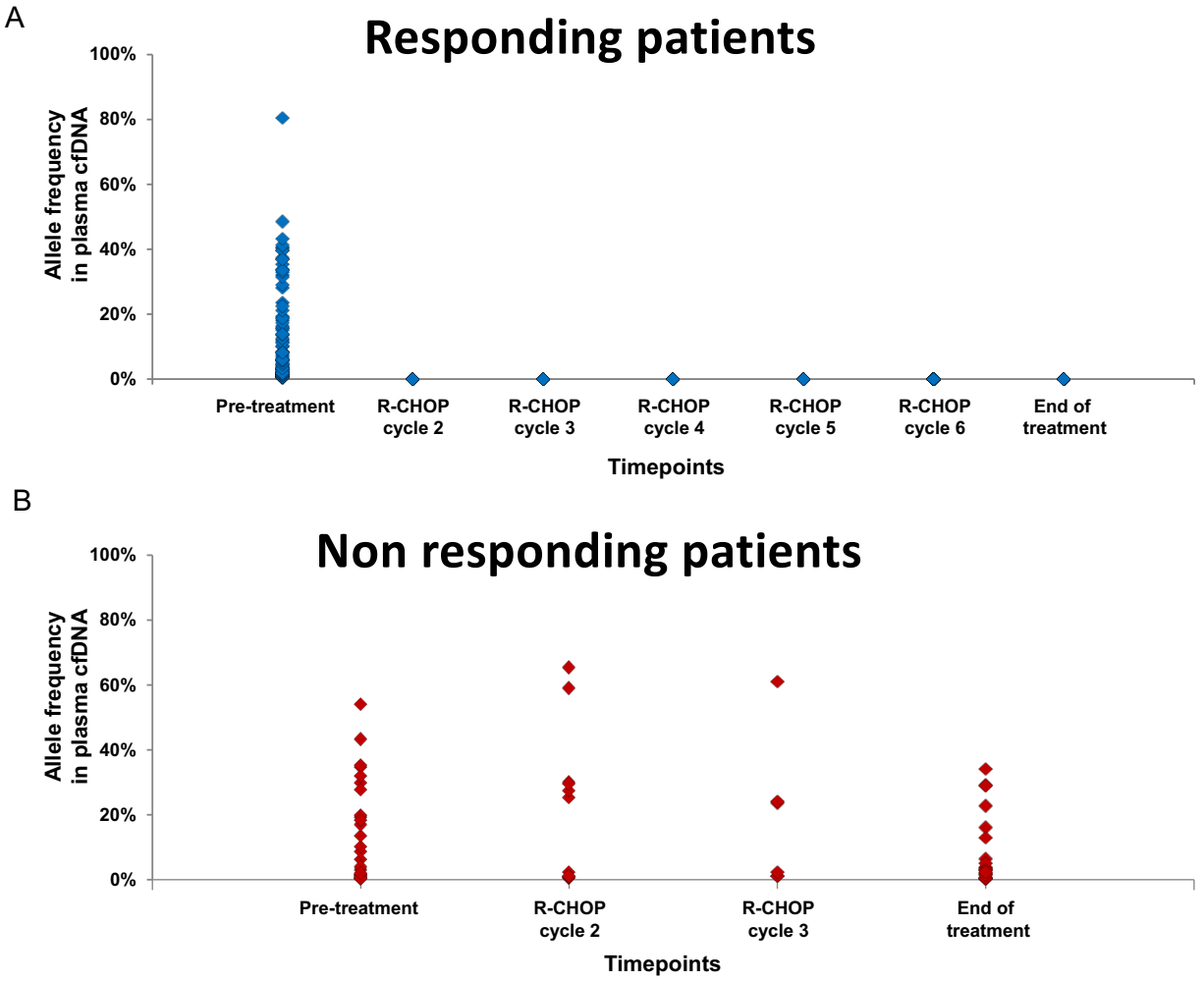
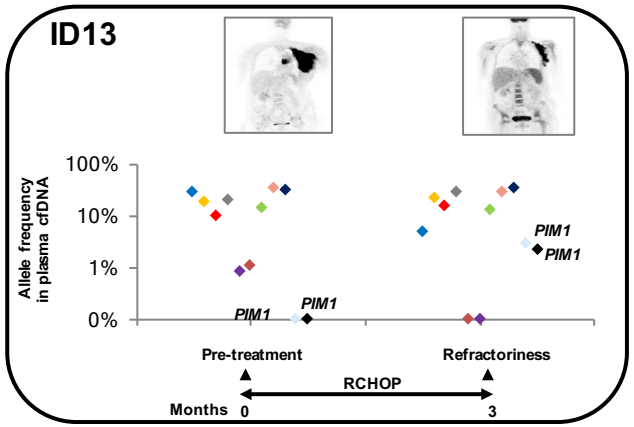
- Genotyping
- **Clonal evolution**
- Outcome prediction and MRD monitoring

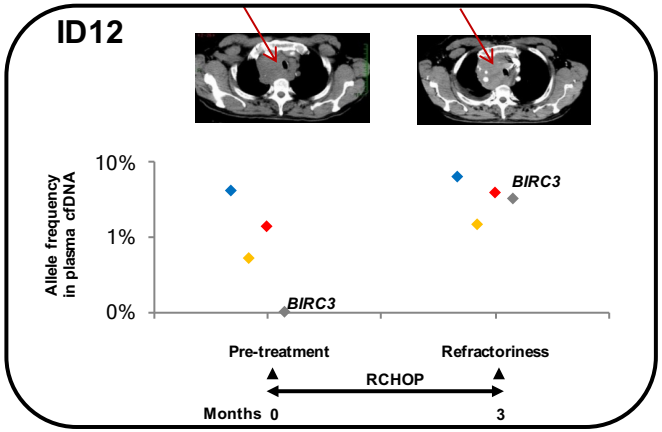
Mutations are cleared from plasma cfDNA in responding DLBCL patients but not in refractory patients treated with R-CHOP

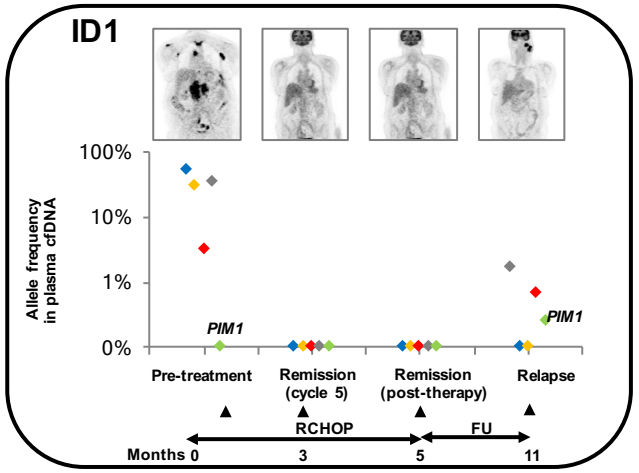


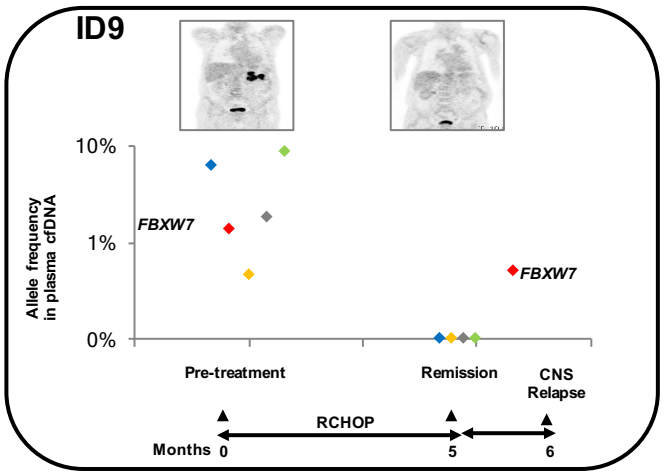
Rossi D et al., *Blood*, 2017

Longitudinal cfDNA genotyping allows real-time monitoring of clonal evolution of DLBCL









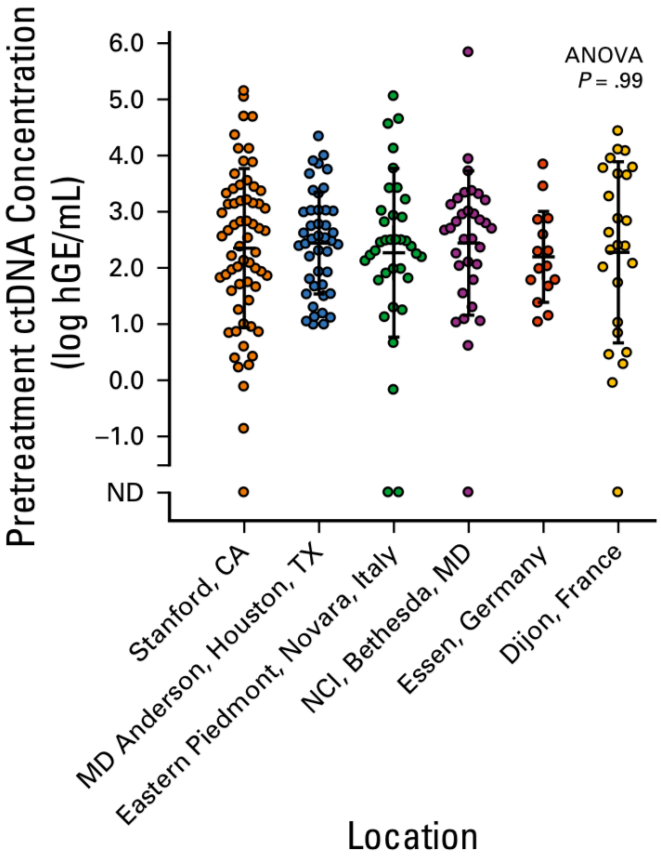
Rossi D, et al., *Blood*, 2017

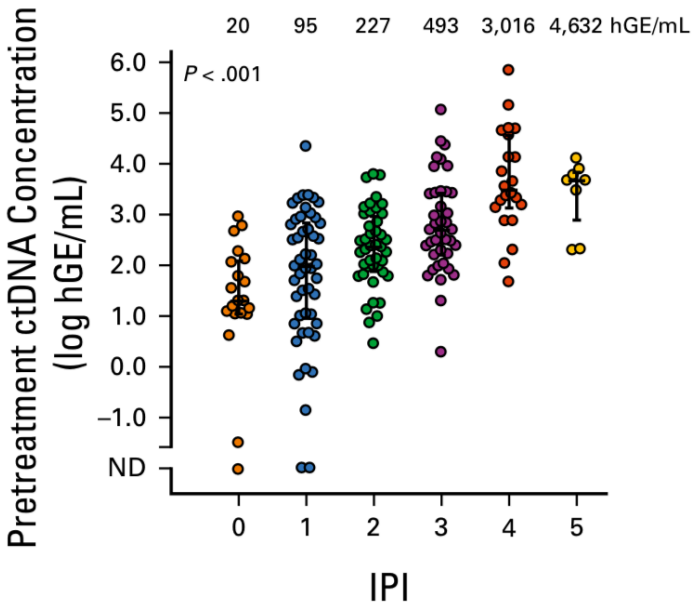
Applications of ctDNA in DLBCL

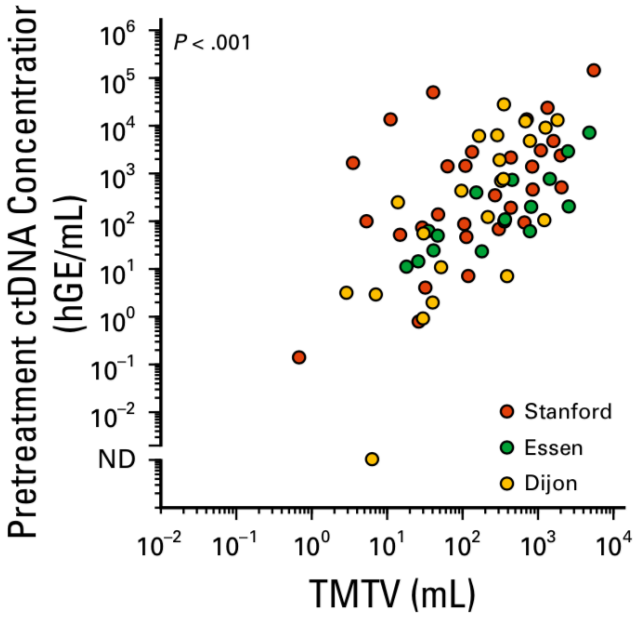
UNIVERSITÀ DEL PIEMONTE ORIENTALE

- Genotyping
- Clonal evolution
- **Outcome prediction and MRD monitoring**

Correlation of ctDNA with IPI and Total Metabolic Tumor Volume (TMTV) in DLBCL

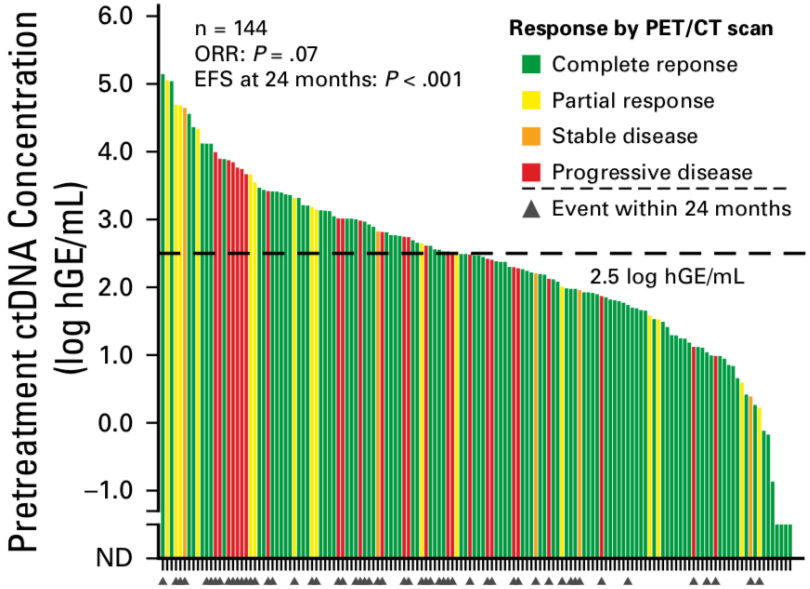


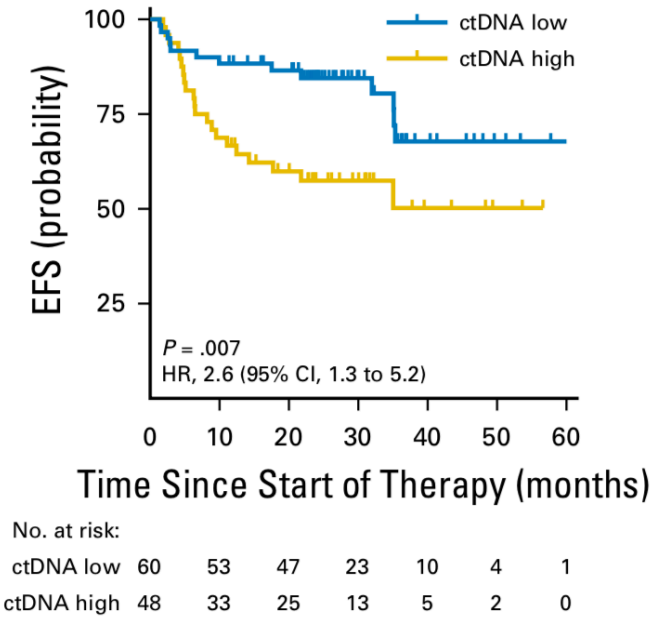




Kurtz DM, et al., *JCO*, 2018

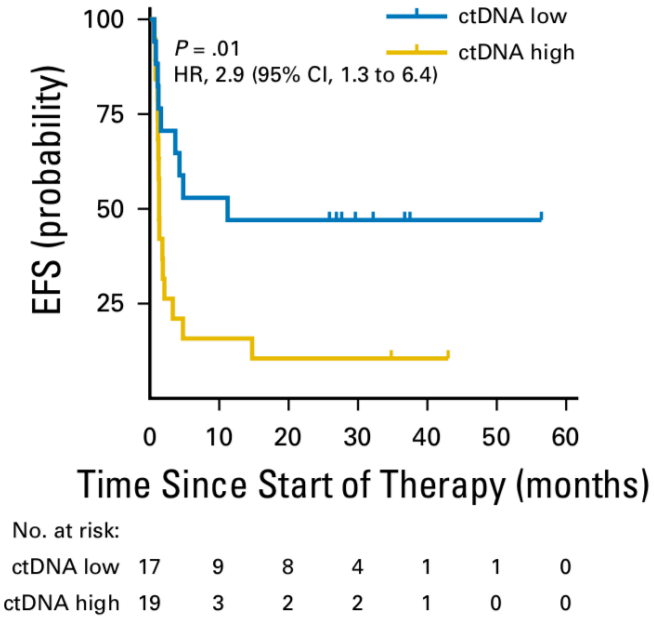
Pretreatment ctDNA is a robust biomarker in DLBCL





No. at risk:

ctDNA low	60	53	47	23	10	4	1
ctDNA high	48	33	25	13	5	2	0

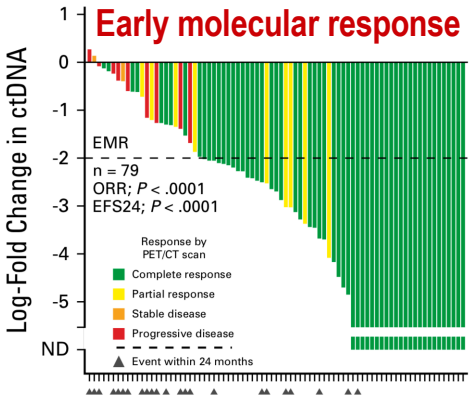


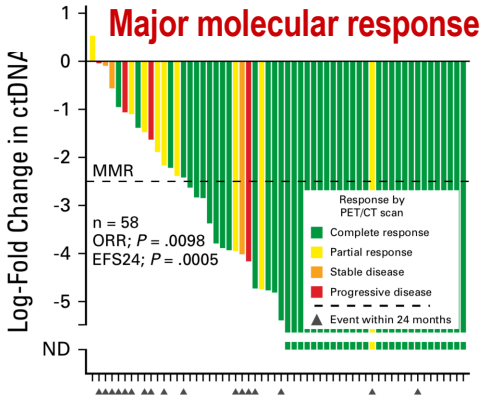
No. at risk:

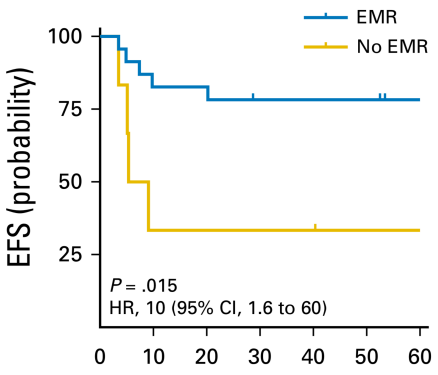
ctDNA low	17	9	8	4	1	1	0
ctDNA high	19	3	2	2	1	0	0

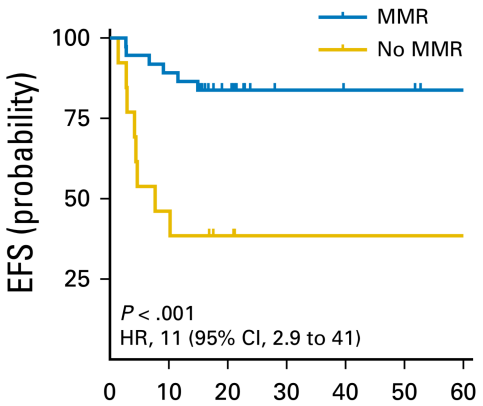
Kurtz DM et al., *JCO*, 2018

ctDNA monitoring during therapy predict outcomes in DLBCL





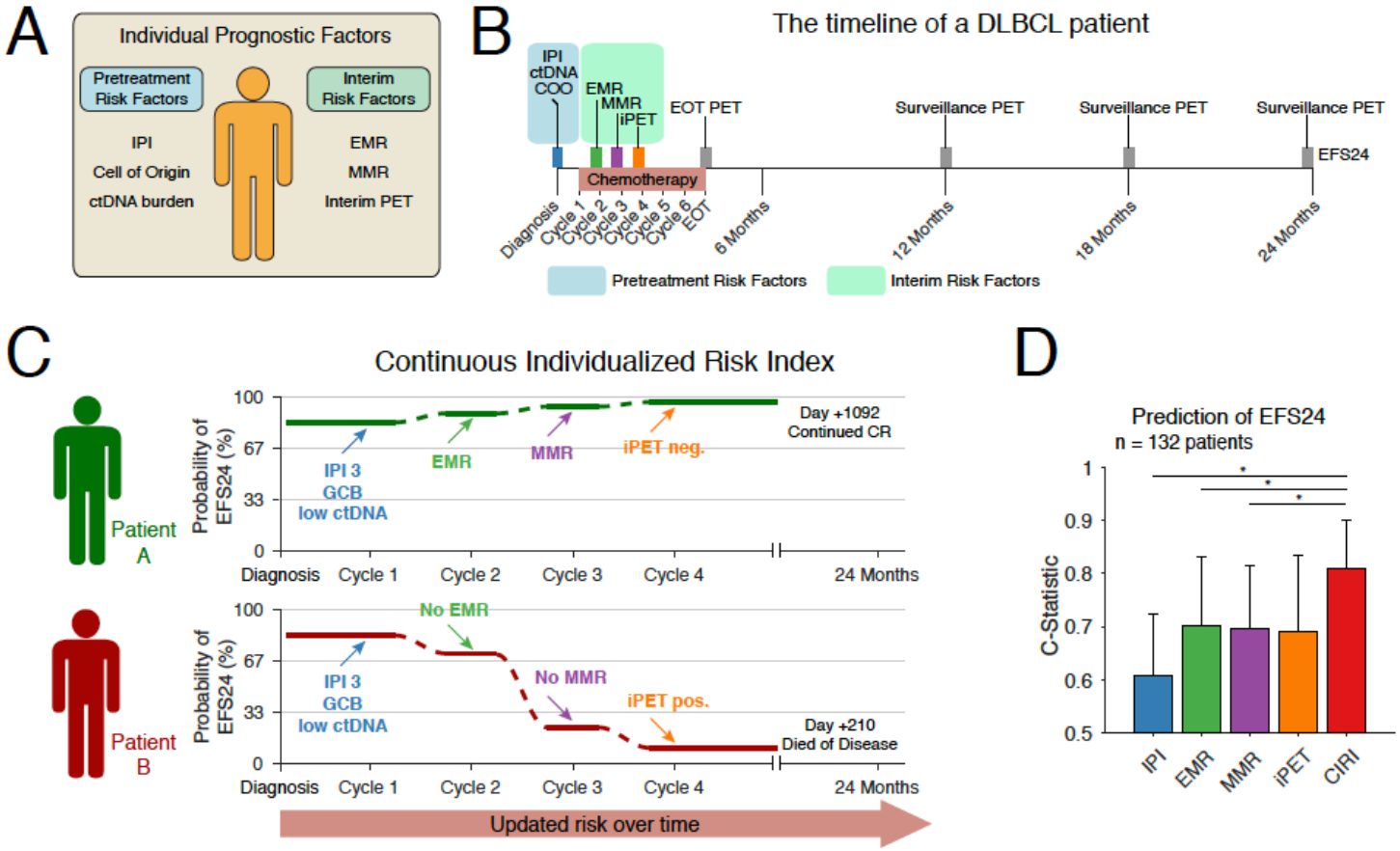




Parameter	Univariable HR (95% CI)	Univariable *P*	Multivariable HR (95% CI)	Multivariable *P*
EFS				
IPI (0 to 5)	1.21 (0.87 to 1.69)	.25	0.93 (0.63 to 1.37)	.71
Pretreatment ctDNA (low *v* high)	2.77 (1.08 to 7.13)	.034*	2.97 (0.92 to 9.62)	.070
Molecular response†	5.93 (2.52 to 13.95)	< .001*	8.58 (3.3 to 22.32)	< .001*
Interim PET (positive *v* negative)	3.74 (1.46 to 9.57)	.006*	3.45 (1.27 to 9.34)	.015*
OS				
IPI (0 to 5)	1.36 (0.82 to 2.23)	.23	1.14 (0.63 to 2.25)	.670
Pretreatment ctDNA (low *v* high)	3.12 (0.65 to 15.05)	.16	1.13 (0.16 to 8.21)	.899
Molecular response†	5.27 (1.41 to 19.78)	.014*	4.15 (1.17 to 15.57)	.029*
Interim PET (positive *v* negative)	22.35 (2.83 to 2868)	< .001*	16.87 (1.96 to 2214)	.005*

Kurtz DM, et al., *JCO*, 2018

CIRI integrated model in DLBCL



Kurtz et al, Cell 2019

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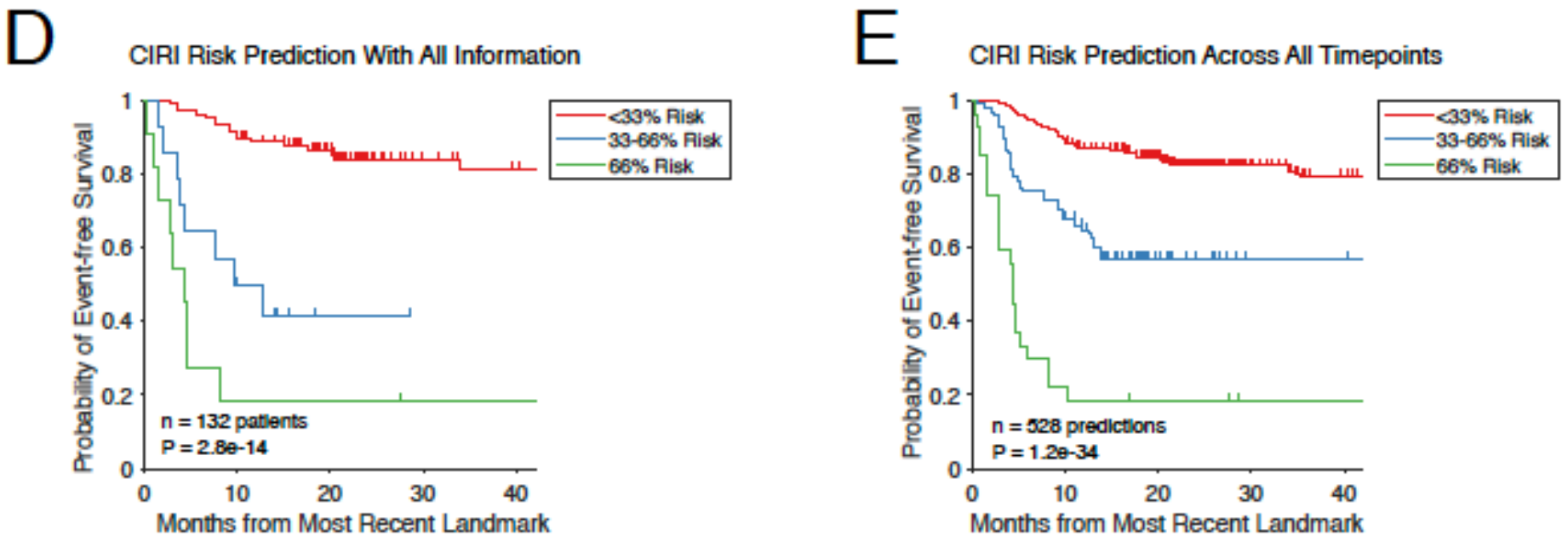
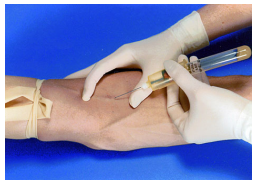
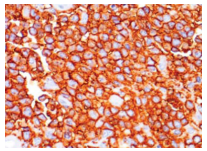


Kurtz et al, Cell 2019

LymphoSIGHT™ platform

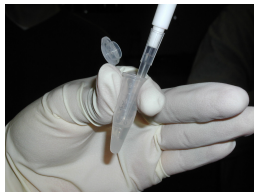
1) Collect 10cc peripheral blood

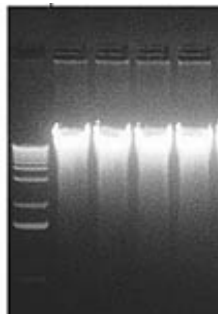




Serum or FFPE biopsy

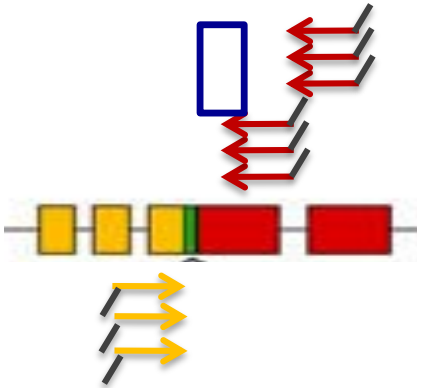
2) Extract DNA





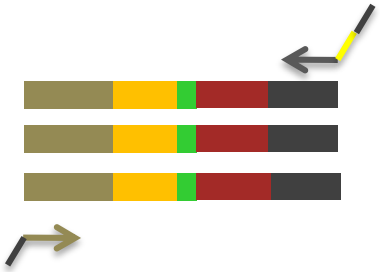
Genomic DNA

3) Amplify VDJ with multiplex PCR



PCR amplicons

4) Prepare for sequencing with common PCR



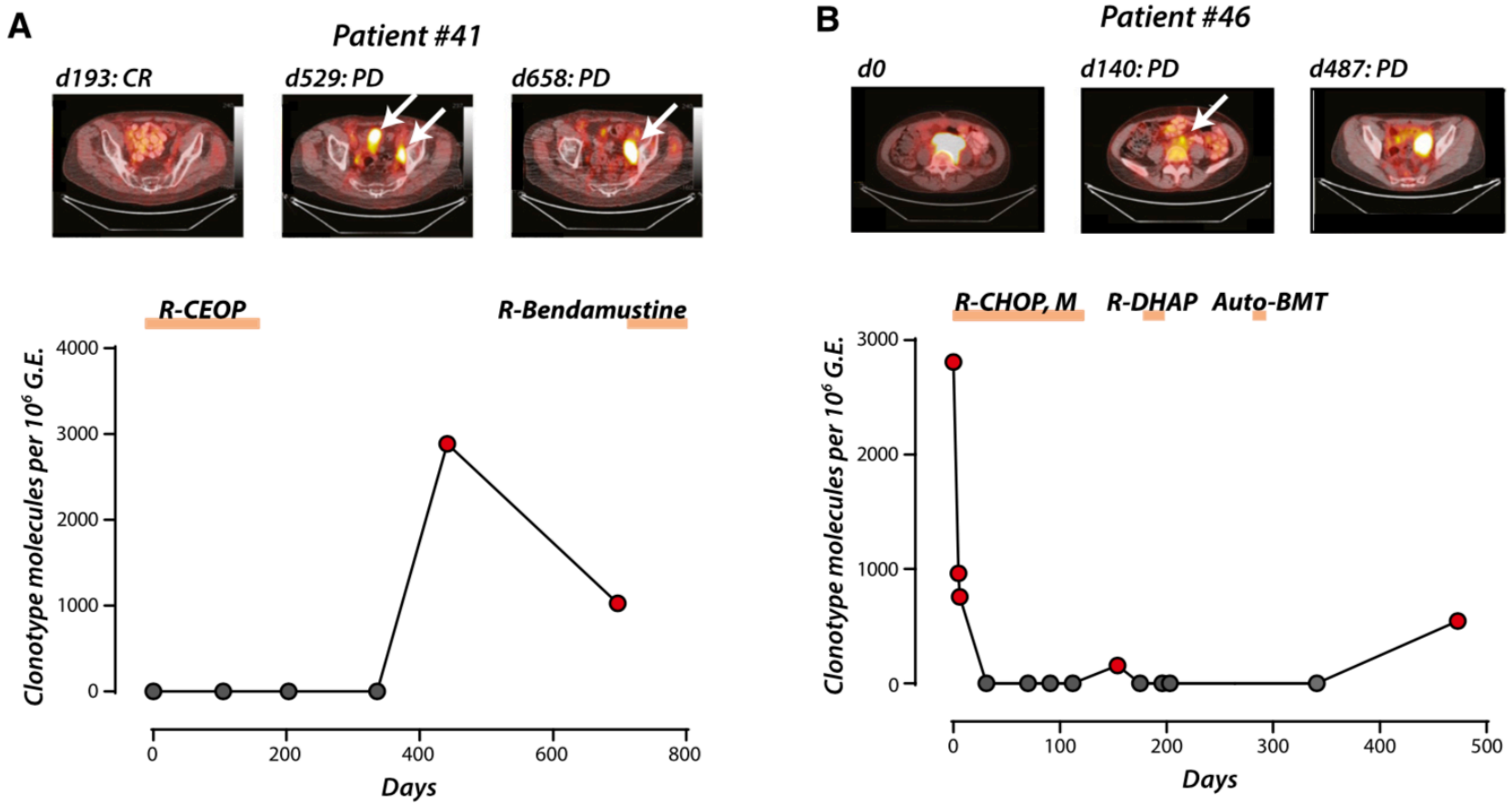
Sequencing library

5) Sequence ~1M 100bp reads

```
CTGGCCCCAGTAGTCATACCAACTAGCG
TTGGCCCCAGAAATCAAGACCATCTAAA
ACGGCCCCAGAGATCGAAGTACCAGTGT
TTGGCCCCAGACGTCCATATTGTAGTAG
CTGGCCCCAGAAGTCAGACCGGCTAACA
```

Sequence data

Noninvasive monitoring of DLBCL by immunoglobulin high-throughput sequencing

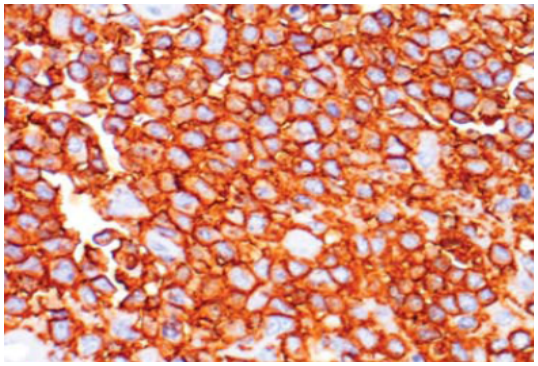


Kurtz DM et al., *Blood* 2015

Diffuse large B-cell lymphoma vs Hodgkin lymphoma

DLBCL

Tumor cells are enriched in the mass



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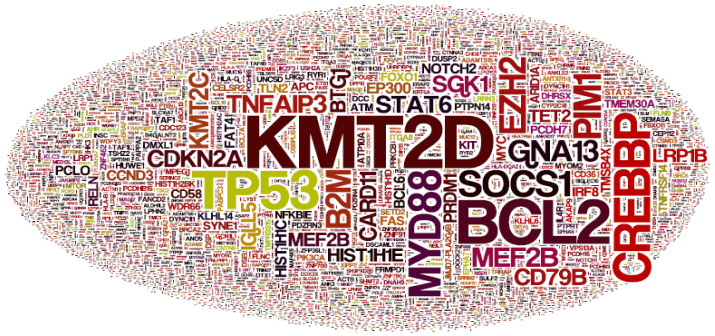

cHL

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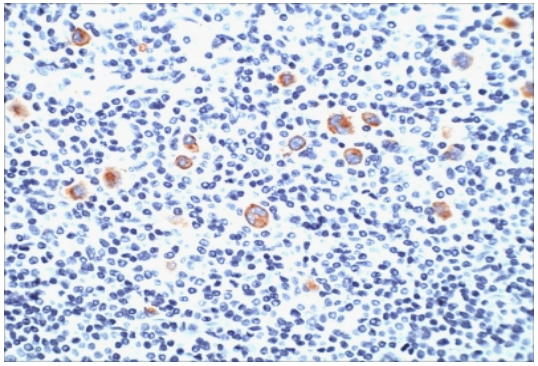

Exome sequencing data from only 10 cases

Pasqualucci L, et al., *Semin Hematol* 2015; Reichel J, et al., *Blood* 2015

Applications of ctDNA in cHL

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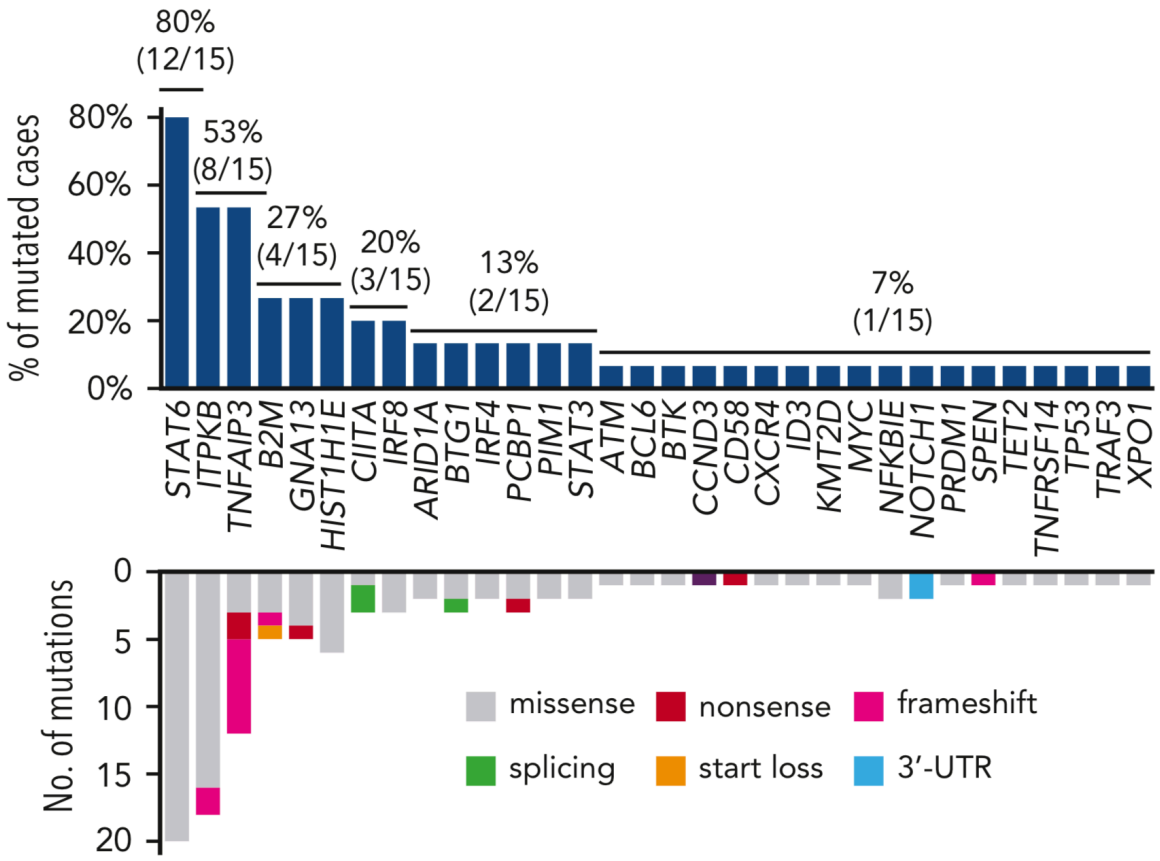
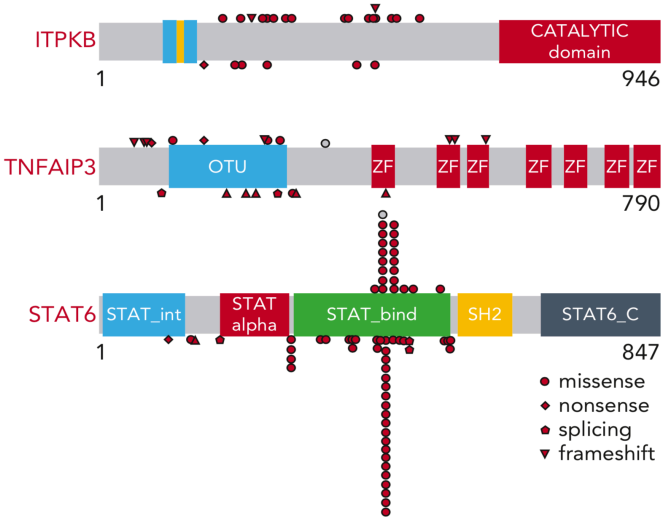
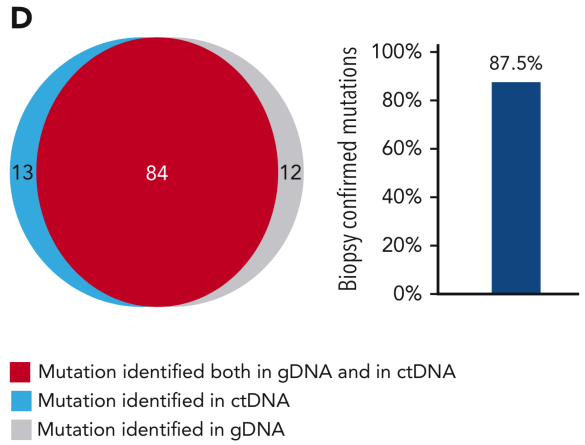
- Clonal evolution
- Outcome prediction and MRD monitoring

The liquid biopsy mirrors the genetics of cHL

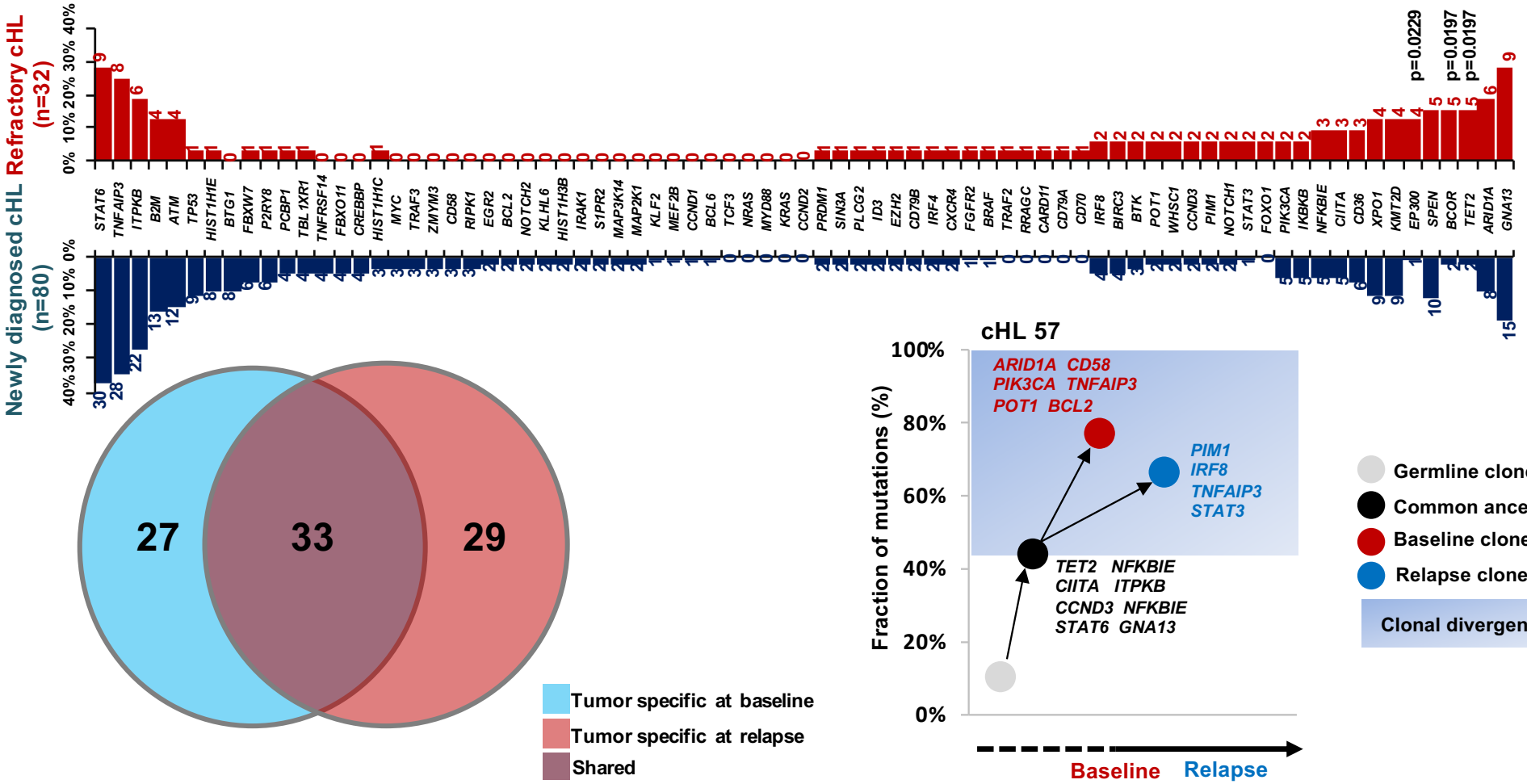






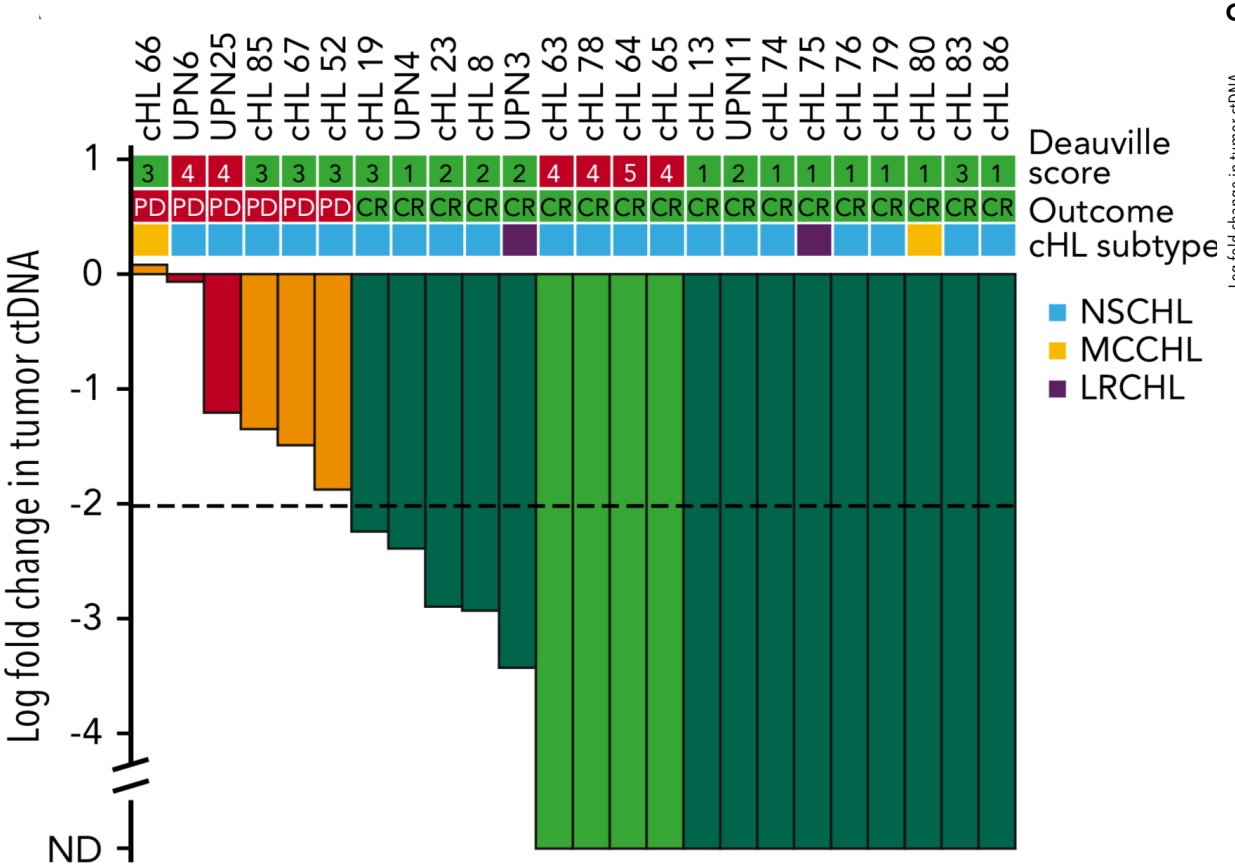
Spina V et al., *Blood*, 2018

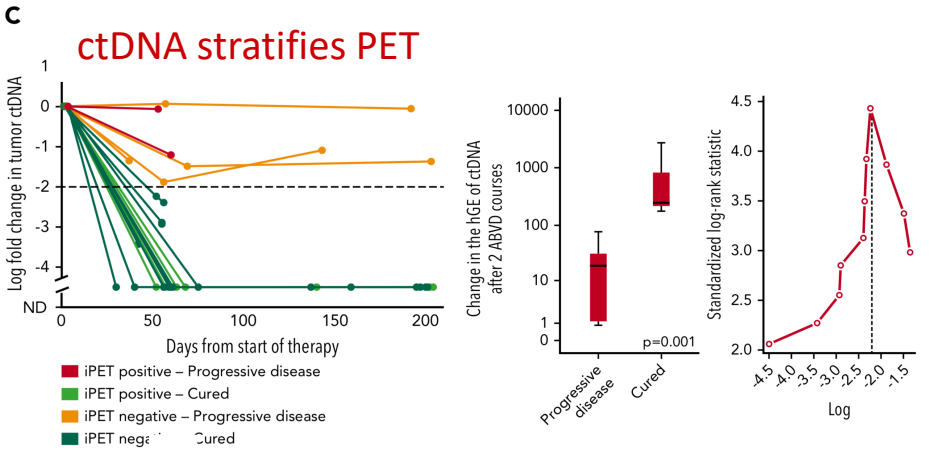
Non invasive monitoring of clonal evolution in refractory cHL

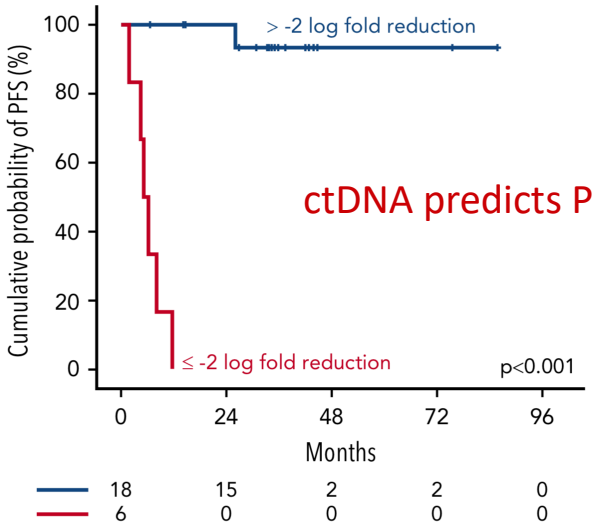


Spina V et al., *Blood*, 2018

Changes in tumor cfDNA complement iPET in cHL







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Conclusions *(i)*: Genotyping and clonal evolution

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- ctDNA is as accurate as genotyping of the diagnostic biopsy to detect somatic mutations both in DLBCL and cHL
- ctDNA genotyping allows the identification of mutations that are otherwise absent in the tissue biopsy conceivably because restricted to clones that are anatomically distant from the biopsy site
- Liquid biopsy may provide a real-time and non-invasive approach to track clonal evolution and emergence of treatment resistant clones in lymphoma
- In the perspective of “precision medicine”, liquid biopsy may allow dynamic monitoring and targeting of DLBCL and cHL

Conclusions *(ii)*: Outcome prediction and MRD monitorig

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- Changes in ctDNA correlate with outcome in DLBCL treated with R-CHOP and in cHL treated with ABVD
- In cHL treated with ABVD, ctDNA MRD analysis may be useful to complement and refine the prognostic value of iPET
- A comprehensive comparison between MRD monitoring by IgH analysis and by CAPP-seq genotyping is needed

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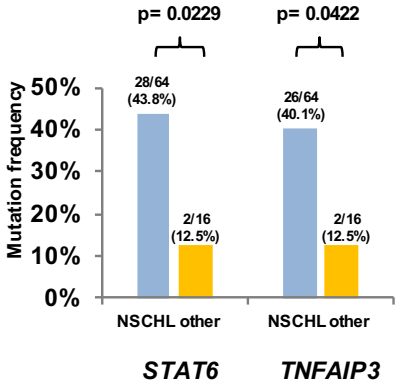
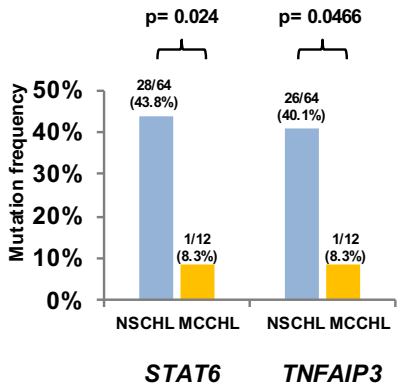
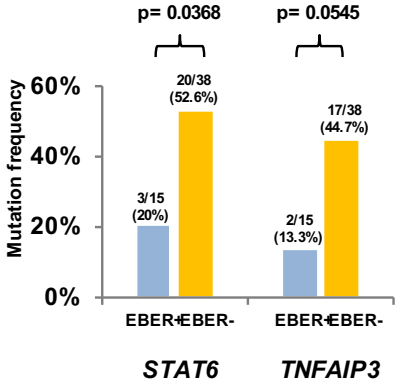
AIRC

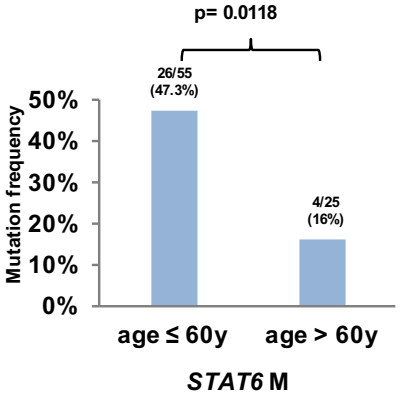
5x1000
x AIRC = RICERCA

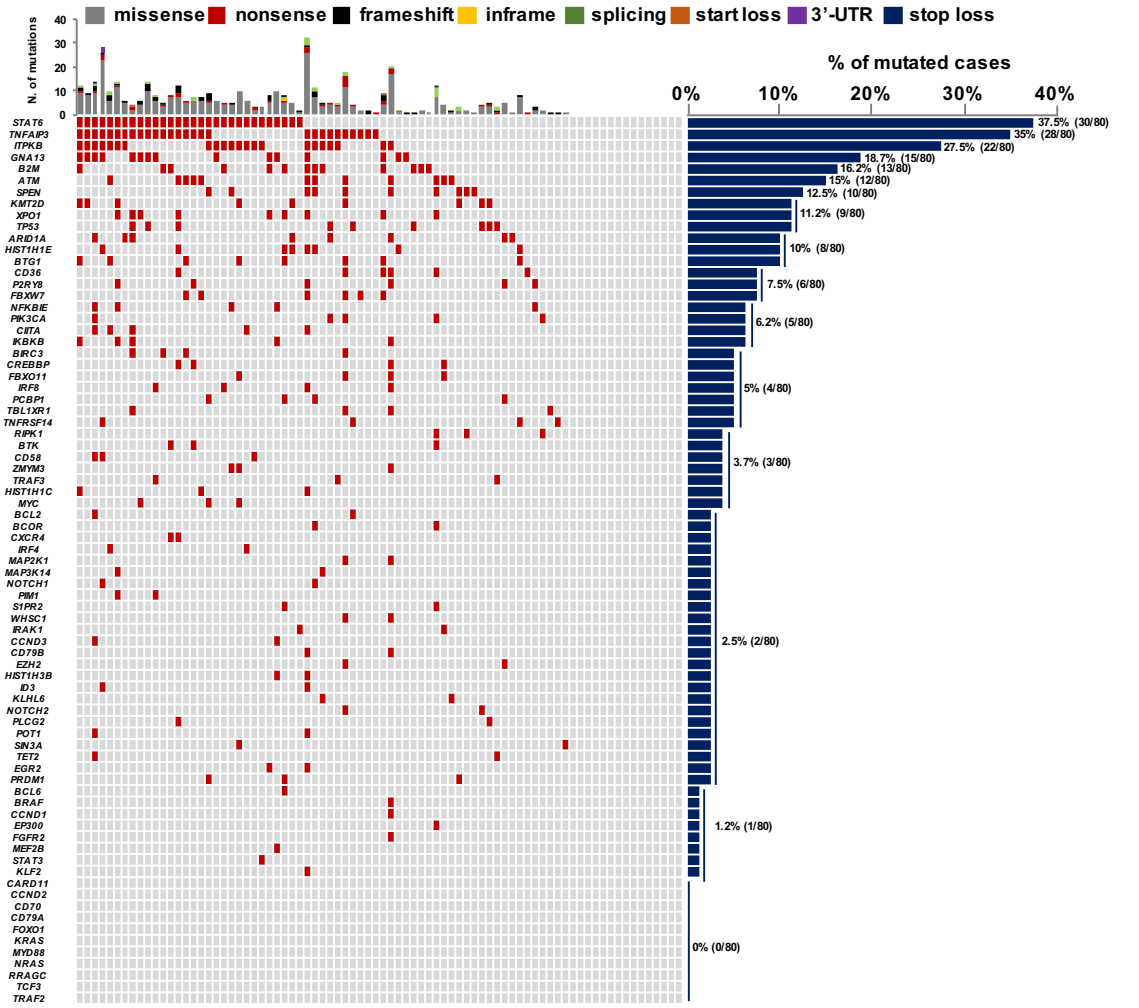
Mutational landscape of newly diagnosed cHL











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“One size fits all” versus Precision Medicine