UPO
UNIVERSITÀ DEL PIEMONTE ORIENTALE

La biopsia liquida nei linfomi

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Disclosures

Abbvie (Advisory Board, Speakers' Bureau)

Astra-Zeneca (Advisory Board)

Beigene (Advisory Board)

Incyte (Advisory Board)

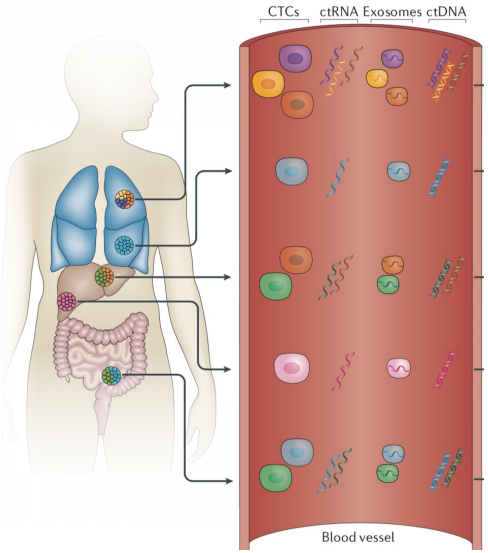
Janssen (Advisory Board, Speakers' Bureau)

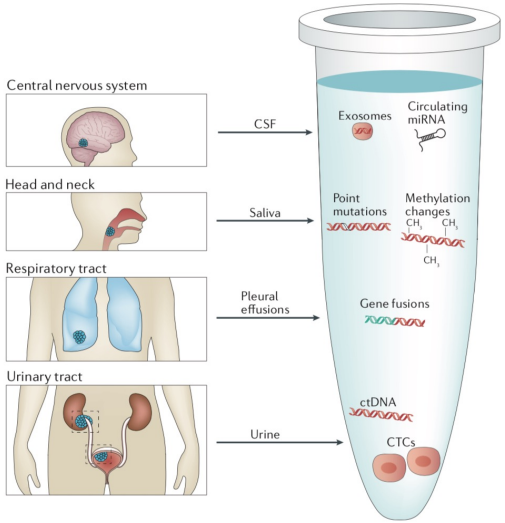
Agenda

- **Liquid biopsy general concepts**
- Liquid biopsy in lymphoma genotyping
- Liquid biopsy for lymphoma prognosis and MRD assessment

The liquid biopsy

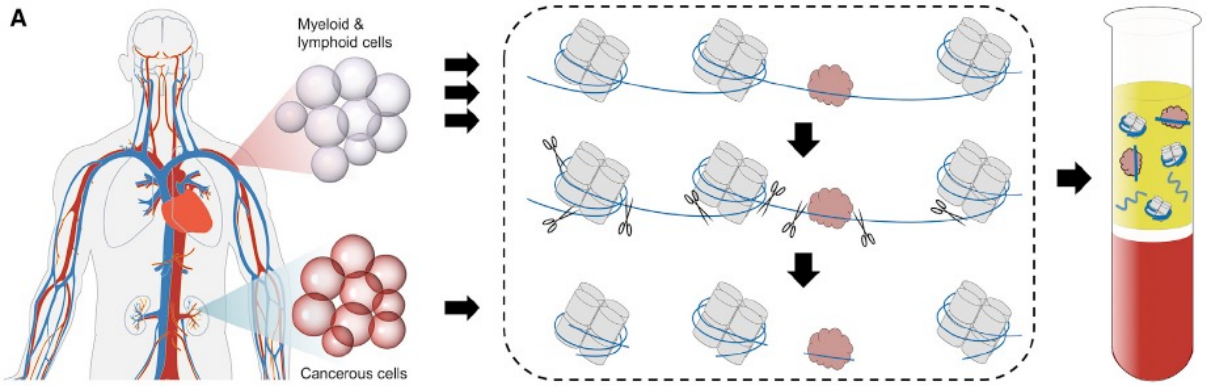
- A broad category of minimally invasive tests done on a sample of blood or other biological fluids
- Liquid biopsy can be used to analyze cell-free DNA (cfDNA), cells and vesicles such as exosomes that can originate from different healty tissues and also from cancers





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

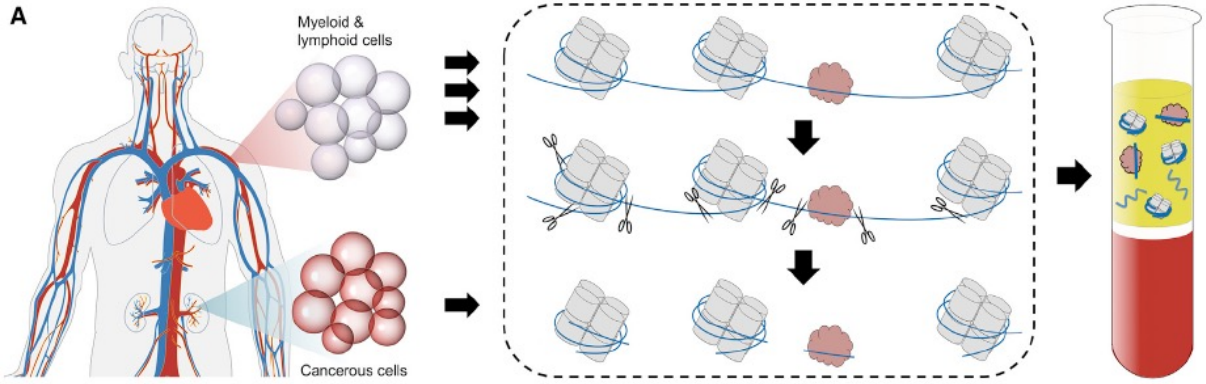
The origin of cfDNA *(i)*



- The presence of fragments of cell-free nucleic acids in human blood was first described in 1948 by Mandel and Métais
- cfDNA comprises double-stranded, short and highly fragmented by nucleases DNA fragments (<200 bp)
- **In healthy individuals, cfDNA derives from apoptosis of normal hematopoietic cells**

Wan JCM, et al., *Nat Rev*. 2017; Snyder MW, et al., *Cell.* 2016

The origin of cfDNA *(ii)*



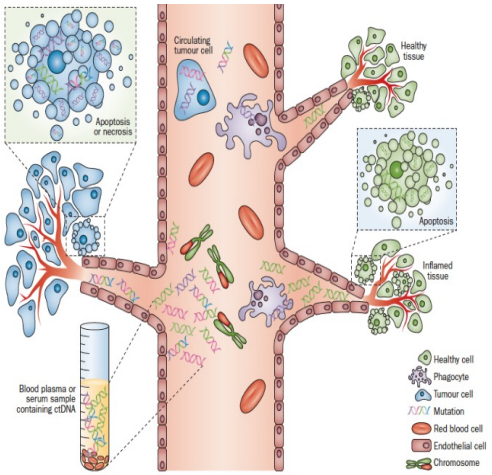
- cfDNA can be elevated in case of non-neoplastic clinical scenarios, such as acute trauma, cerebral infarction, exercise, and infection
- **In tumor patients, cfDNA is released by apoptotic cells and the fraction of cell-free DNA that originates from tumor cells is called ctDNA (for circulating tumor DNA)**
- **The half-life of cfDNA in the circulation is between 16 minutes and 2.5 hours, which enables cfDNA analysis to be considered as a 'real-time' snapshot of disease burden**

Wan JCM, et al., *Nat Rev*. 2017; Snyder MW, et al., *Cell.* 2016

Liquid Biopsy vs Tissue Biopsy

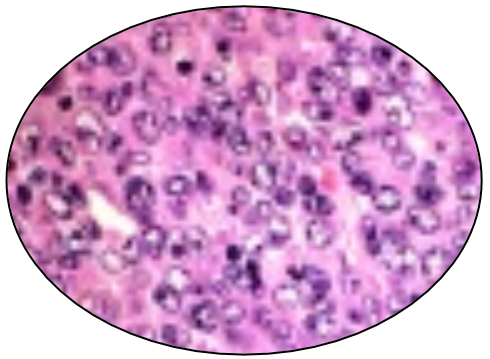
Liquid biopsy:

- allows early disease detection
- enables assessment of tumor heterogeneity and monitoring of tumor dynamics
- in solid cancers, allows evaluation of metastasis in real-time and monitoring of the treatment response



Tissue biopsy during clinical course:

- may not reflect current disease condition
- may not be feasible based on patient conditions or tumor accessibility
- impractical for periodic monitoring for progression/recurrence



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

Agenda

- Liquid biopsy general concepts
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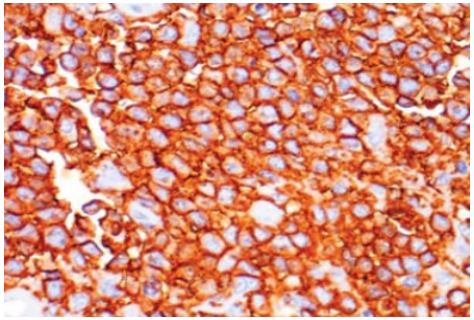
- Liquid biopsy for lymphoma prognosis and MRD assessment

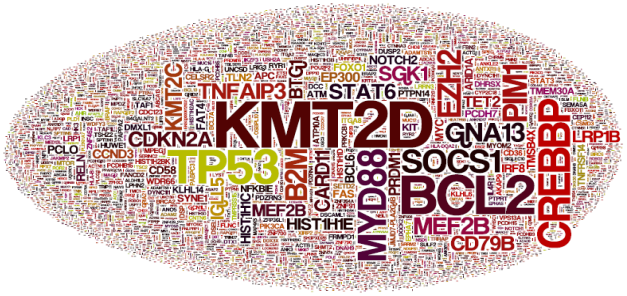
Diffuse large B-cell lymphoma vs Hodgkin lymphoma

DLBCL

Tumor cells are enriched in the mass

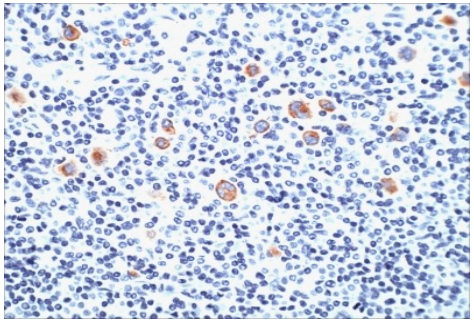


Exome sequencing data from >1000 cases



cHL

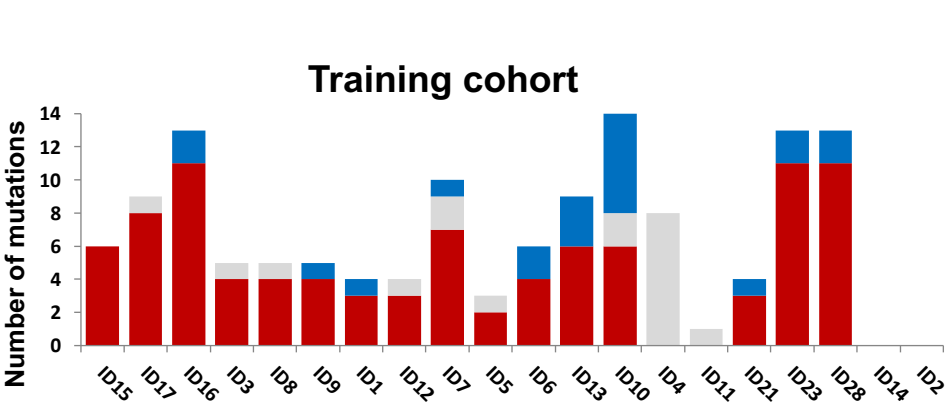
Tumor cells are rare in the mass

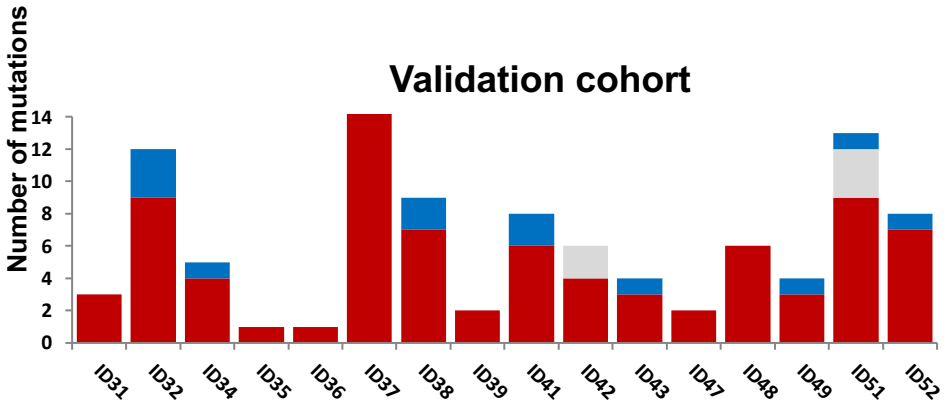


Exome sequencing data from only 10 cases

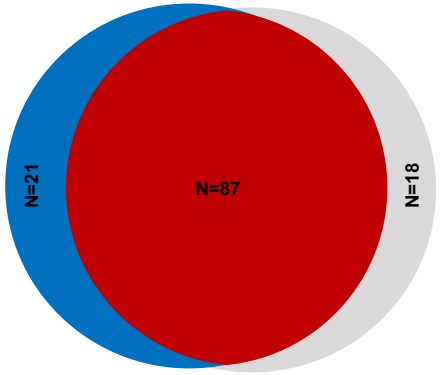
Pasqualucci L, et al., *Semin Hematol* 2015; Reichel J, et al., *Blood* 2015

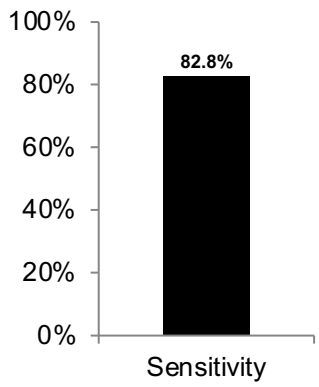
Plasma cfDNA genotyping vs tumor gDNA genotyping

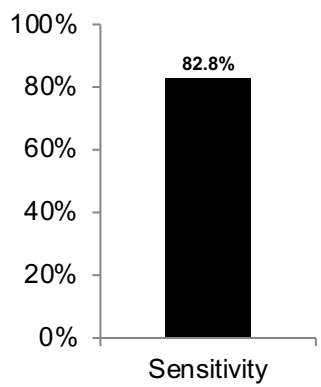


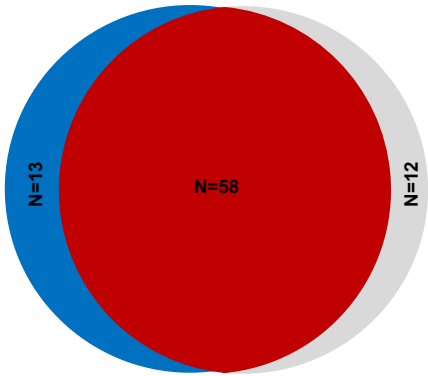


Mutation identified both in gDNA and in cfDNA | Mutation identified in gDNA only | Mutation identified in cfDNA only





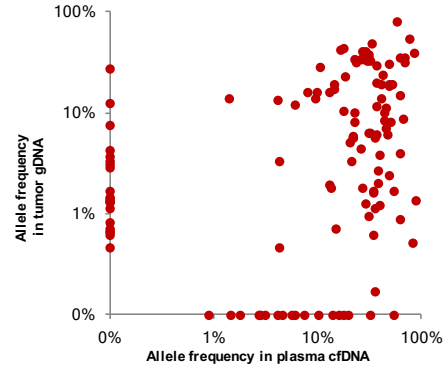


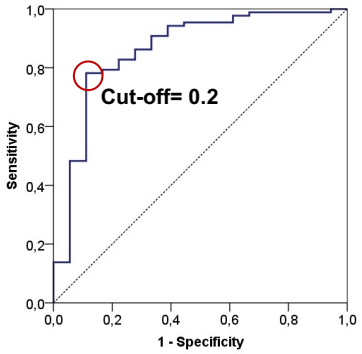


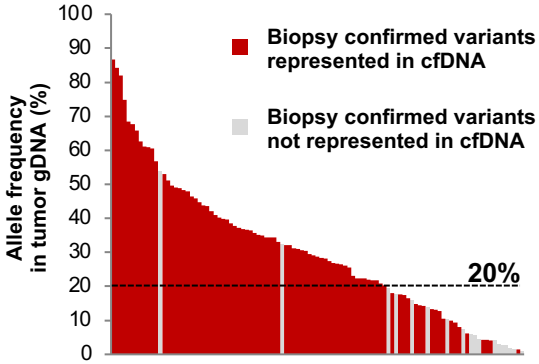
- Most DLBCL mutations (in red) are identified both in the tissue biopsy and on the liquid biopsy
- However, a fraction of DLBCL mutations are detectable only in the liquid biopsy

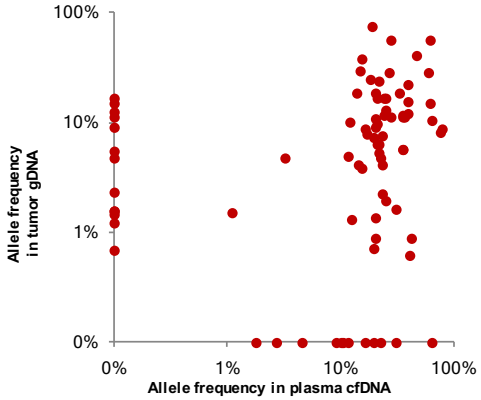
Rossi D, et al., *Blood*, 2017

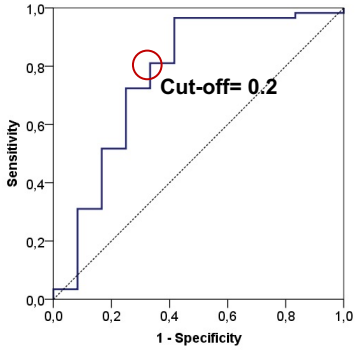
cfDNA genotyping has an optimal sensitivity for mutations represented in >20% of the tumor alleles

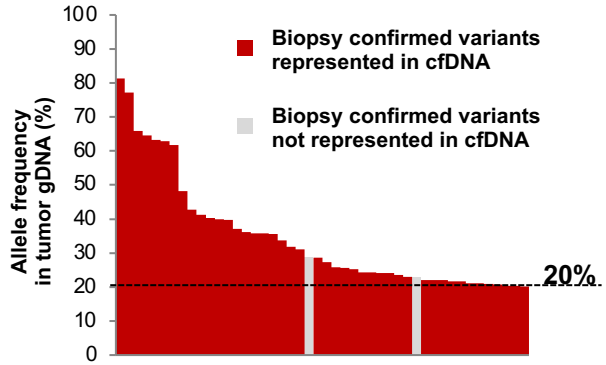






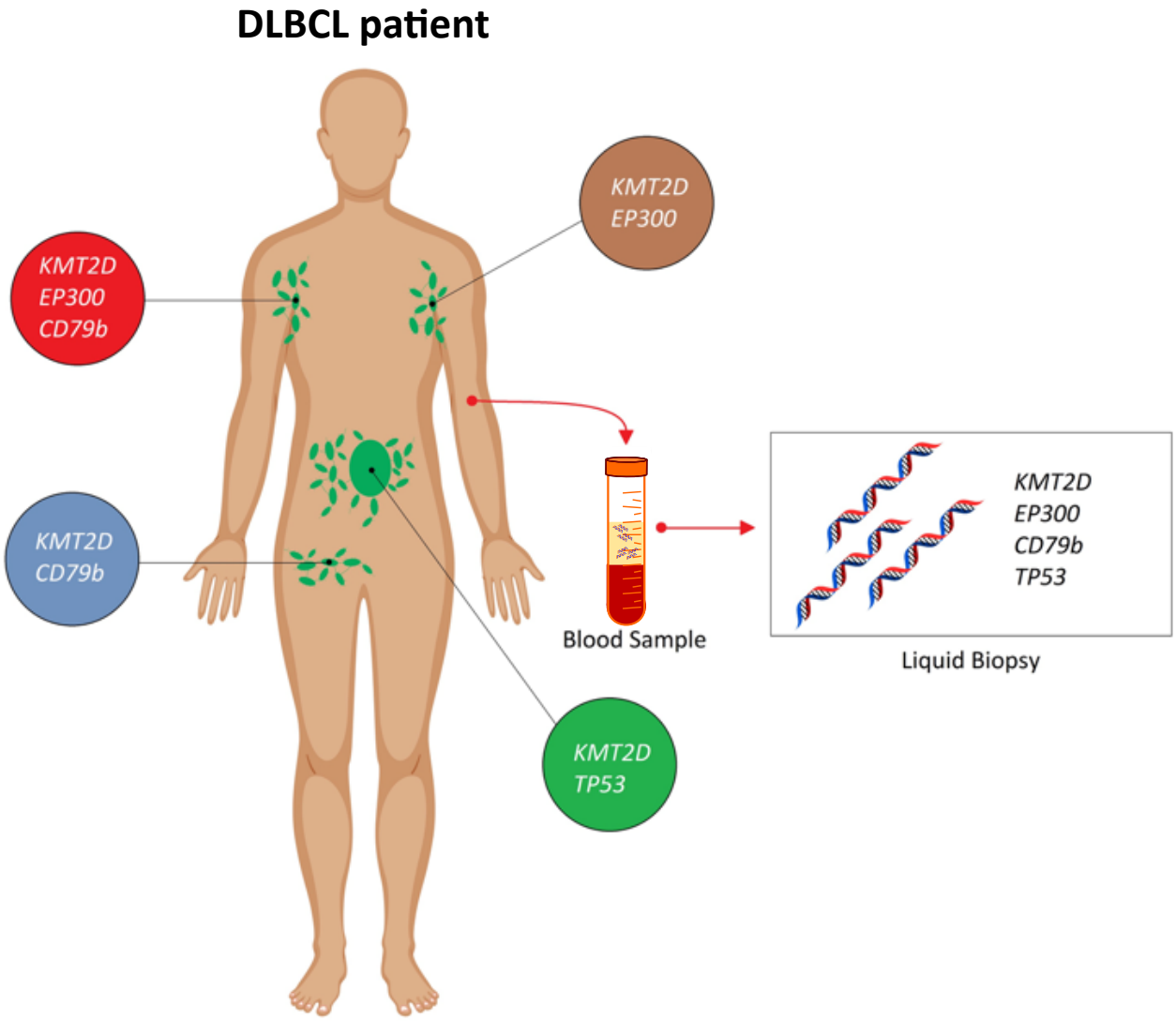






Rossi D, et al., *Blood*, 2017

Liquid biopsy may allow a non-invasive and comprehensive disease genotyping of DLBCL



Diffuse large B-cell lymphoma vs Hodgkin lymphoma

DLBCL

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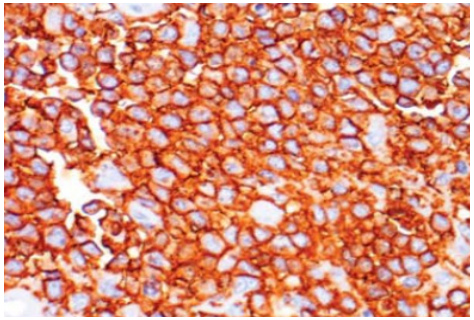
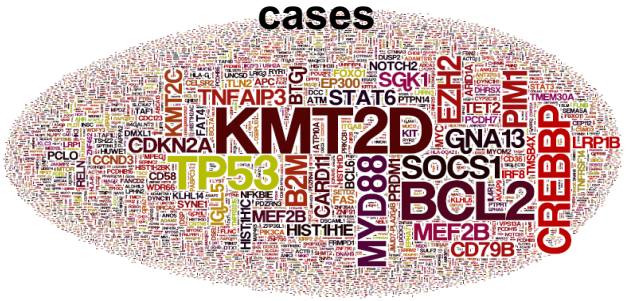


Exome sequencing data from >1000 cases



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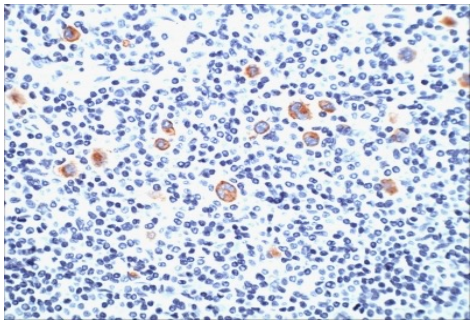

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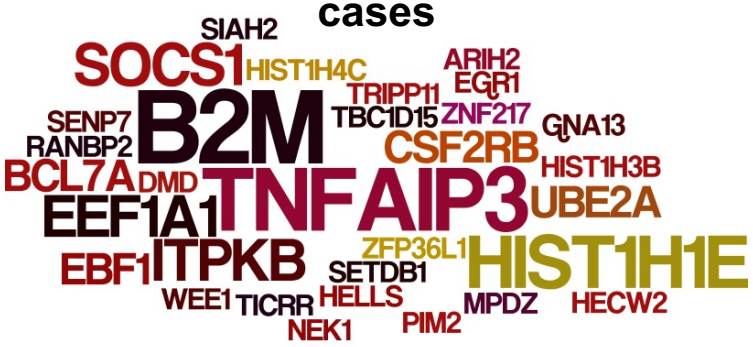
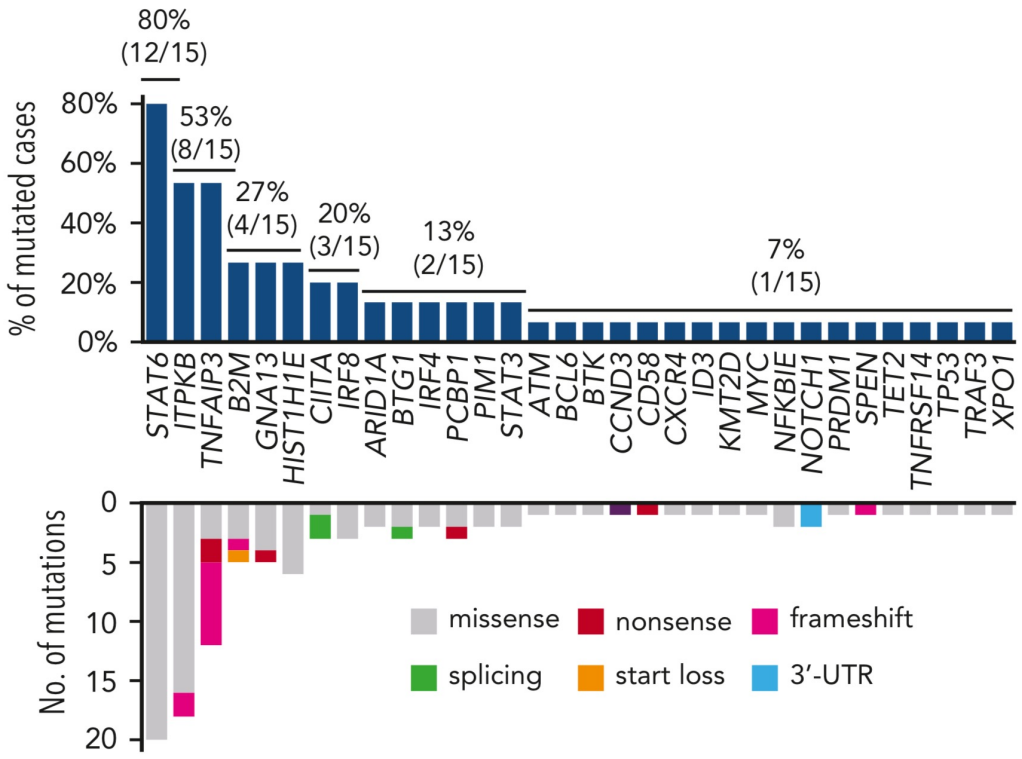
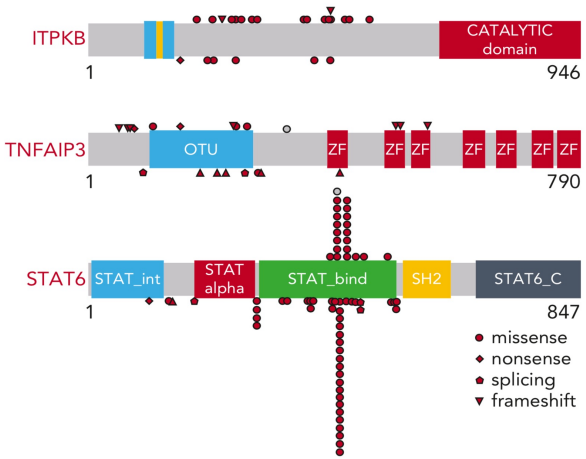
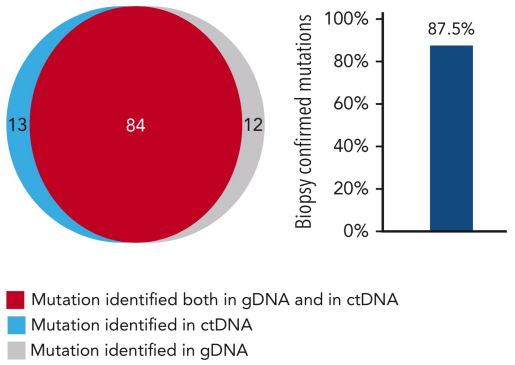


Pasqualucci L, et al., *Semin Hematol* 2015; Reichel J, et al., *Blood* 2015

The liquid biopsy mirrors the genetics of cHL







Spina V, et al., *Blood*, 2018

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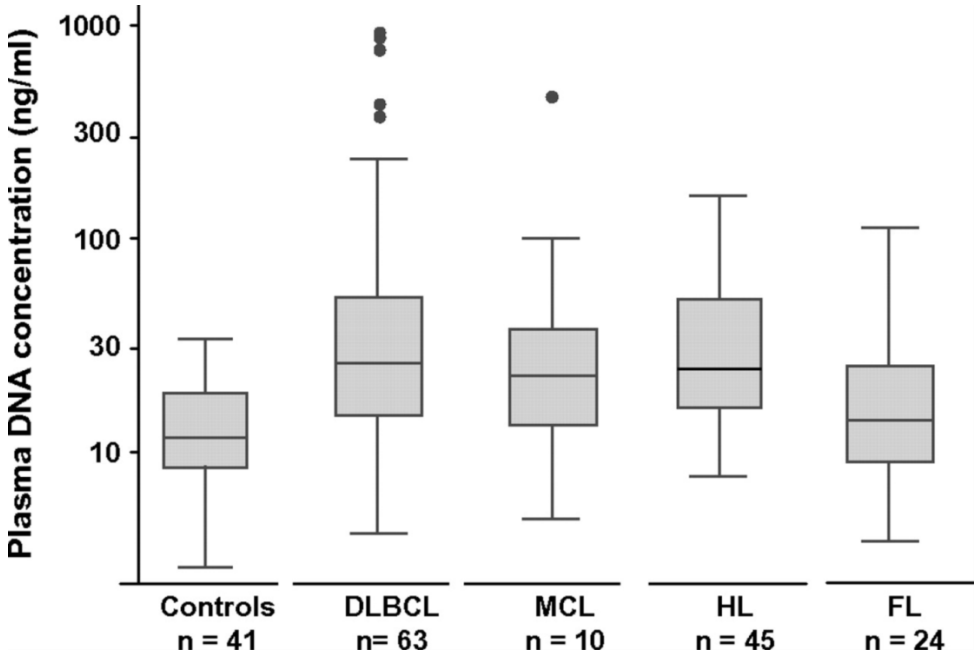
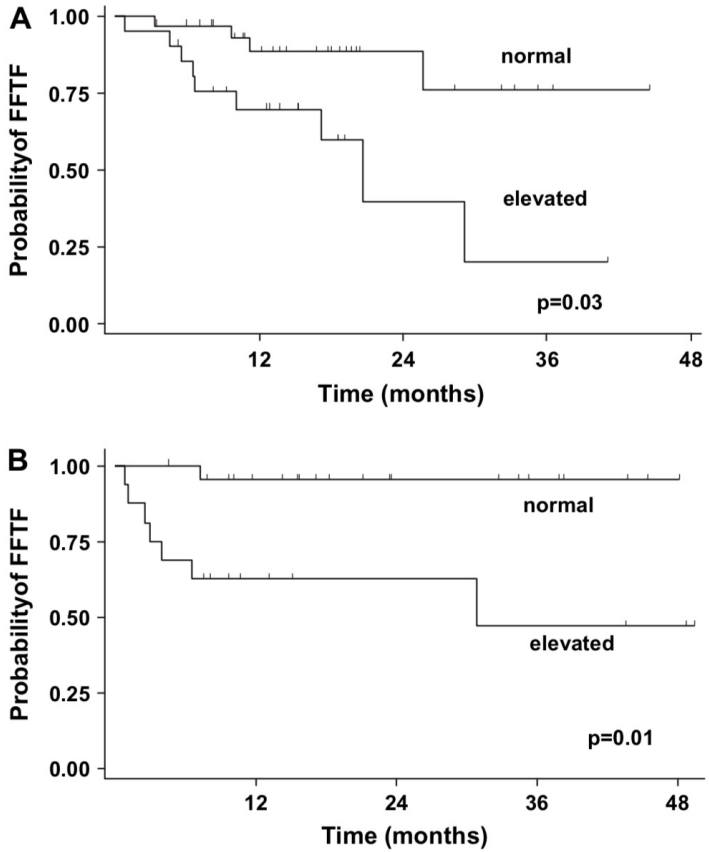
- **Liquid biopsy for lymphoma prognosis and MRD assessment**

cfDNA concentration and prognosis in lymphomas

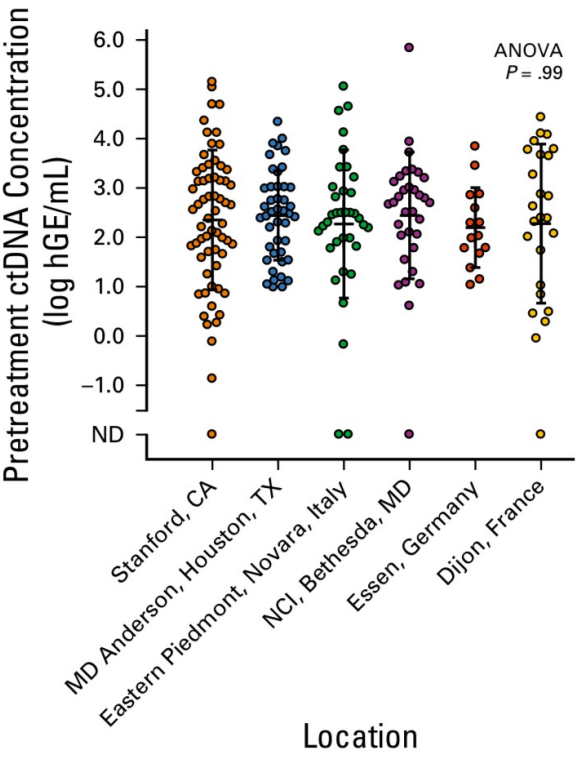


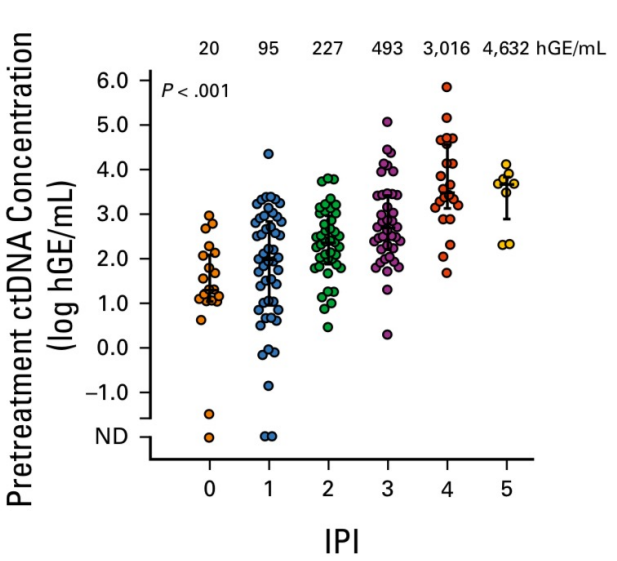


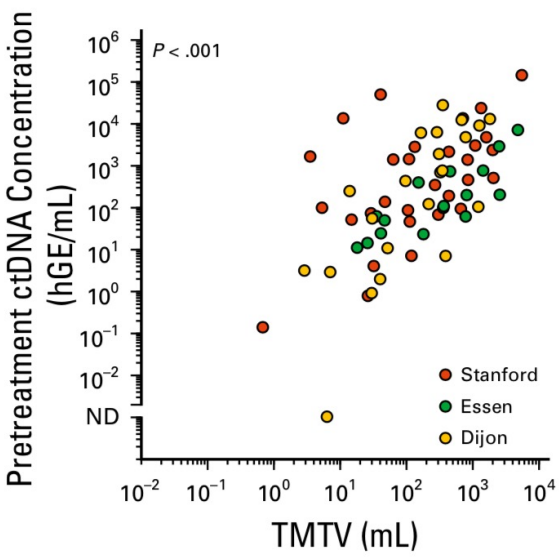
- Seminal studies documented high levels of cfDNA in lymphoma patients and provided a proof of concept for subsequent studies in different histological types of the disease

Hohaus S, et al., *Ann Oncol*. 2009

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





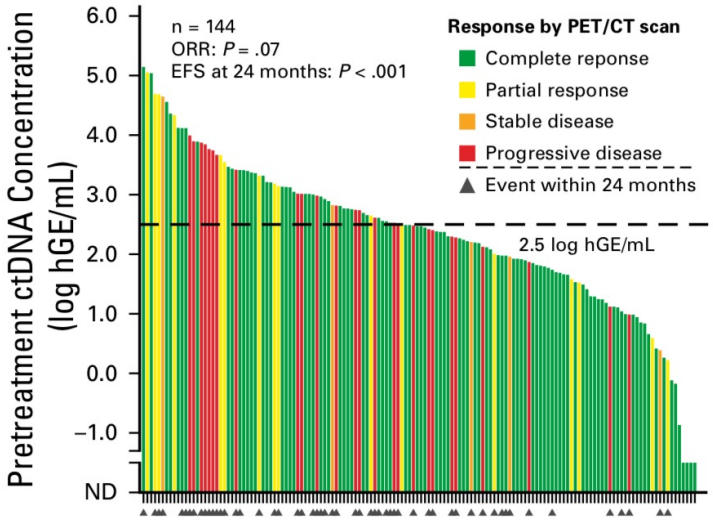


- The quantity of ctDNA positively correlates with the International Prognostic Index (IPI) and with the total metabolic tumor volume (TMTV) evaluated by PET scan

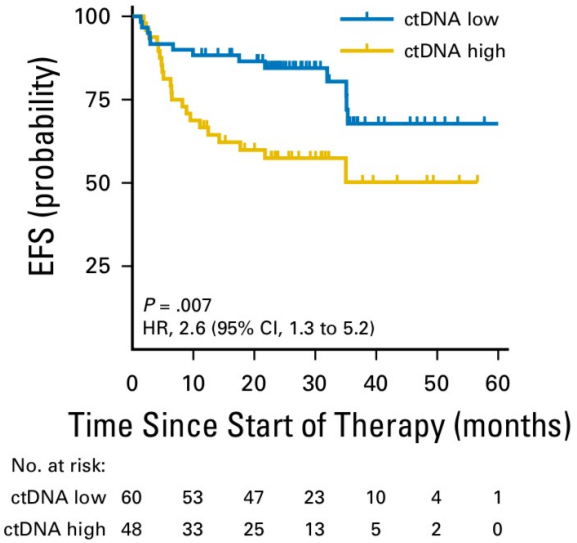
- Therefore, the quantity of ctDNA correlates with DLBCL aggressiveness and extension at diagnosis

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

Pretreatment ctDNA is a robust biomarker in DLBCL

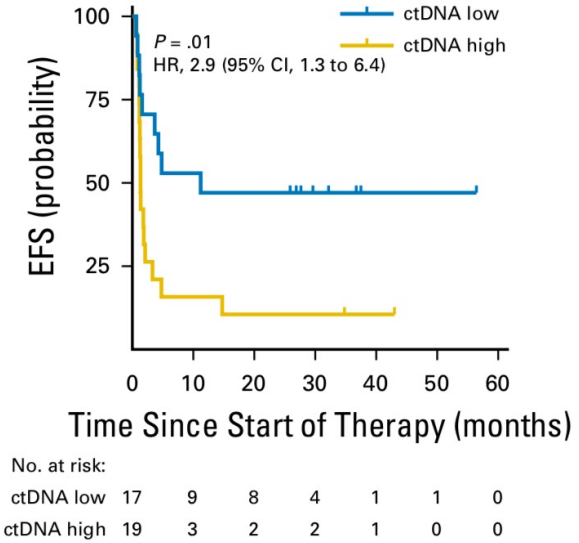


First line DLBCL



No. at risk:							
ctDNA low	60	53	47	23	10	4	1
ctDNA high	48	33	25	13	5	2	0

R/R DLBCL

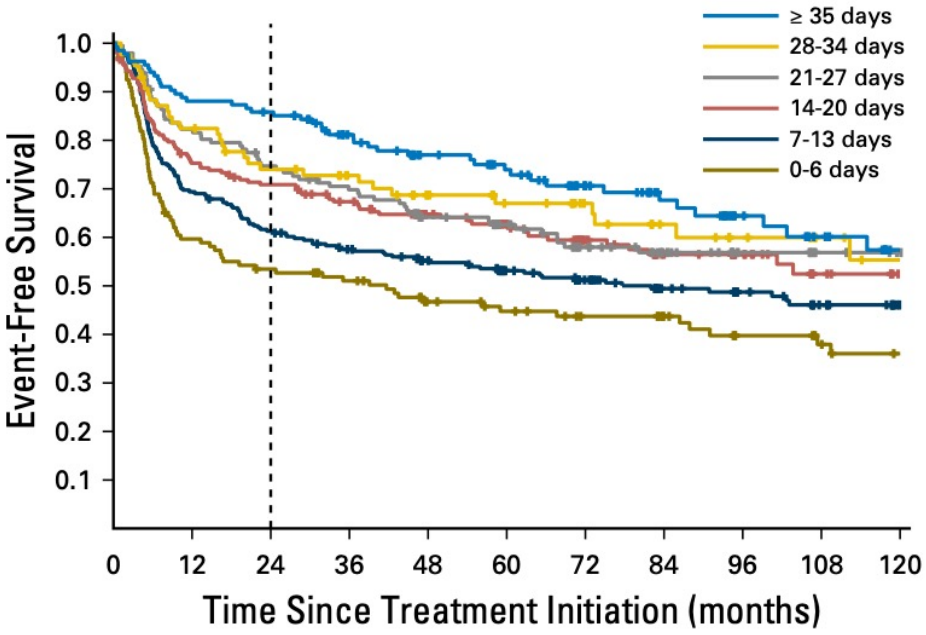


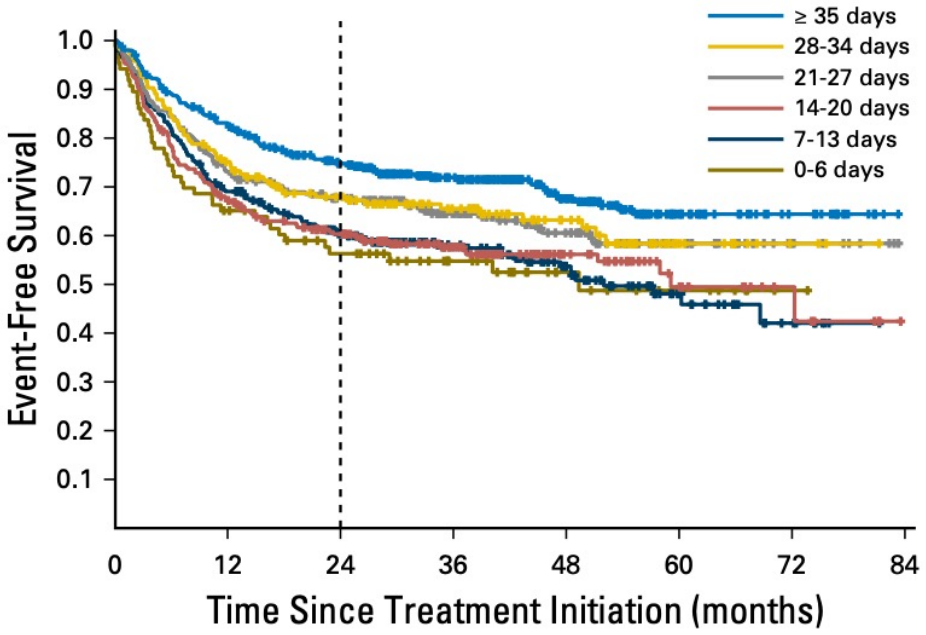
No. at risk:							
ctDNA low	17	9	8	4	1	1	0
ctDNA high	19	3	2	2	1	0	0

- Pre-treatment levels of ctDNA correlate with Event Free Survival (EFS) in both first line and in relapsed/refractory DLBCL

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

Shorter DTI predict inferior outcome in DLBCL

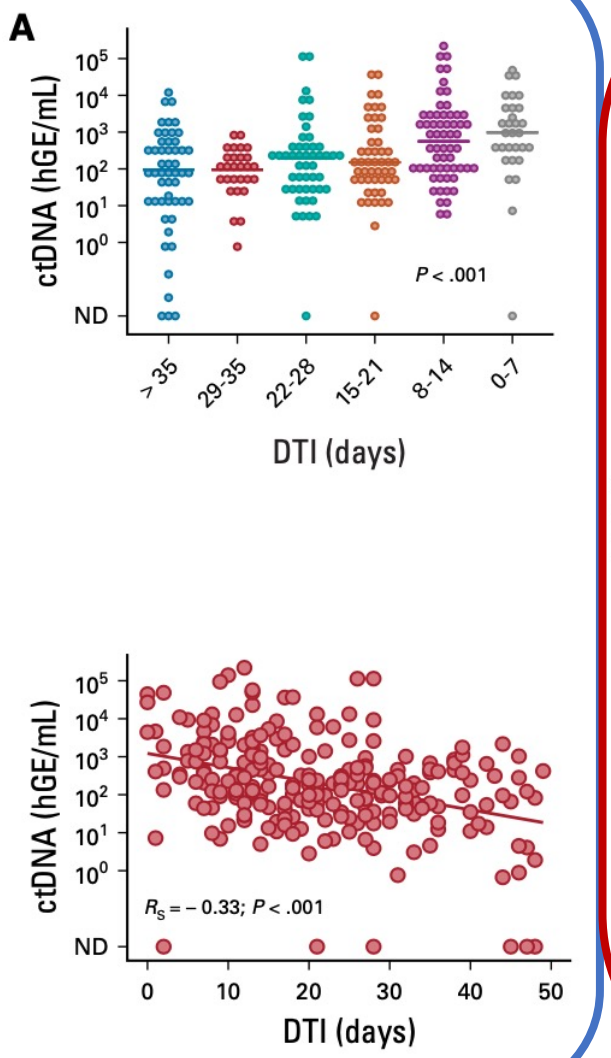


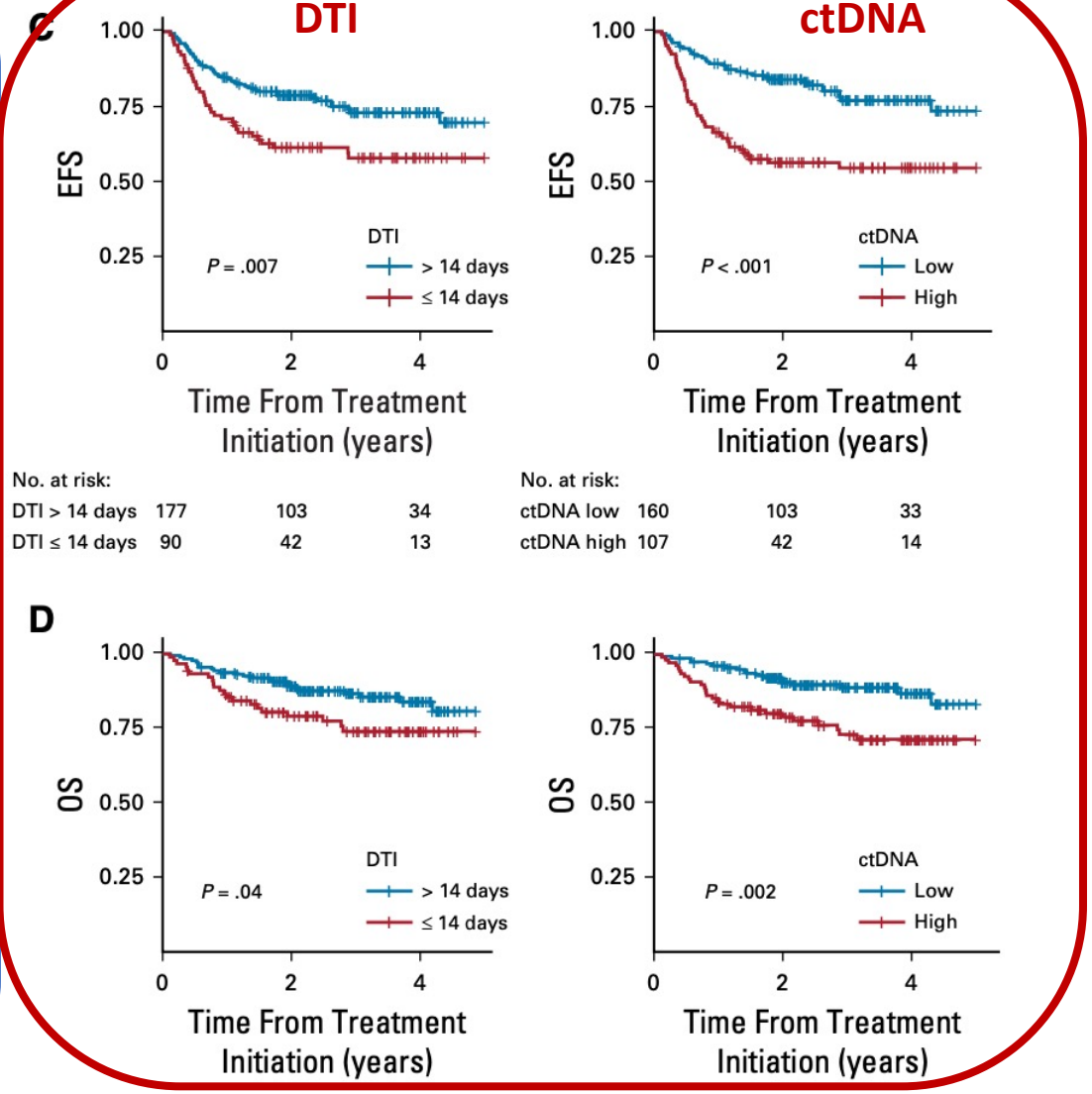


Short diagnosis-to-treatment interval (DTI) is associated with adverse clinical risk factors and inferior event-free survival in DLBCL patients

Maurer, *et al., JCO.* 2018

ctDNA and DTI predict outcome in DLBCL

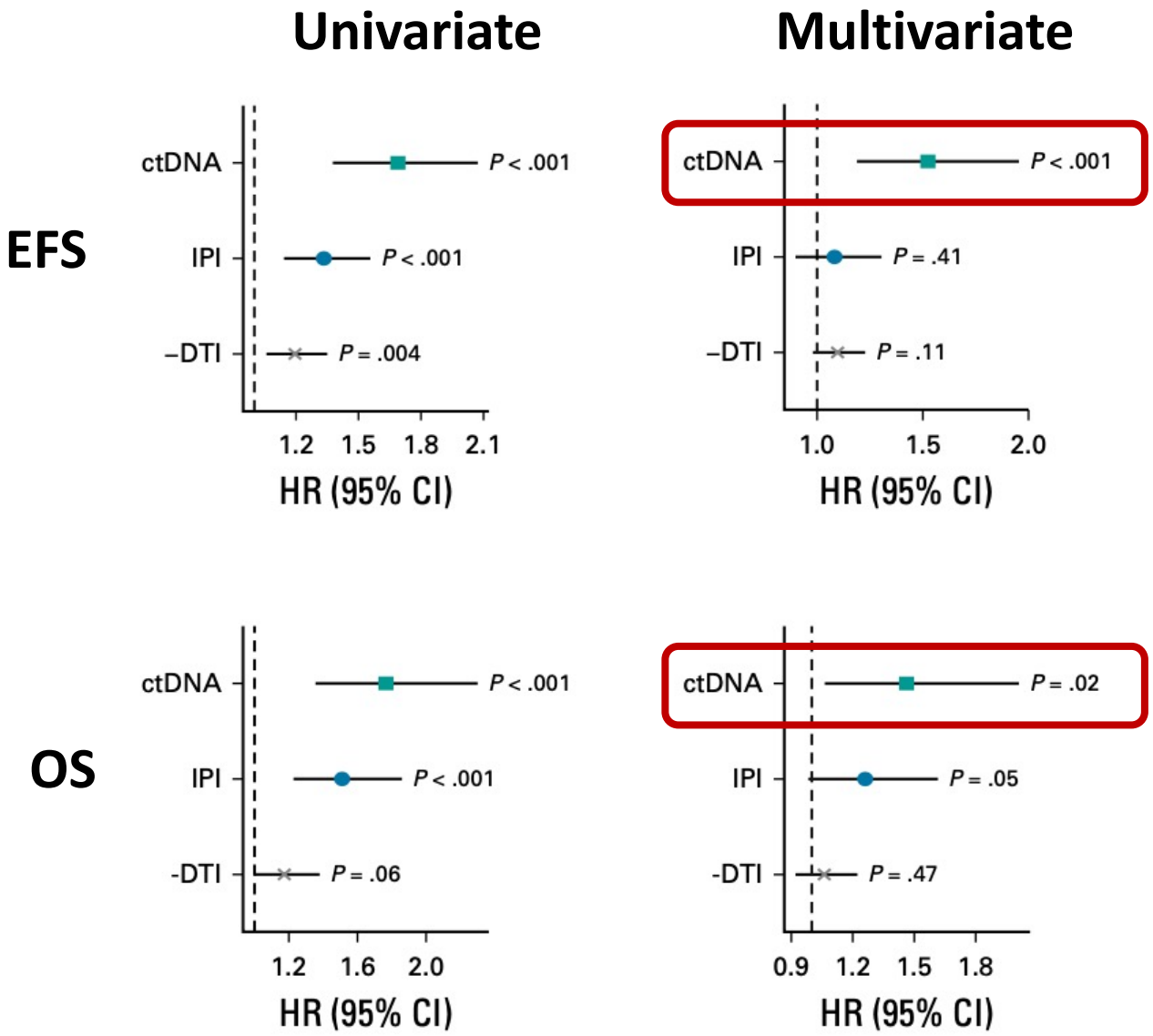




- Shorter DTI is associated with higher amount of ctDNA
- Both ctDNA levels and DTI predict EFS and OS

Kurtz, *et al., JCO.* 2021

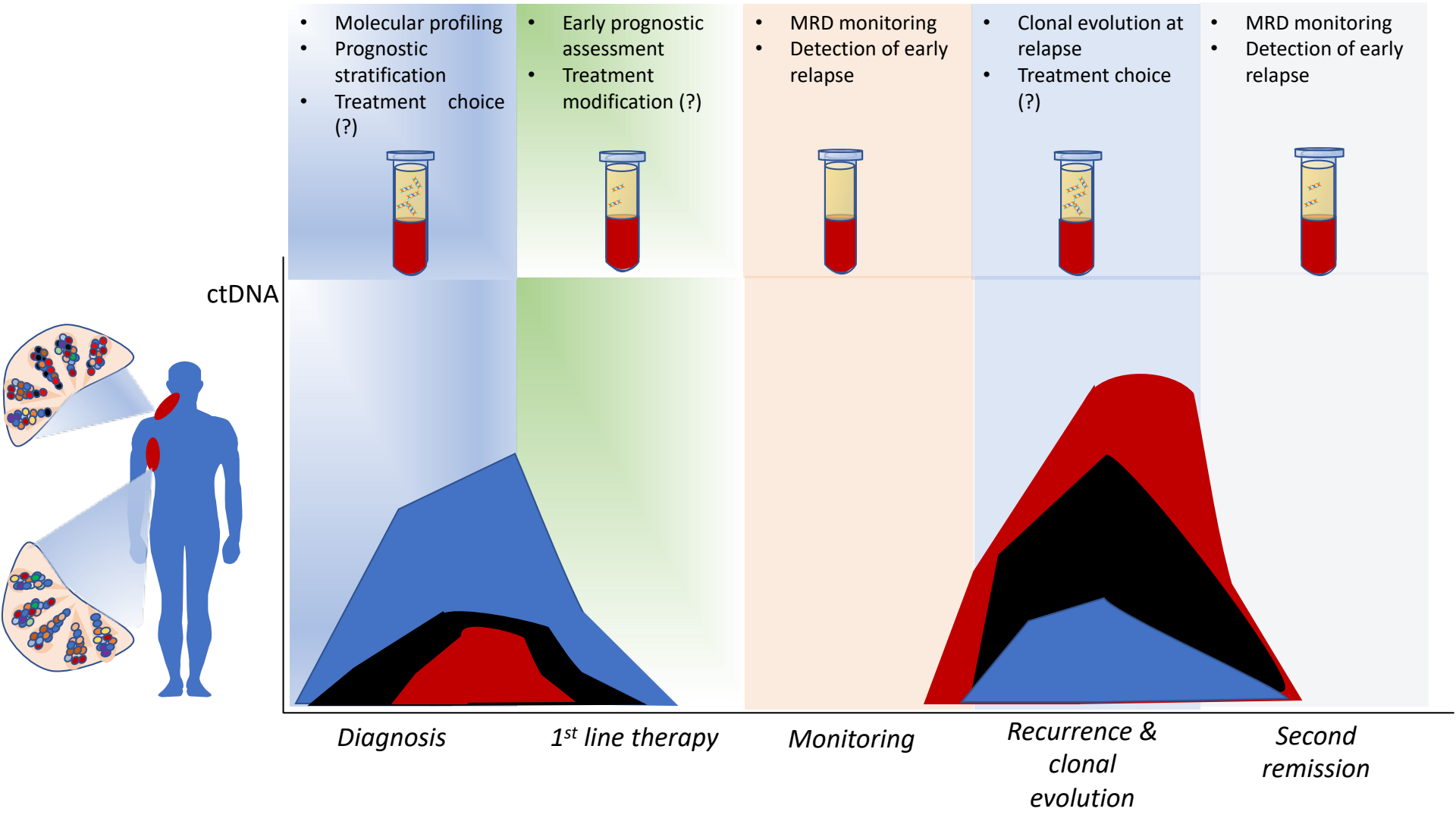
ctDNA retained prognostic value in multivariate analysis



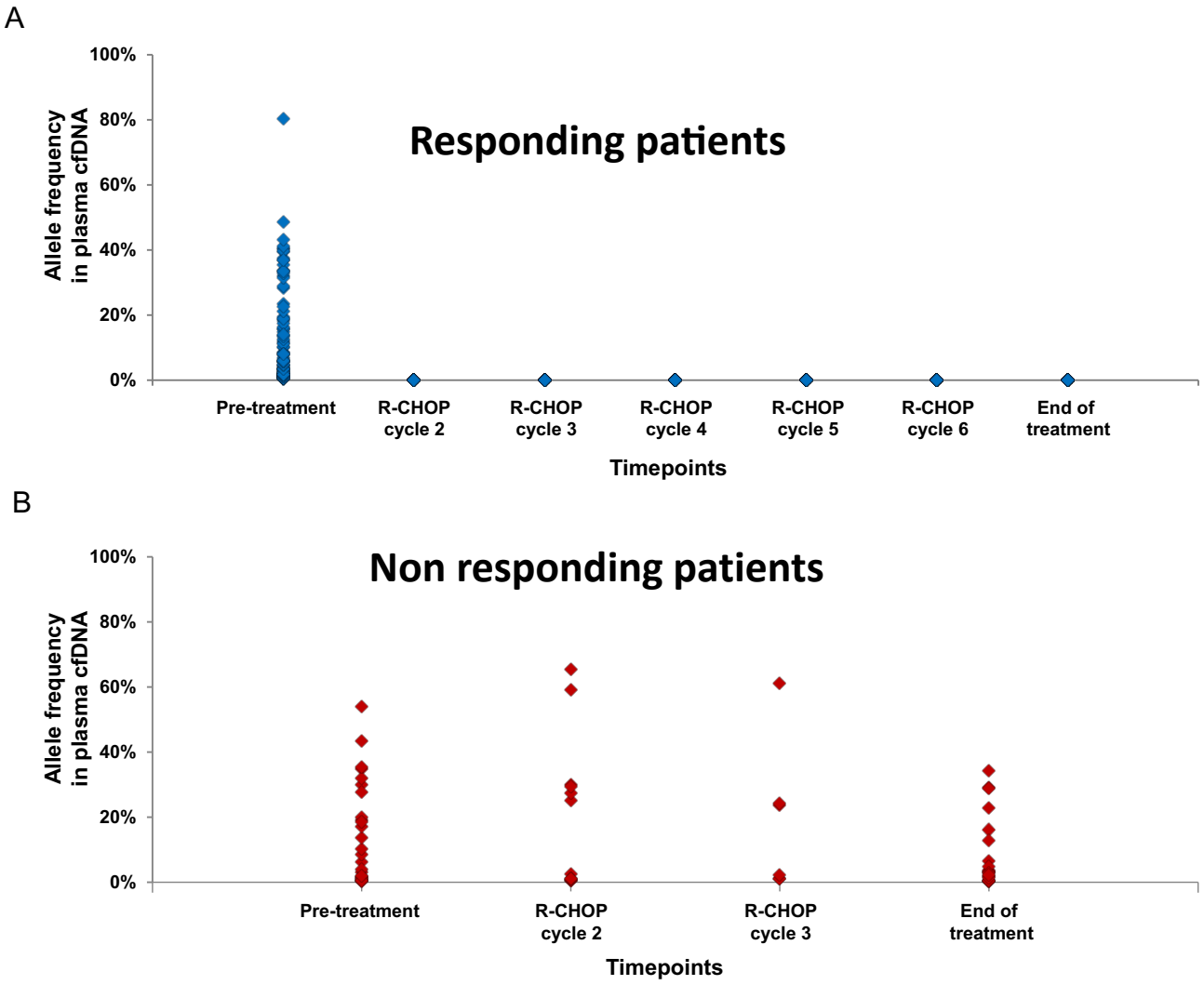
Pre-treatment ctDNA levels have utility for quantifying and guarding against selection biases in prospective DLBCL clinical trials.

Kurtz, *et al., JCO.* 2021

Using liquid biopsy as a non-invasive tool to monitor the disease during the course of therapy

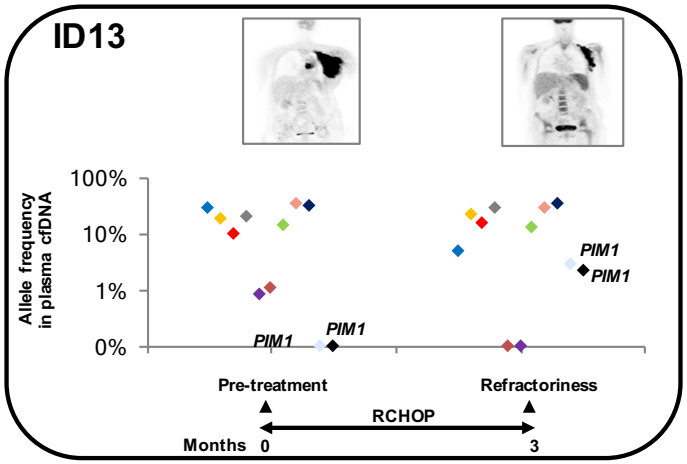


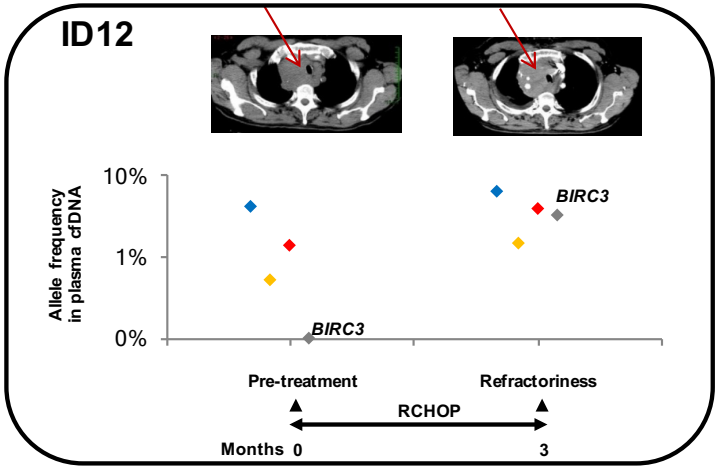
Mutations are cleared from plasma cfDNA in responding DLBCL patients but not in refractory patients

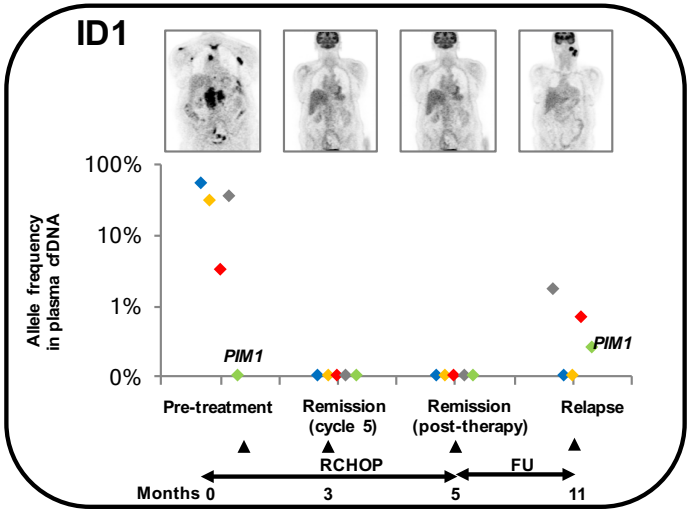


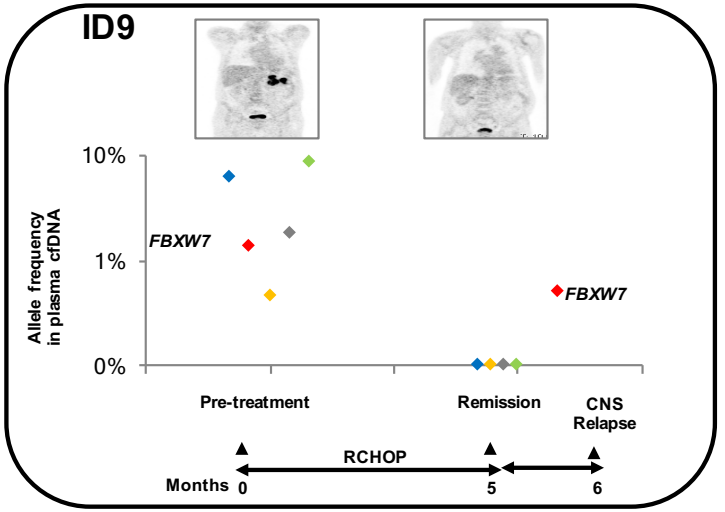
Rossi D, et al., *Blood*, 2017

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



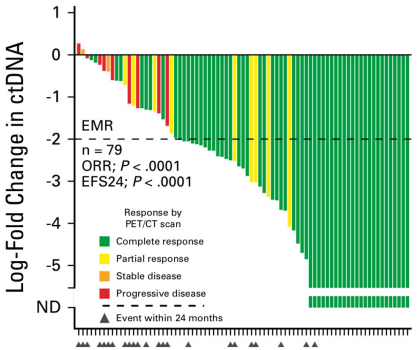


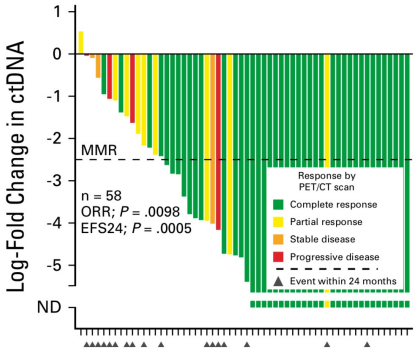


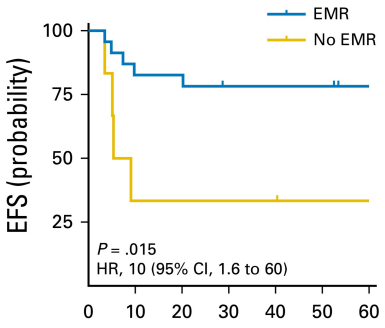


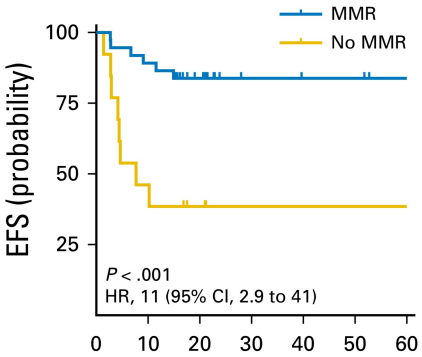
Rossi D, et al., *Blood*, 2017

ctDNA monitoring during therapy predict outcomes in DLBCL









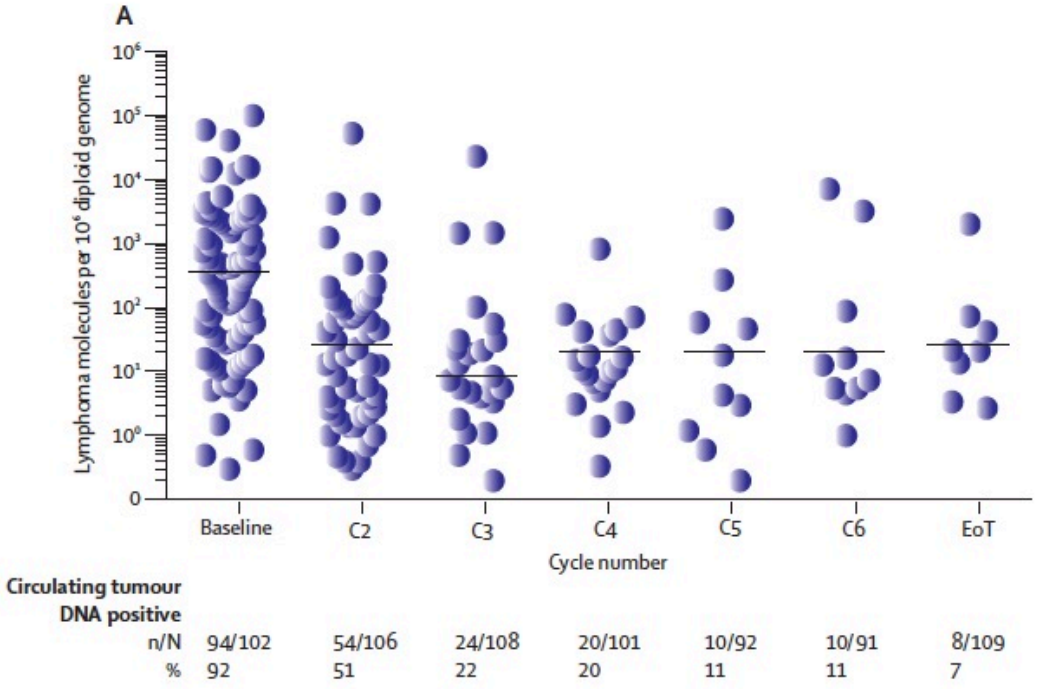
	Univariable		Multivariable	
Parameter	HR (95% CI)	*P*	HR (95% CI)	*P*
EFS				
IPI (0 to 5)	1.21 (0.87 to 1.69)	.25	0.93 (0.63 to 1.37)	.71
Pretreatment ctDNA (low *v* high)	2.77 (1.08 to 7.13)	.034*	2.97 (0.92 to 9.62)	.070
Molecular response†	5.93 (2.52 to 13.95)	< .001*	8.58 (3.3 to 22.32)	< .001*
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*

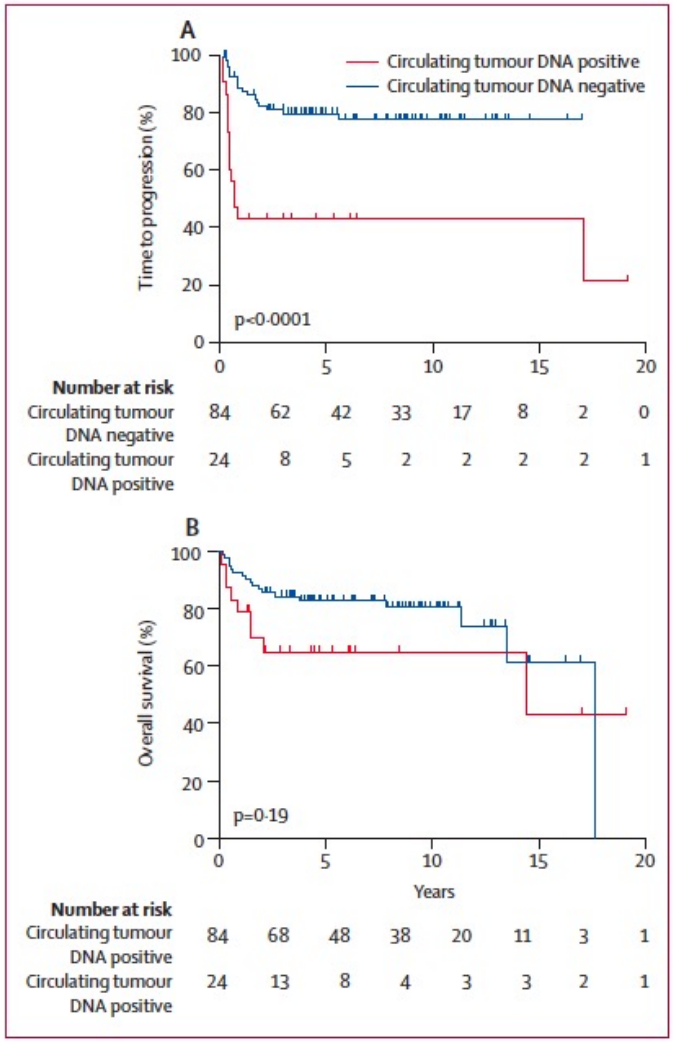
- A 2 log drop in ctDNA levels after 1 course of therapy (Early Molecular Response, EMR) is inpendently associated with improved EFS

- A 2 log drop in ctDNA levels after 2 courses of therapy (Major Molecular Response, MMR) is inpendently associated with improved EFS

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

Postivity for VDJ rearrangement in plasma decreases during DLBCL treatment with R-CHOP

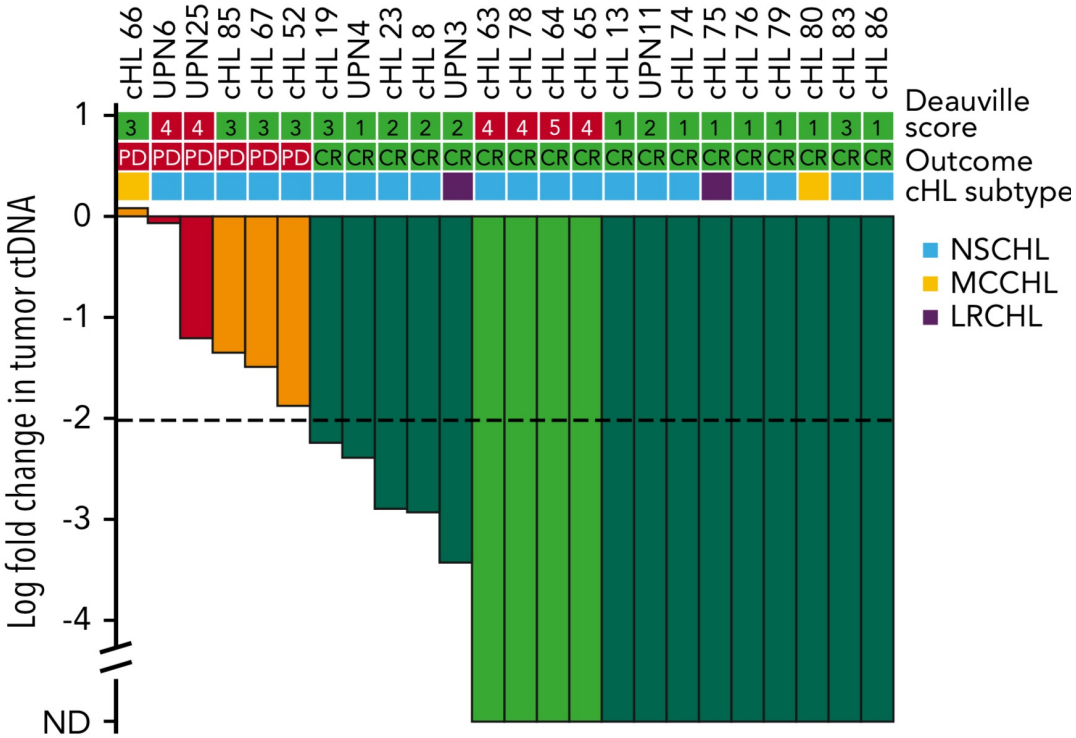


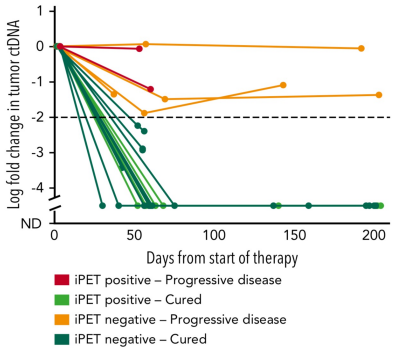


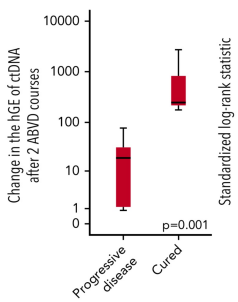
- cfDNA levels by the analysis of IGHV decrease after R-CHOP therapy
- Persistence of cfDNA during the course of therapy associated with worse outcome

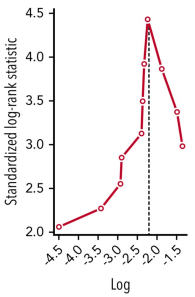
Roschewsky M, et al., *Lancet* 2015

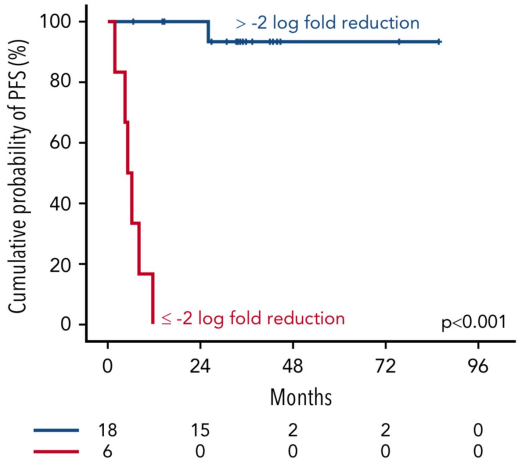
Changes in tumor cfDNA complement iPET in cHL





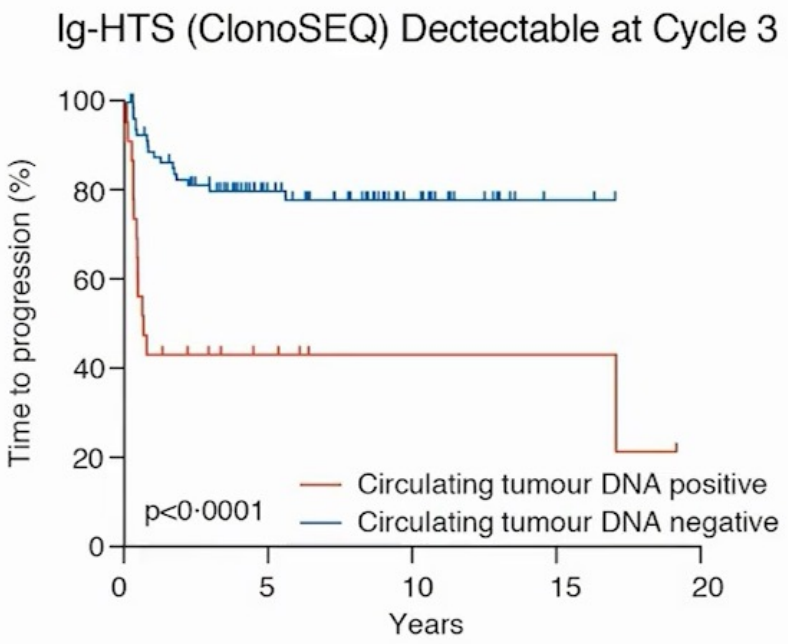




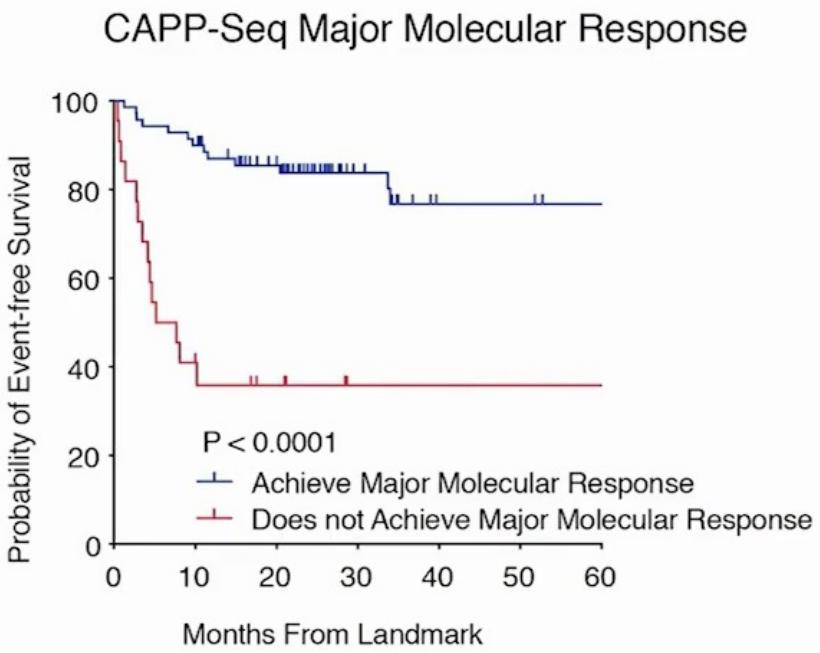


Spina V, et al., *Blood*, 2018

How sensitive is sensitive enough?



Roschewski MR et al. *Lancet Oncology*. 2015.

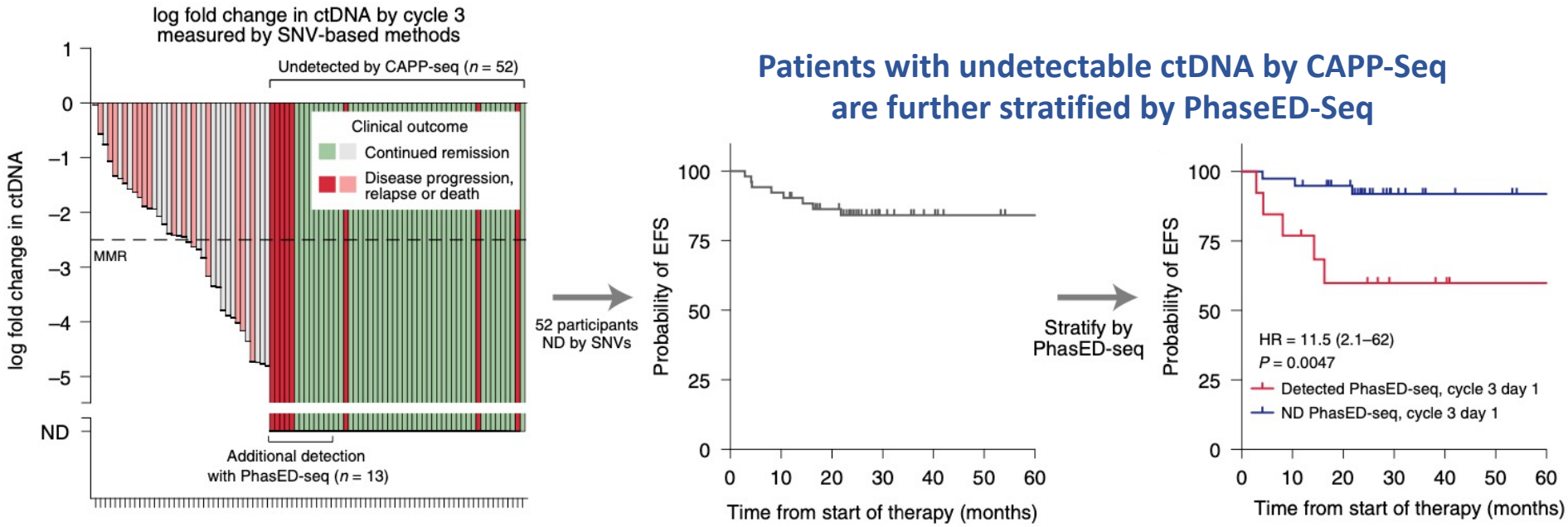


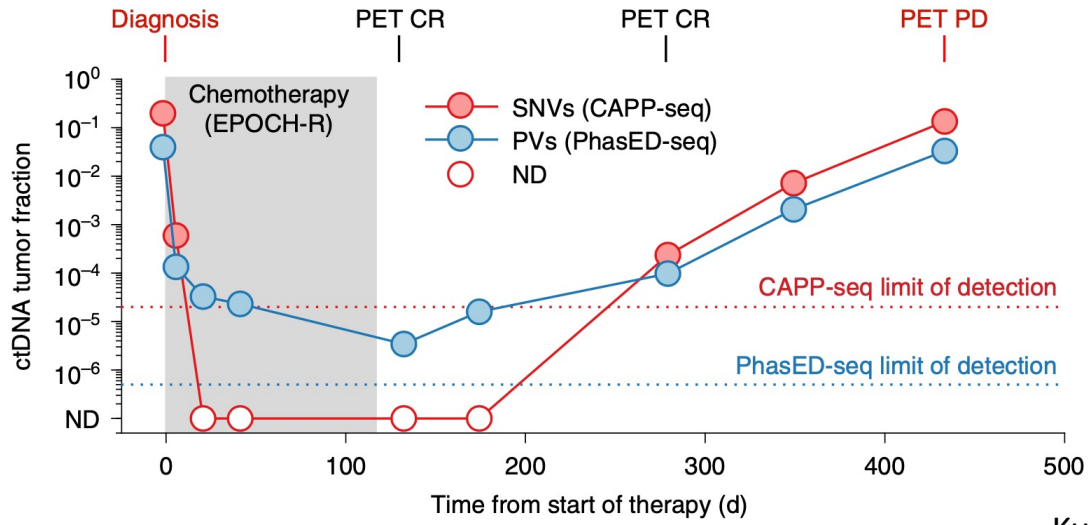
Kurtz et al, *JCO*. 2018

- Similar results have been obtained using IgHTS/clonoSEQ and CAPP-Seq
- Approximately 20% of patients with undetectable ctDNA experience a relapse

Roschewsky M, et al., *Lancet* 2015; Kurtz *et al., JCO*. 2018; Alizadeh ICML 2021.

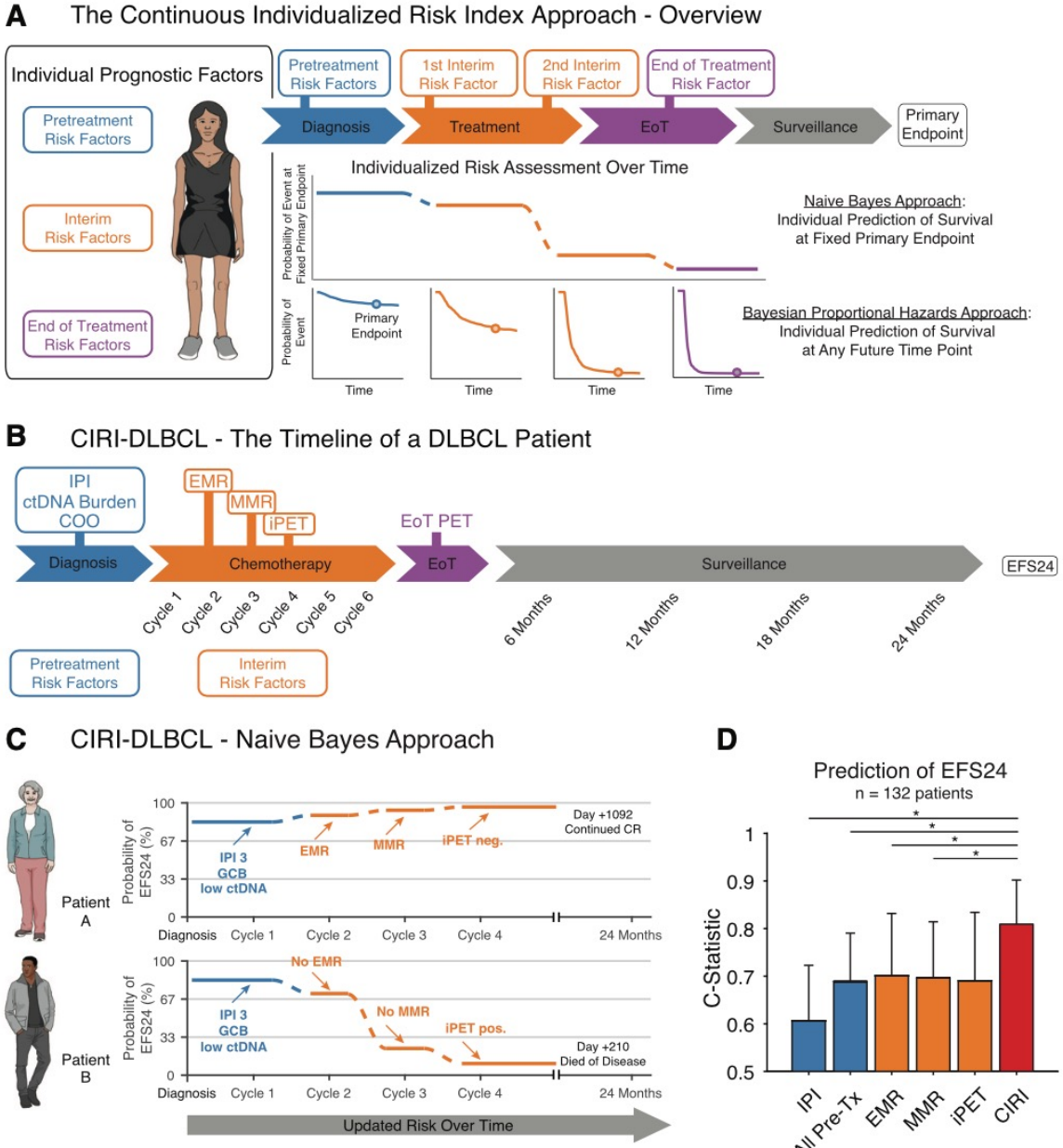
ctDNA detection by PhaseED-Seq improves outcome prediction





Kurtz *et al., Nat Biotechnol. 2021.*

Dynamic risk assessment of outcome using CIRI



The dynamic risk assessment will facilitate personalized medicine and enable innovative therapeutic paradigms.

CIRI: Continuous Individualized Risk Index

Kurtz, *et al., Cell.* 2019

Summing up

- 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
- Quantitative levels of ctDNA change rapidly after therapy and dynamic ctDNA responses are strongly prognostic of survival
- Circulating tumor DNA can be detected prior to clinical relapse but requires more sensitive techniques