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Plasma Cell Free DNA Markers as Diagnostic Tools in Lymphomas

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Hematology
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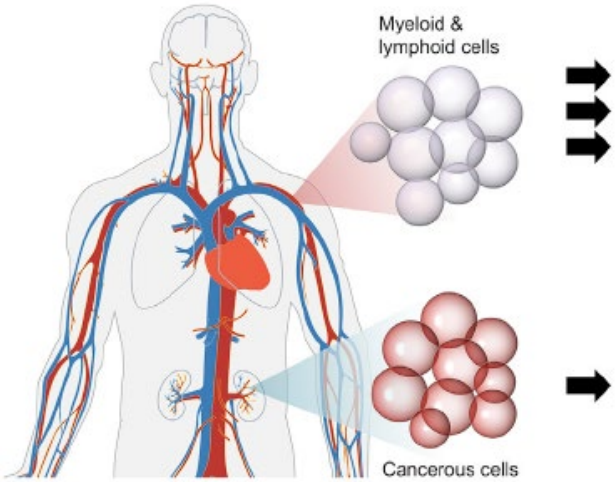
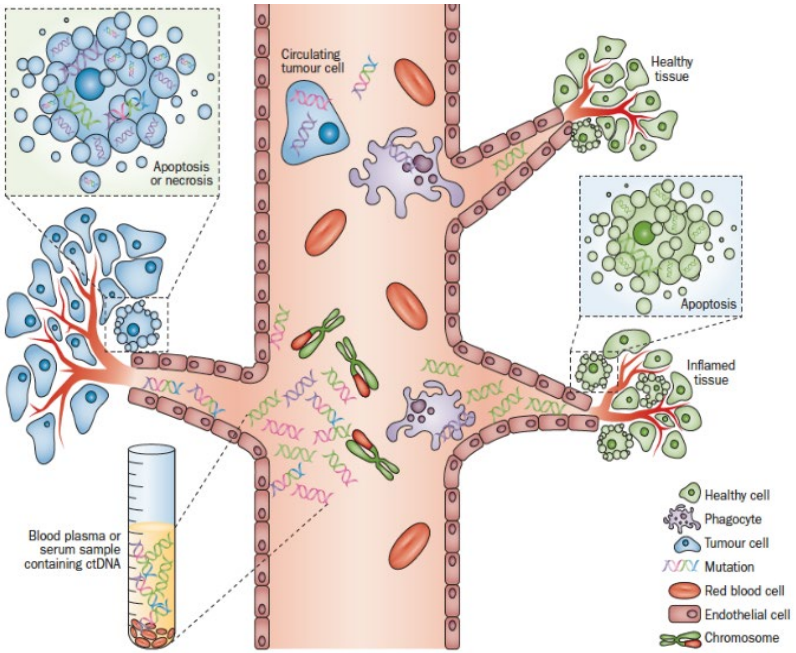
Bellinzona - Switzerland

Conflict of interest

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

Cell free DNA (cfDNA) - Circulating tumor DNA (ctDNA)

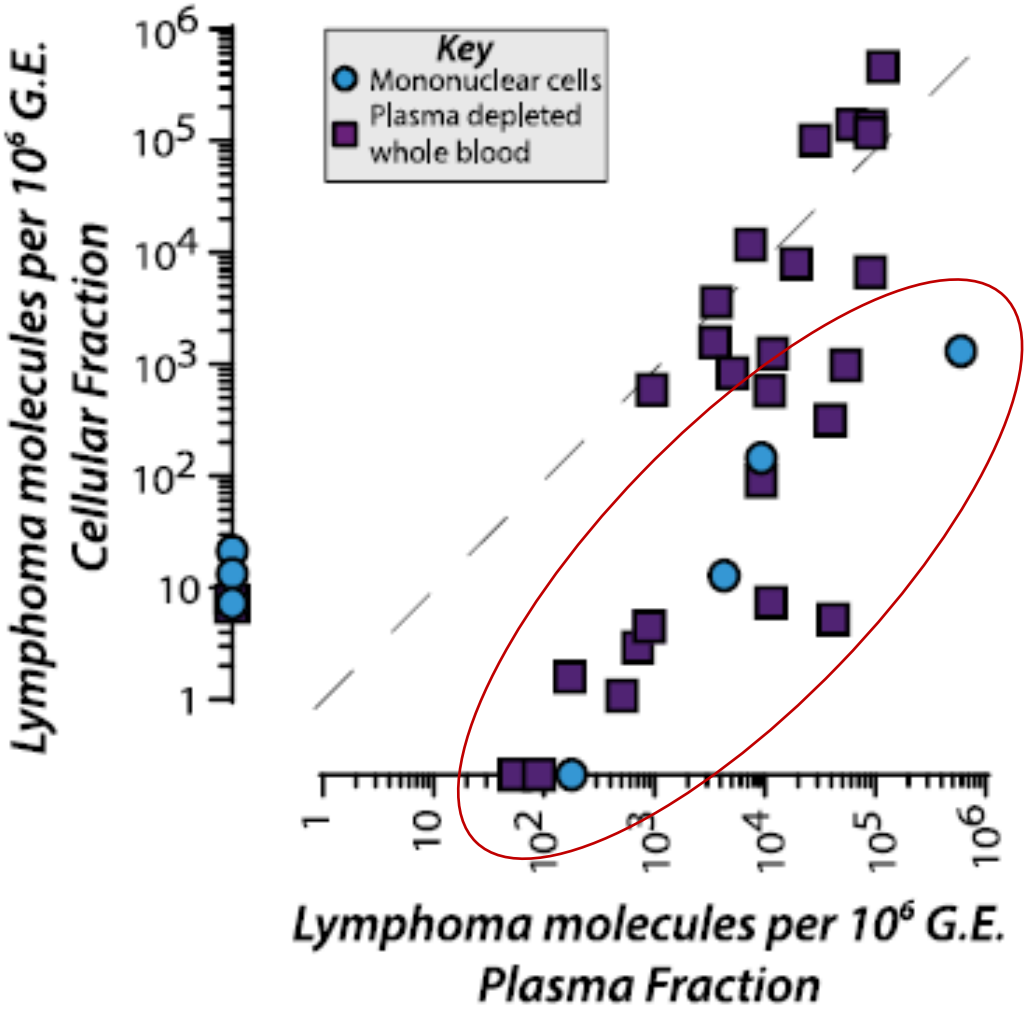




- In healthy individuals cfDNA derives from apoptosis of normal hematopoietic cells
- In tumor patients ctDNA is released by tumor apoptotic cells
- ctDNA is distinguished from other cfDNA by the presence of somatic mutations representative of tumor biology absent in normal cells

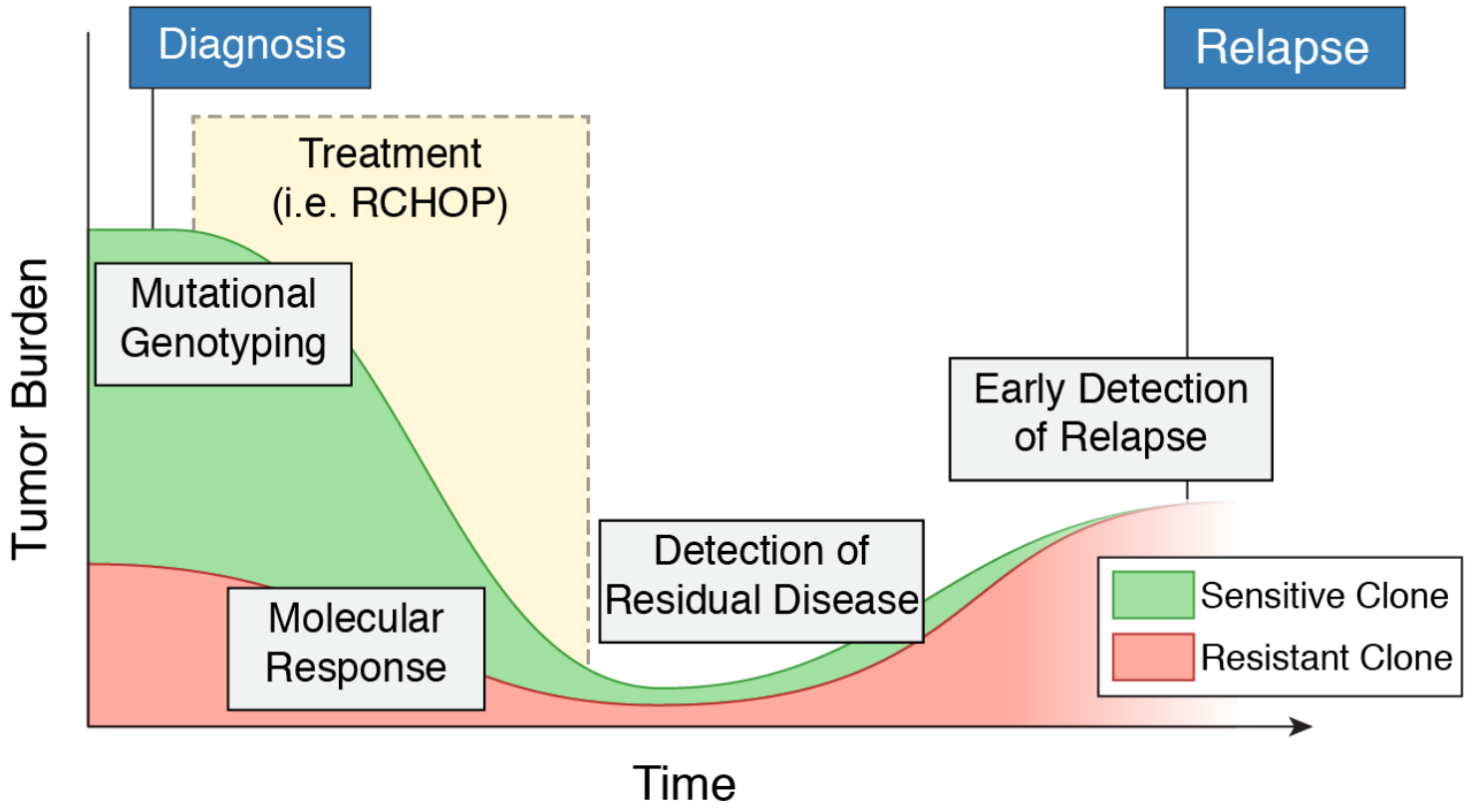
Adapted from Crowley, E. et al. Nat. Rev. Clin. Oncol. 2013.

In aggressive lymphomas tumor DNA is 150-fold more abundant in plasma than PBMC

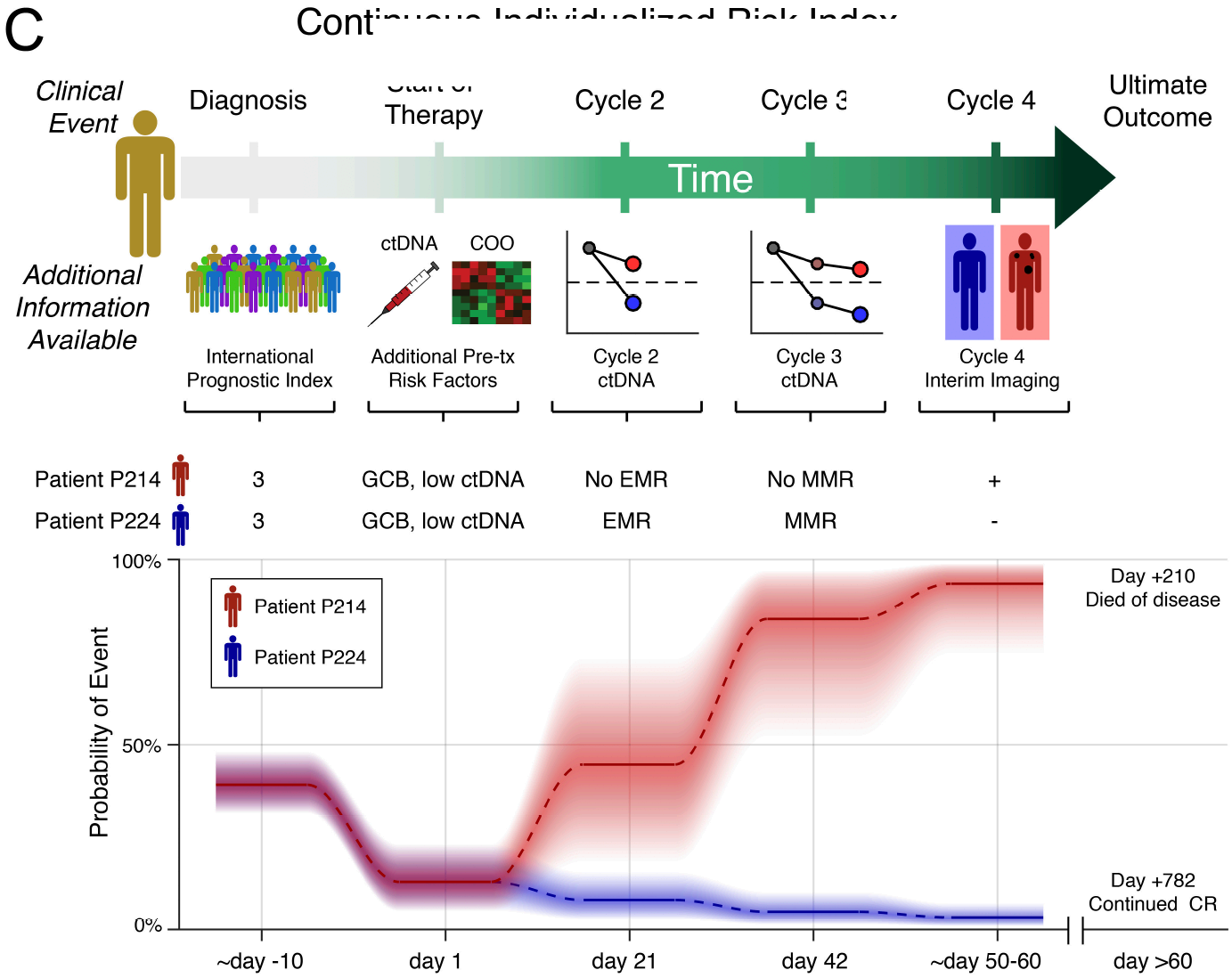


Kurtz DM et al. Blood, 2015
Herrera AF et al. Br J Haematol 2016

Breadth of Potential Applications for Liquid Biopsies

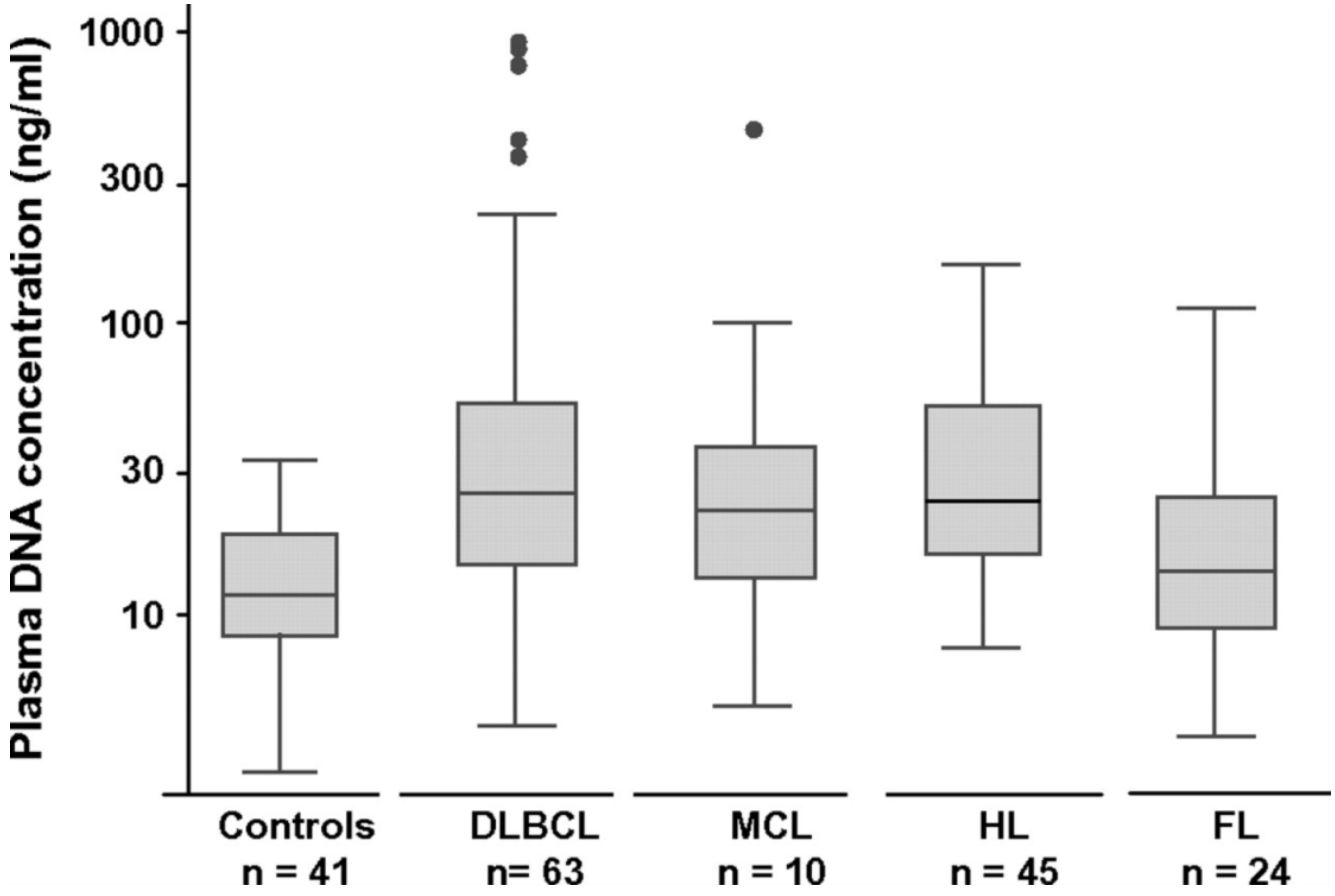


Dynamics of ctDNA predicts outcome continuously in DLBCL



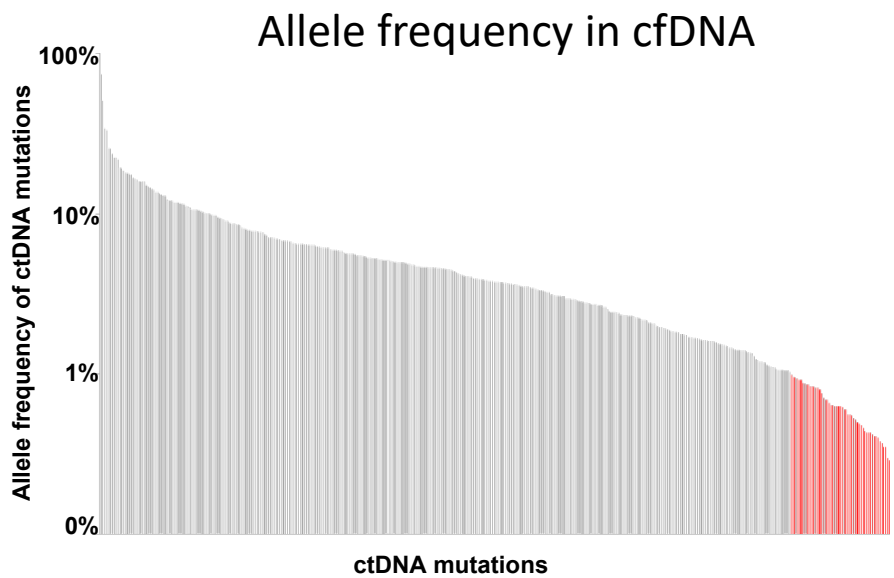
Kurtz et al. *Cell.* 2019 Jul 25;178(3):699-713

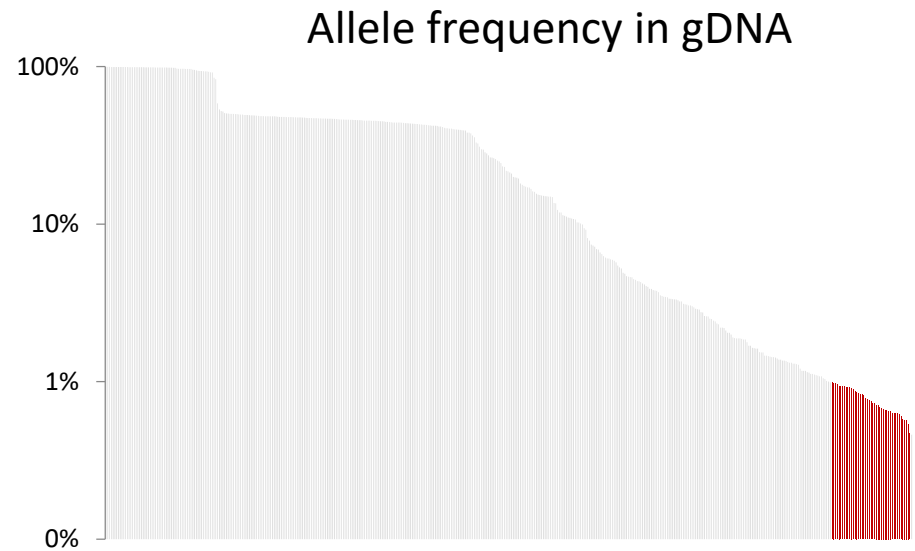
cfDNA circulates in small amounts

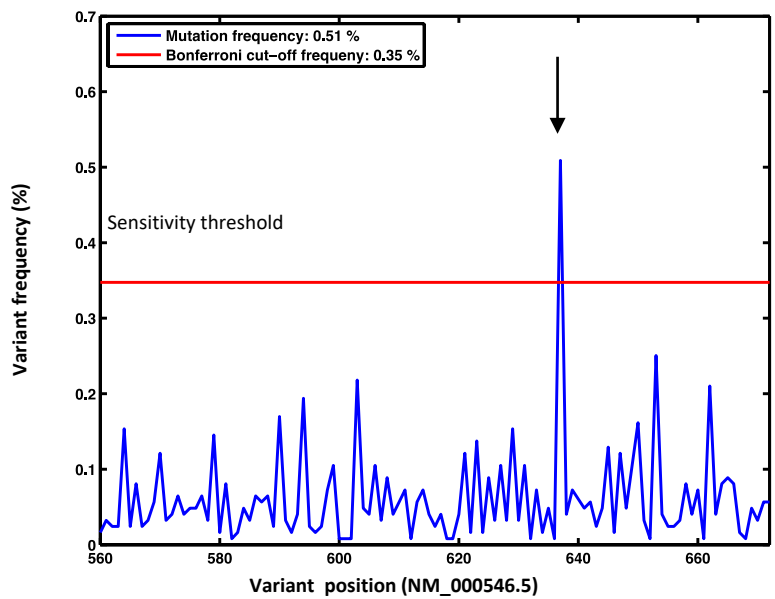


Hohaus S et al. Ann Oncol. 2009;20(8):1408-1413

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

Error Suppression Improves ctDNA Detection

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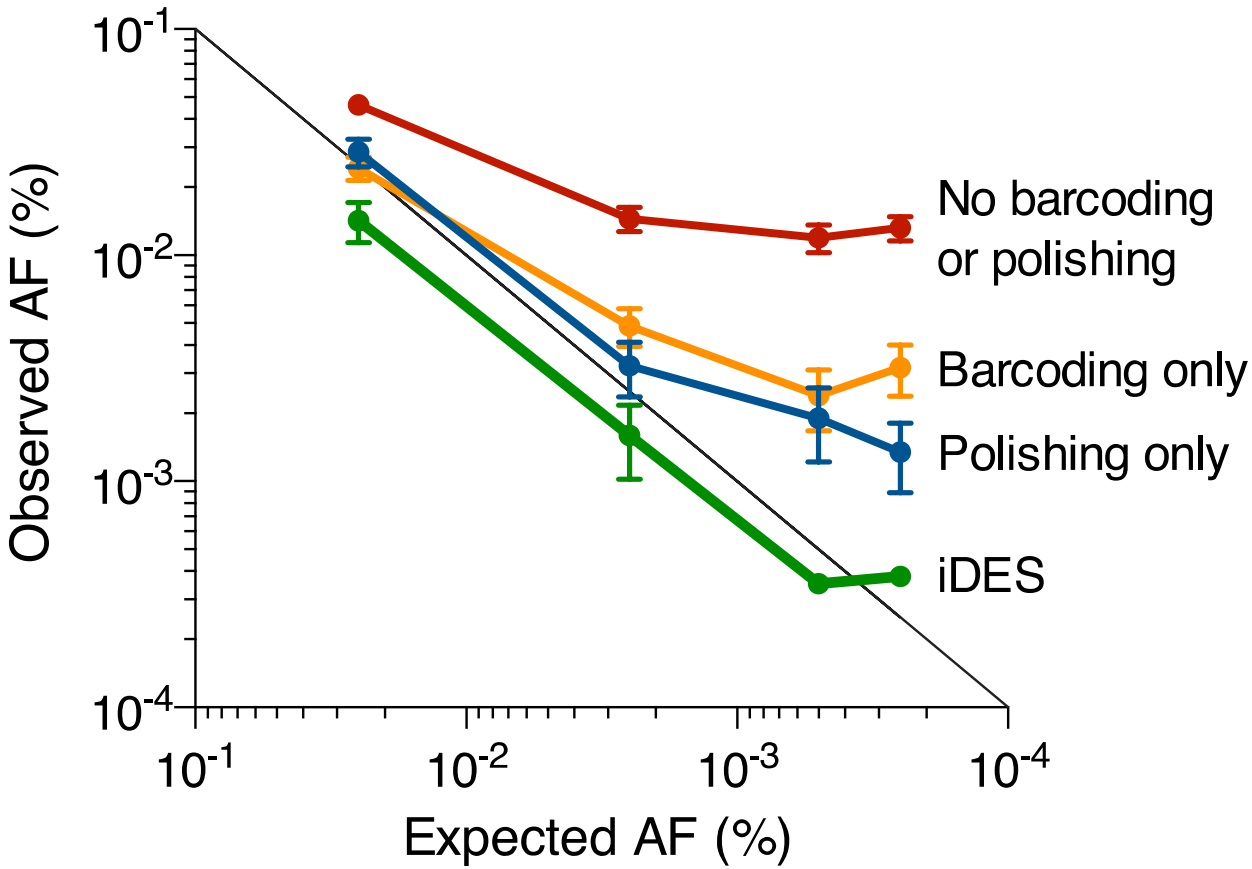
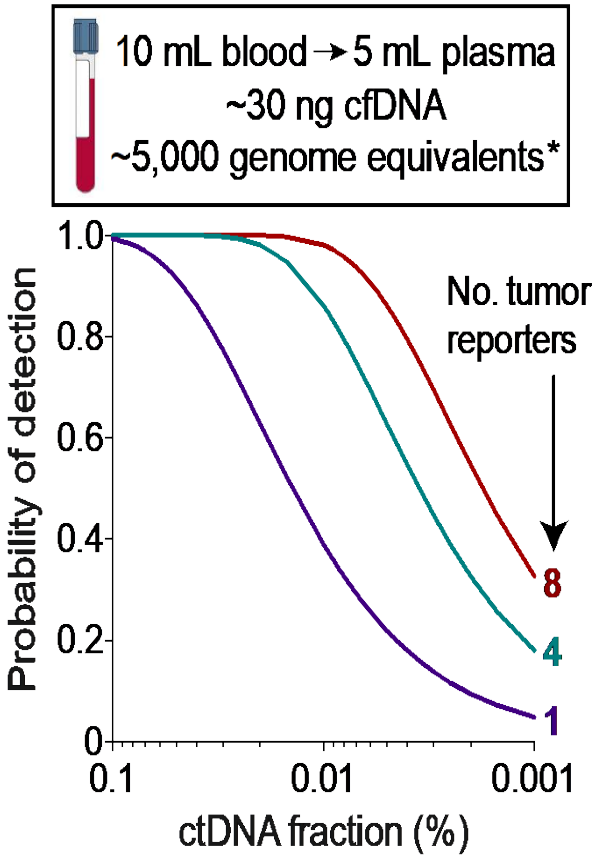
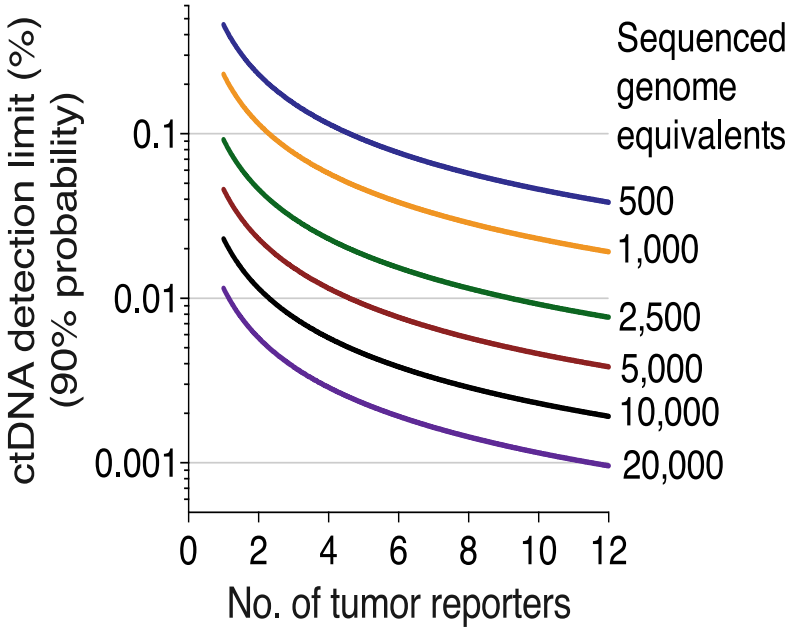


Newman, Lovejoy, and Klass et al. (2016) *Nature Biotechnology*

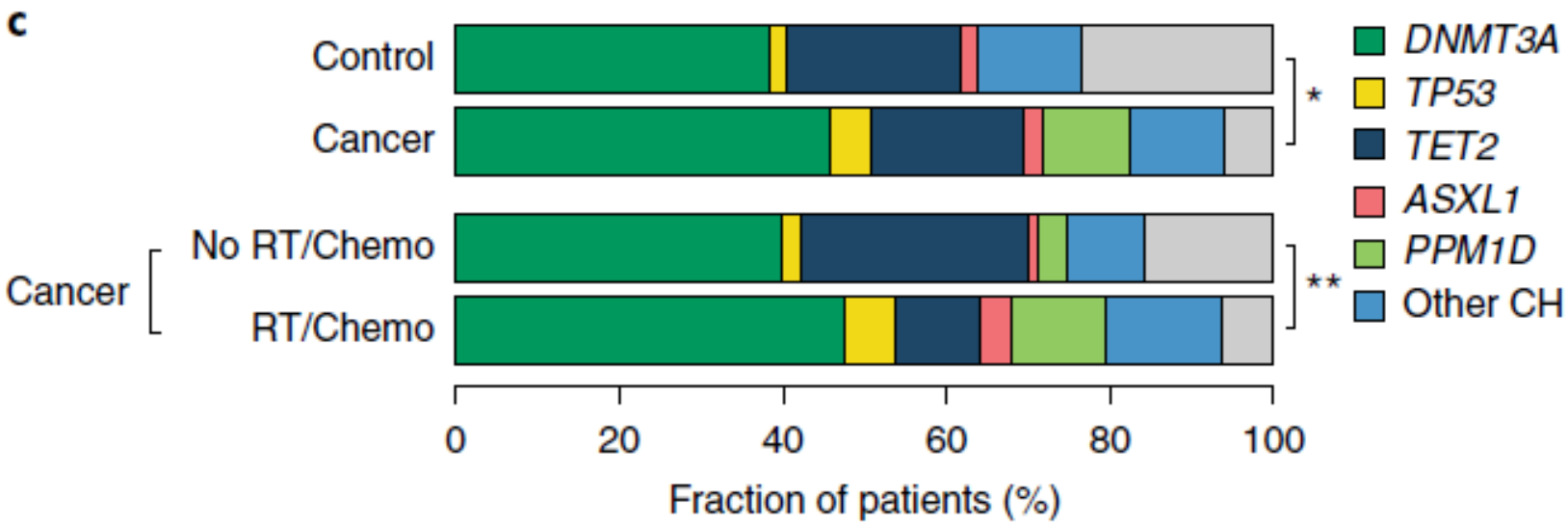
Technicalities that improve ctDNA detection



*assuming 50% recovery rate



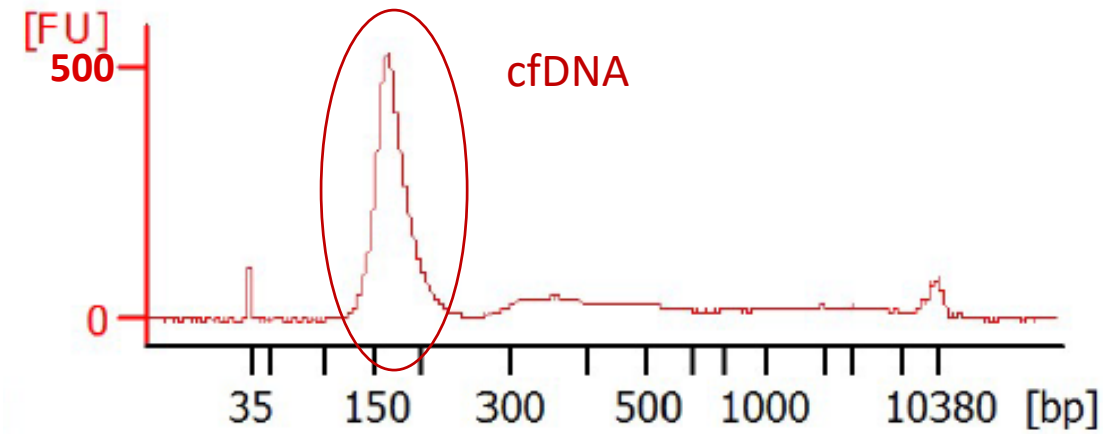
Analysis of paired germline DNA from blood cells suppresses
The biological background



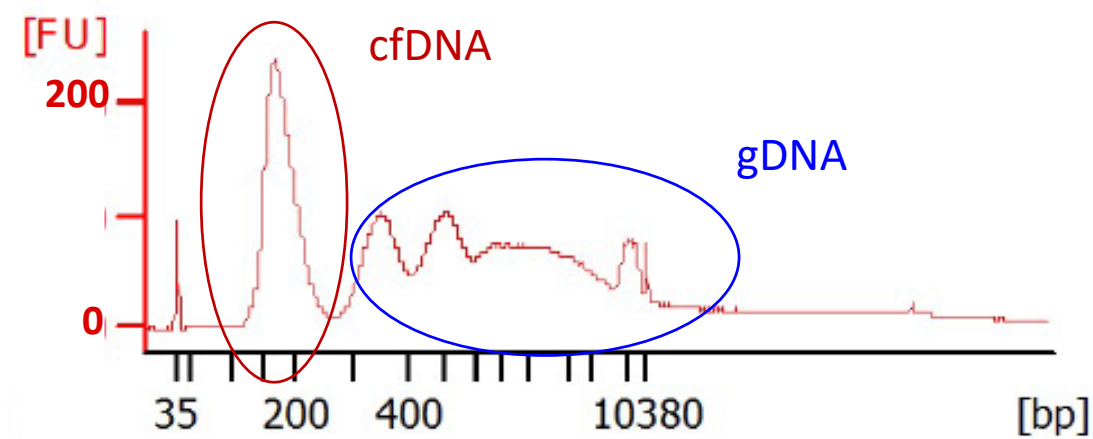
Razavi et al. Nature Medicine 2019

Pre-analytics is critical

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



cfDNA of poor quality: gDNA contamination



Plasma tubes

K2EDTA

Streck BCT

Roche CFD

- K2EDTA
  - Lowest cost
  - Must be processed within 6 hours
- Streck BCT
  - Higher cost
  - Can be at room temperature for up to 7 days
  - Glass
- Roche CFD
  - Higher cost
  - Can be at room temperature for up to 7 days
  - Plastic

LyV4.0 ctDNA CAPP-seq assay

Target region

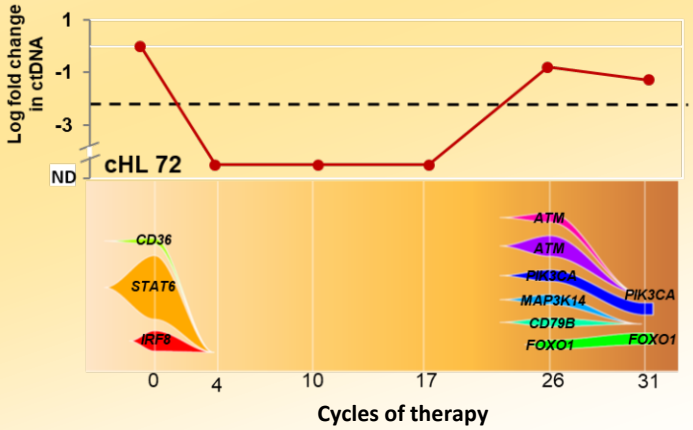
- ~ 340 kb
- 103 genes recurrently mutated in CLL and lymphomas
- 30 non-coding region targeted by aberrant somatic hypermutation

Ultra deep sequencing

- Paired cfDNA and gDNA from granulocytes
- Coverage ≥ 2000× in ≥ 80% of the target region

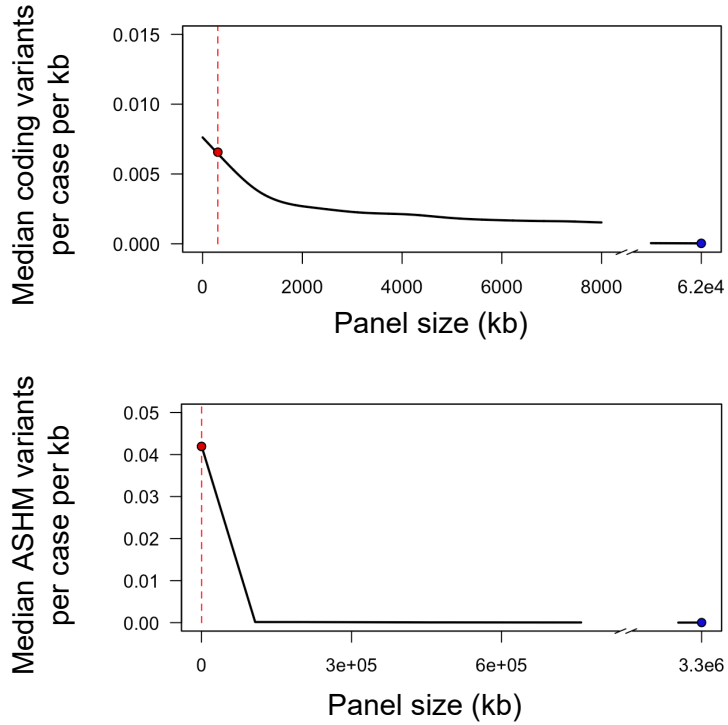
Bioinformatics

- Error suppression pipeline



Technical validation of the LyV4.0 ctDNA CAPP-seq assay for genotyping and residual disease quantification

A



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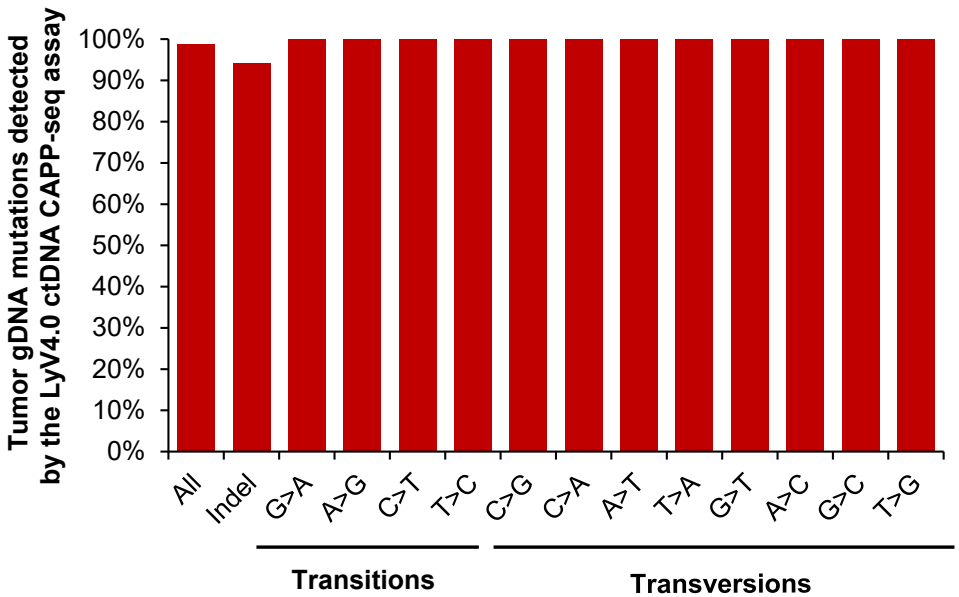

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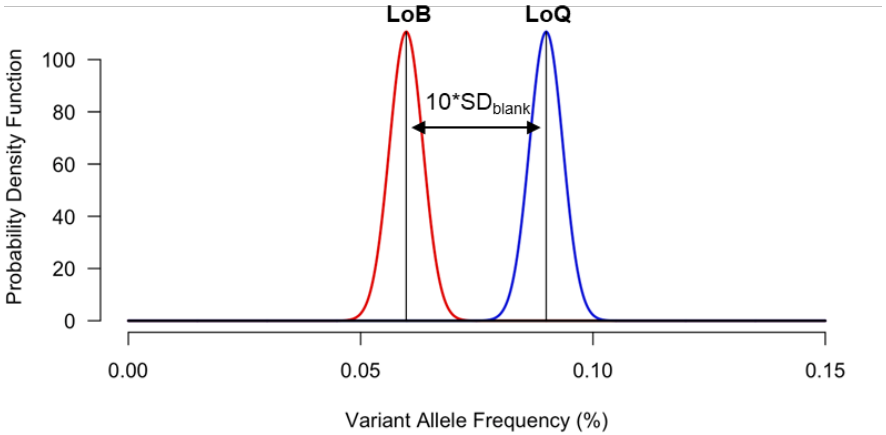

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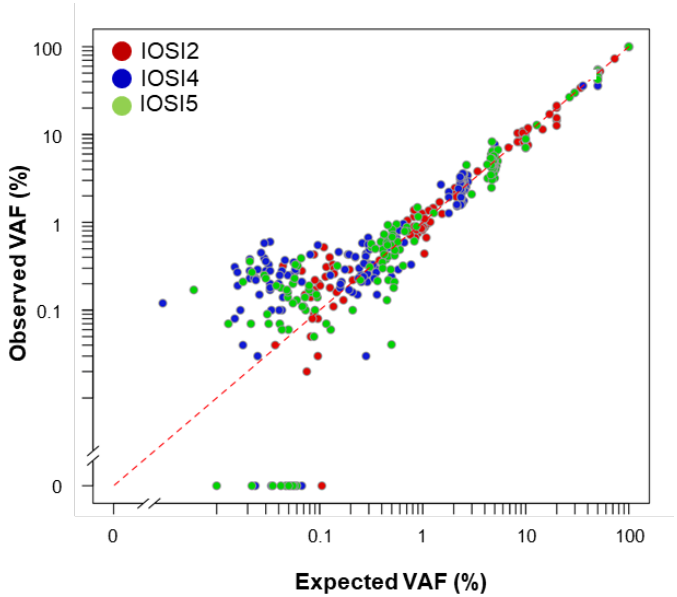
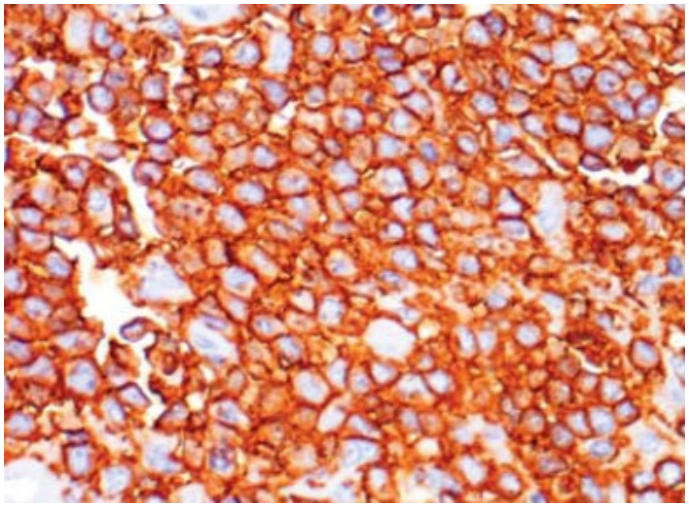


Opportunities from ctDNA genotyping in cHL

iOR

DLBCL

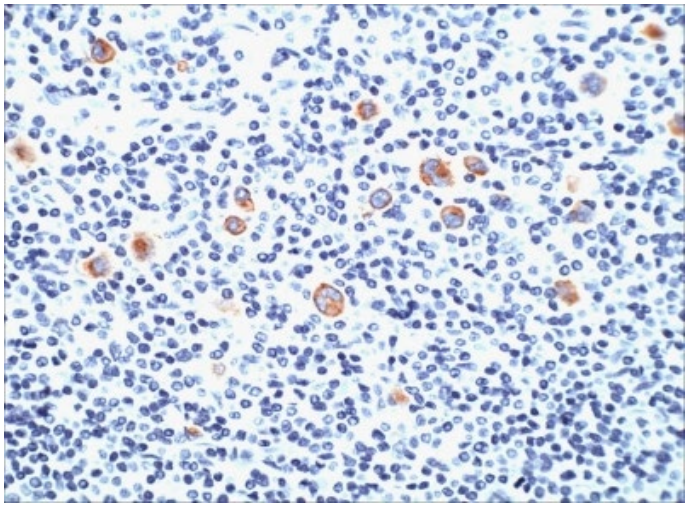
Tumor cells are enriched in the mass



Sequencing data from >1000 cases

cHL

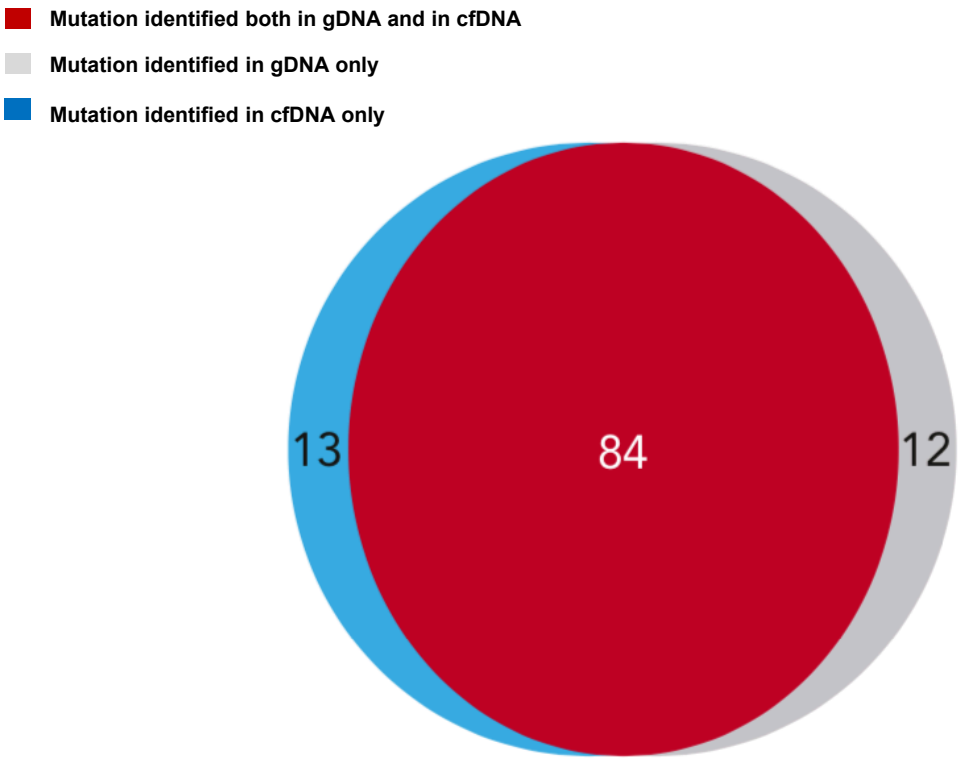
Tumor cells are rare in the mass

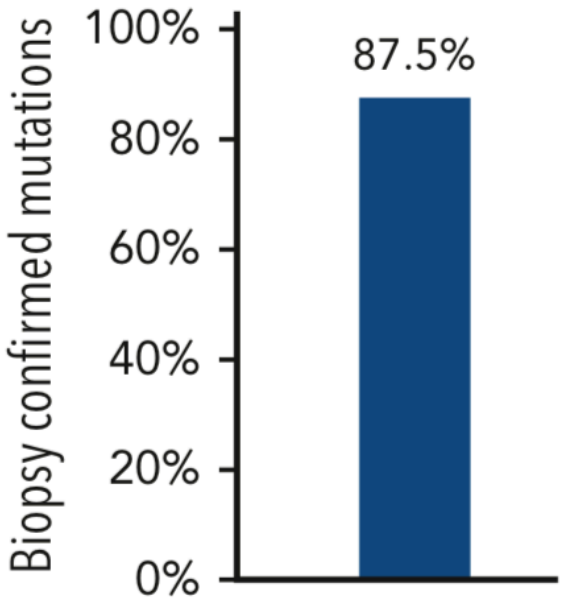


Sequencing data from 44 cases

Tiacci E et al. Blood 2018
Reichel J, et al. Blood 2015

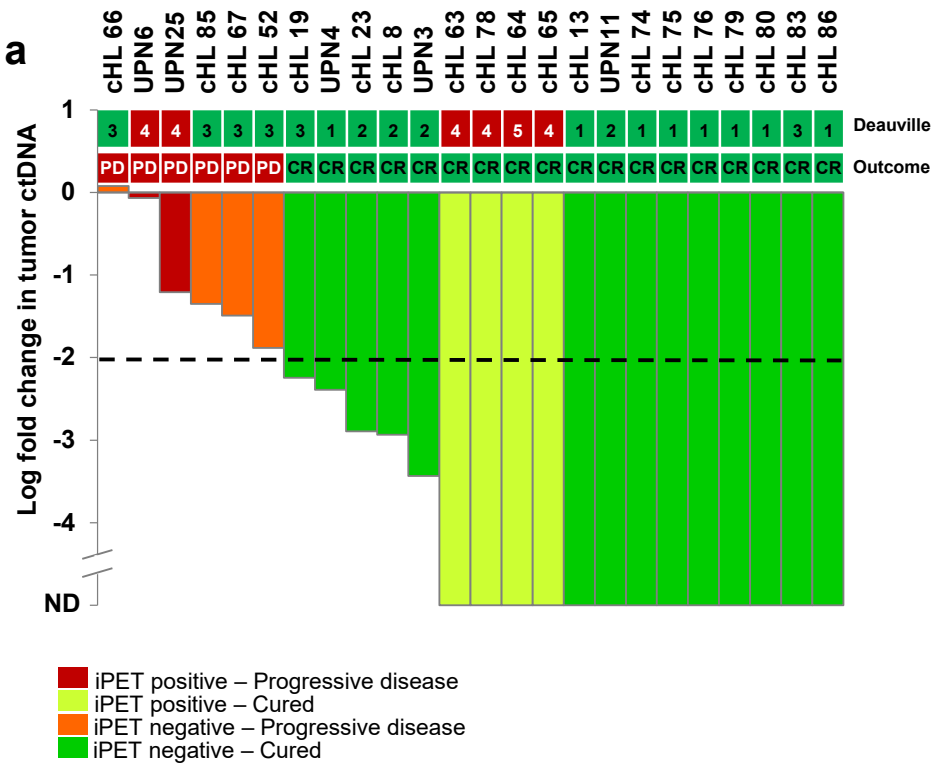
Plasma Can Identify Unique Mutations in HL

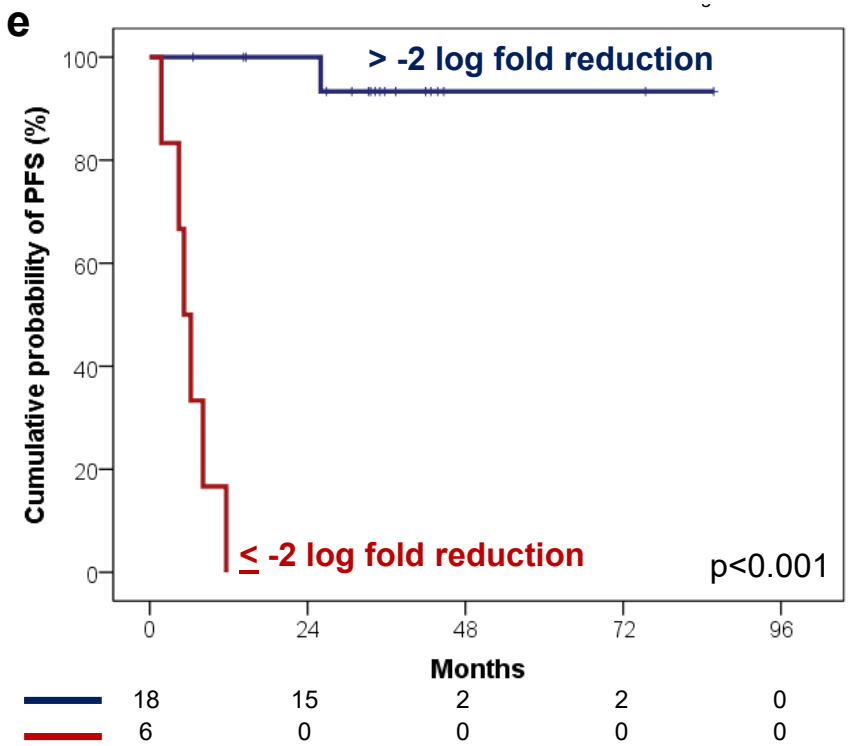




Spina et al. *Blood* 2018 May 31;131(22):2413-2425

ctDNA complements iPET in cHL





Spina V, et al. Blood 2018

Plasma Can Identify Unique Mutations in DLBCL

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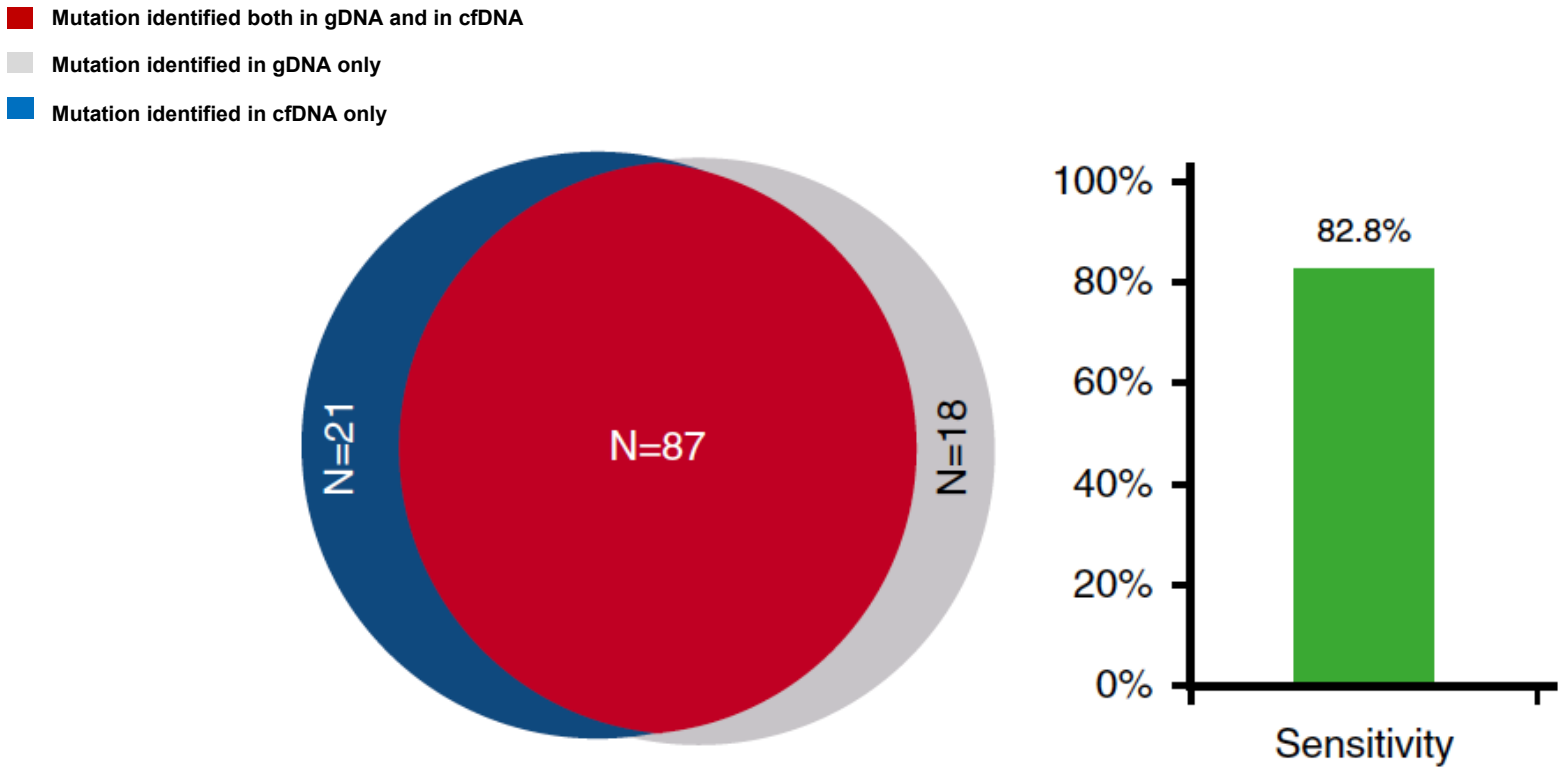
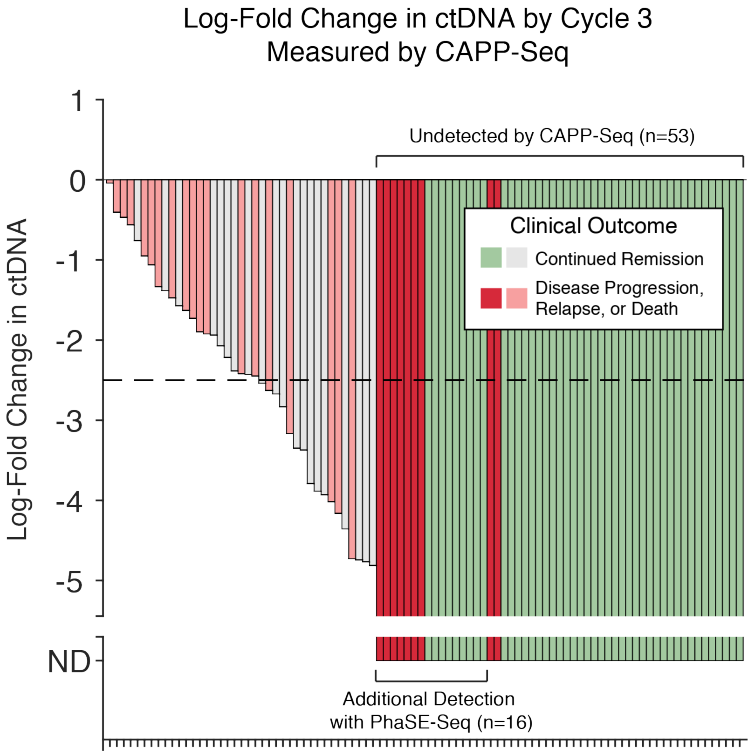
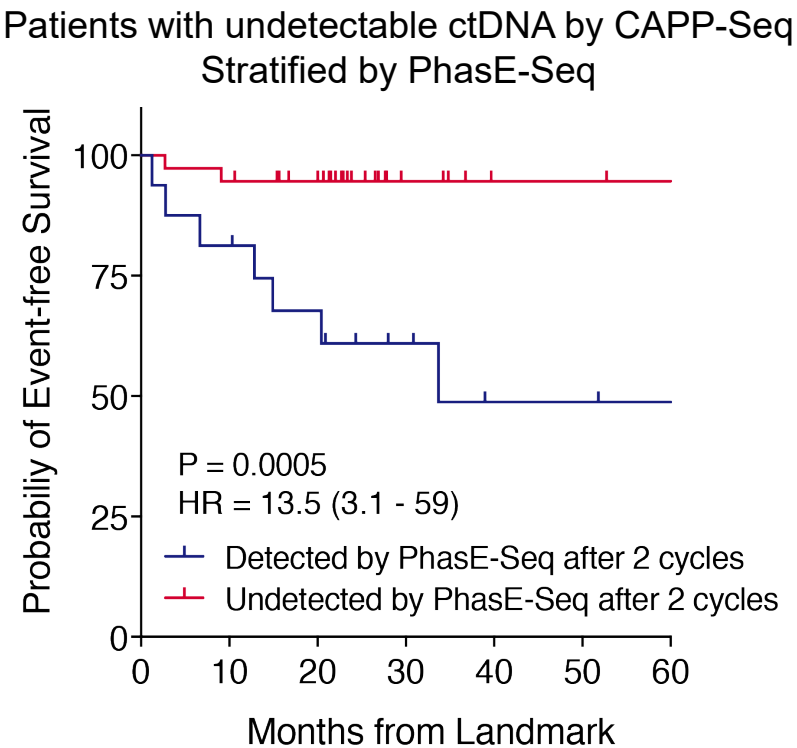


Rossi et al. *Blood.* 2017 Apr 6;129(14):1891-1893

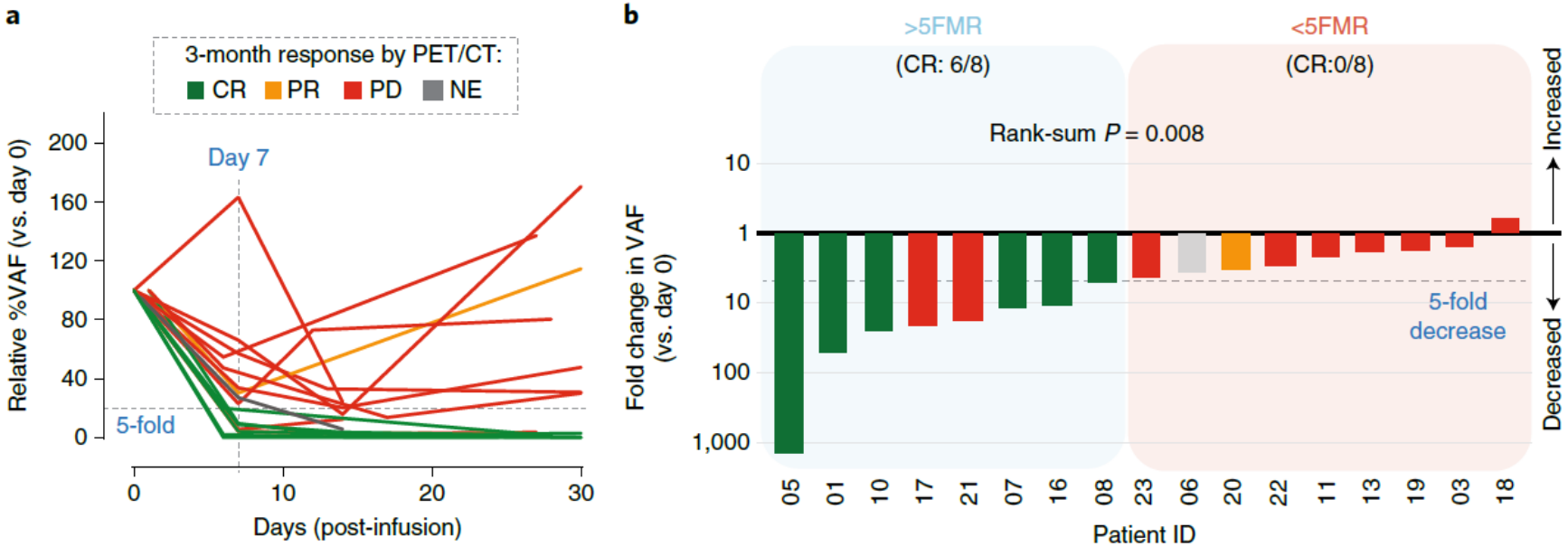
ctDNA complements iPET in DLBCL





Kurtz DM et al, *ASH* 2019

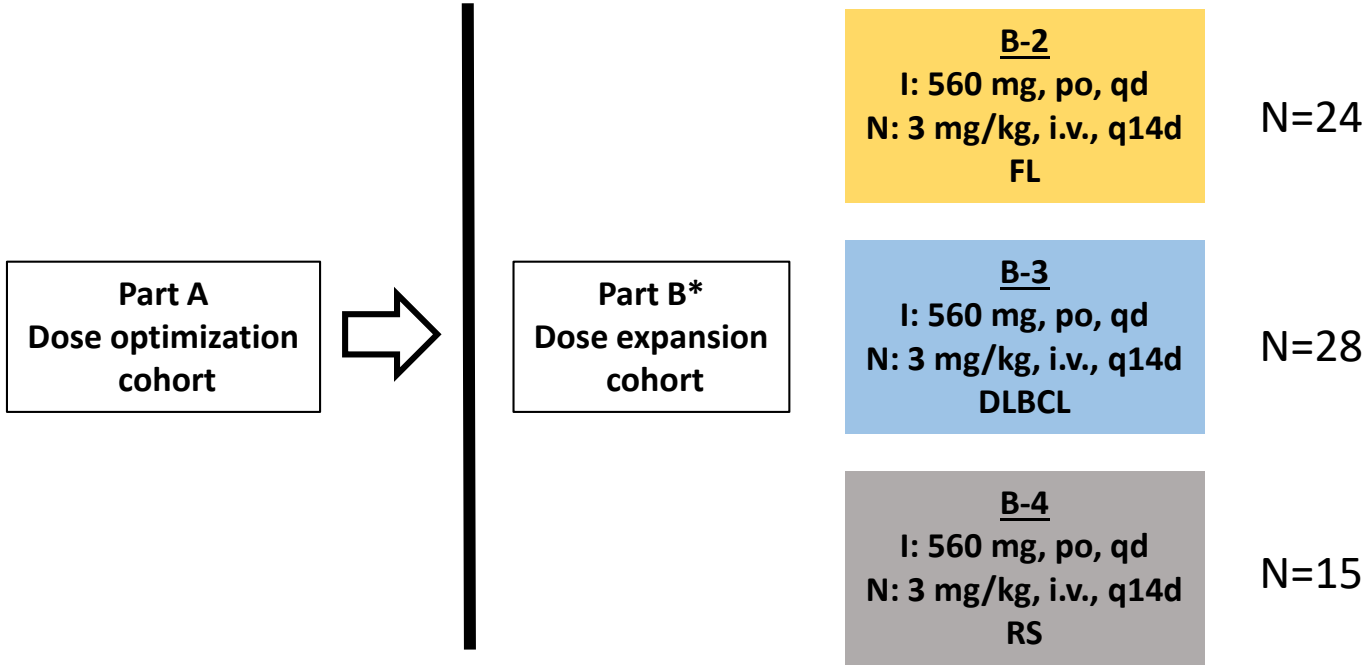
Response dynamics within the first week of treatment provides insights into CAR T cell clinical activity



Deng Q, Nat Med. 2020

Clinical validation

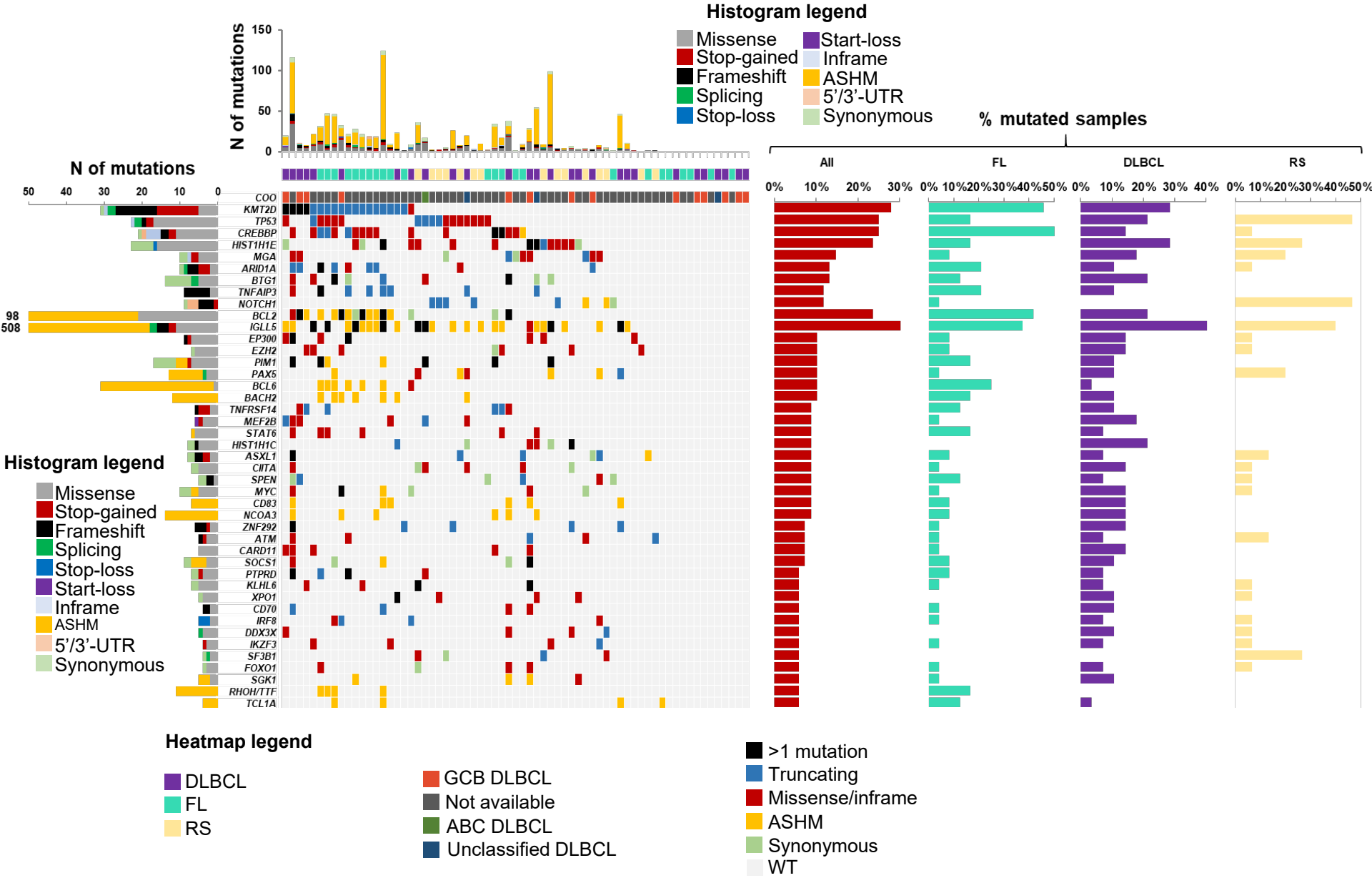
LYM1002 study



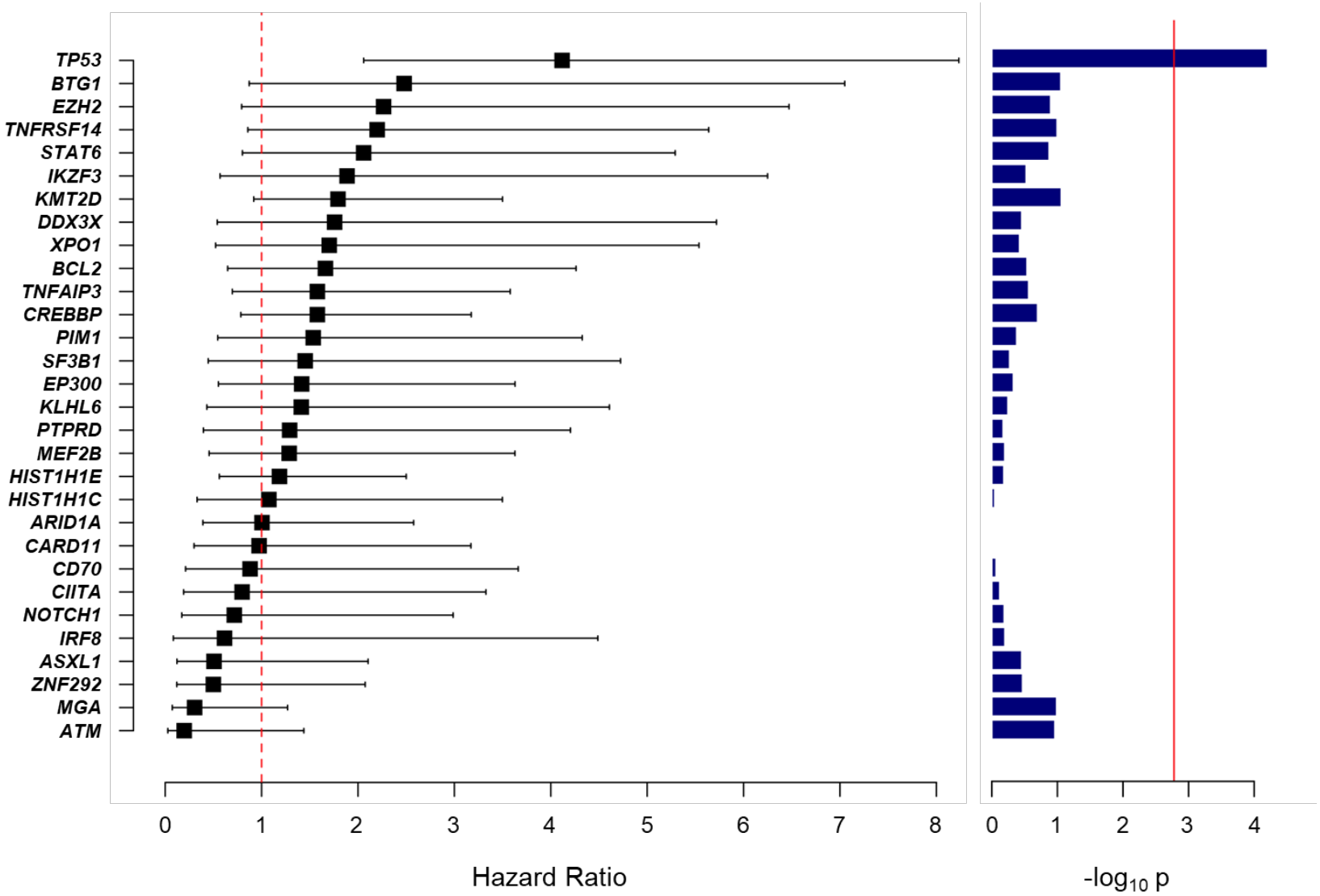
ClinicalTrials.gov Identifier: NCT02329847
Younes A, et al. *Lancet Haematol.* 2019;6(2):e67-e68.

*Cohort B1 CLL/SLL was not included in this analysis.
DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; I, ibrutinib; iv, intravenous; N, nivolumab; po, oral; RS, Richter syndrome; qd, once daily; q14d, every 2 weeks.

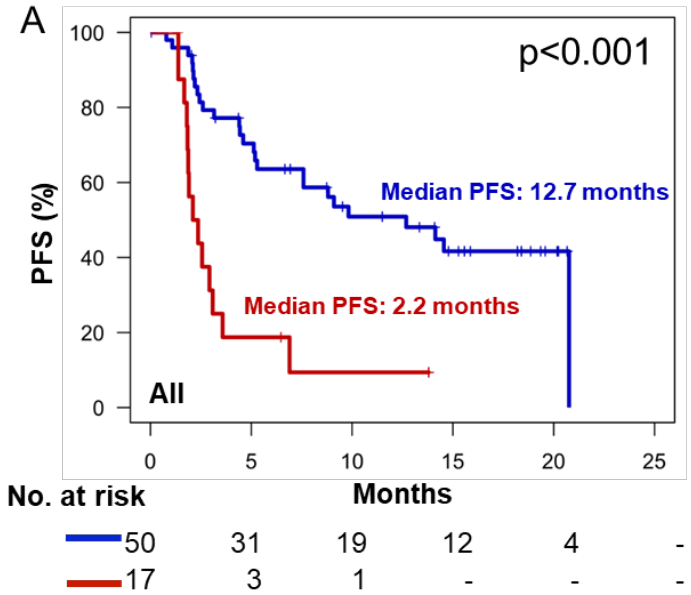
Pre-treatment mutation profile of lymphoma treated in the LYM1002 trial

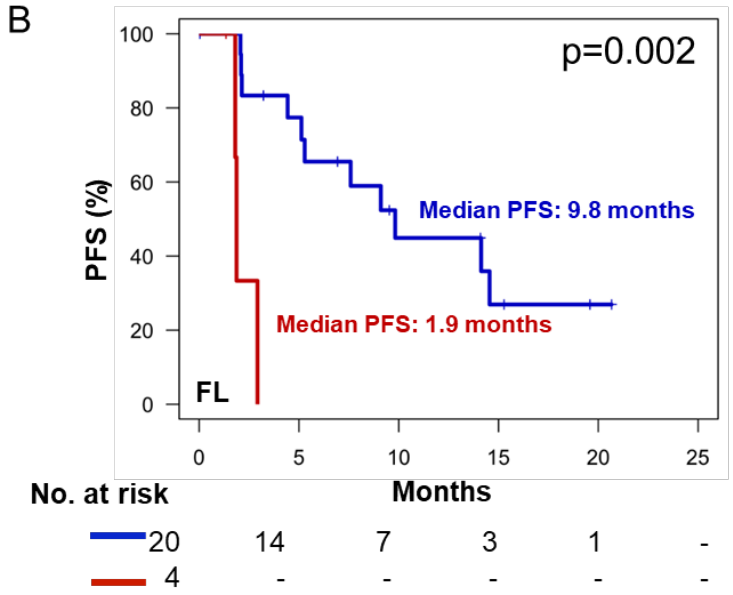


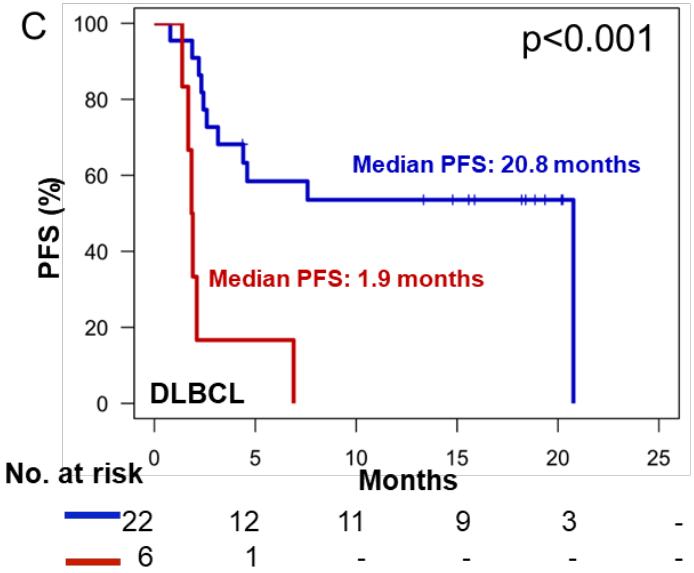
Prognostic value of pretreatment ctDNA

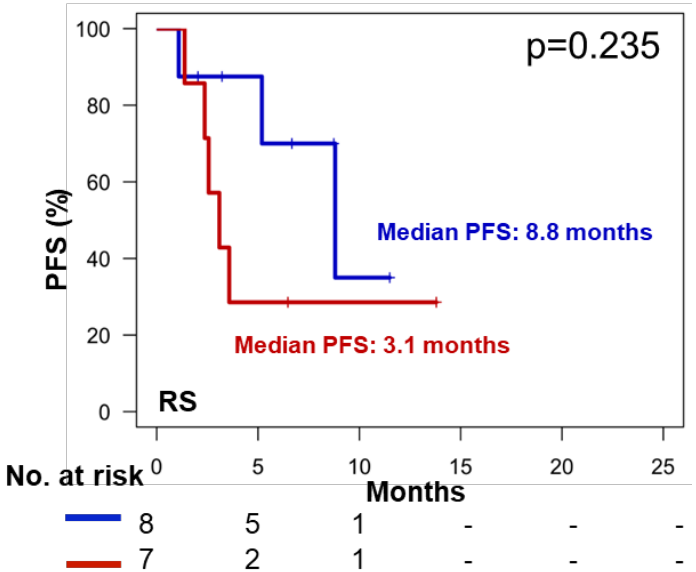


Shorter PFS in patients having *TP53* mutations in pre-treatment ctDNA

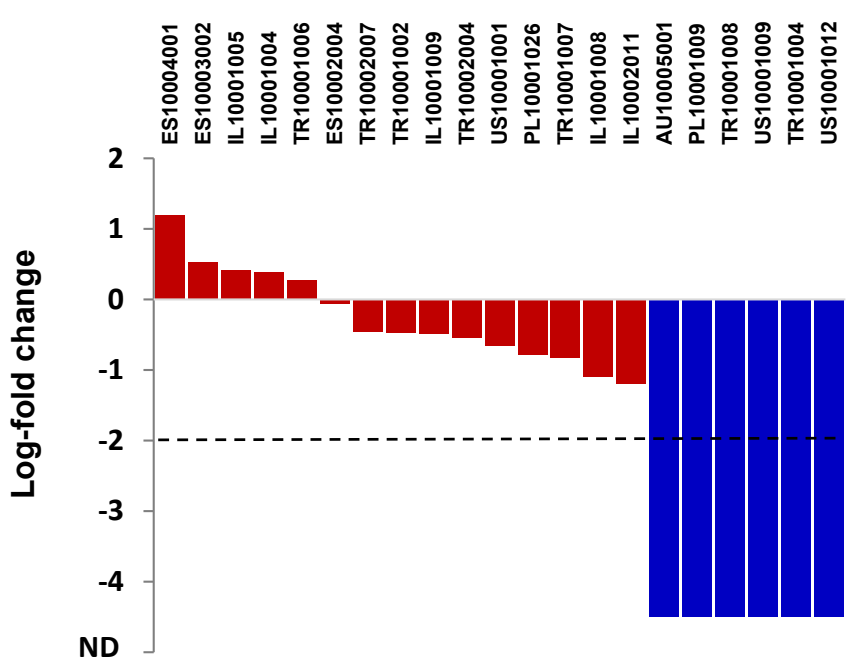


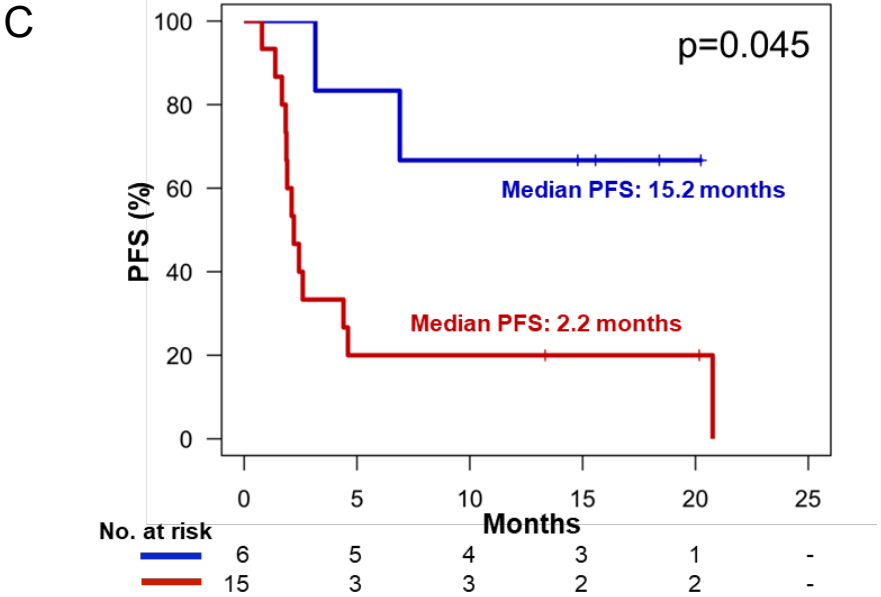


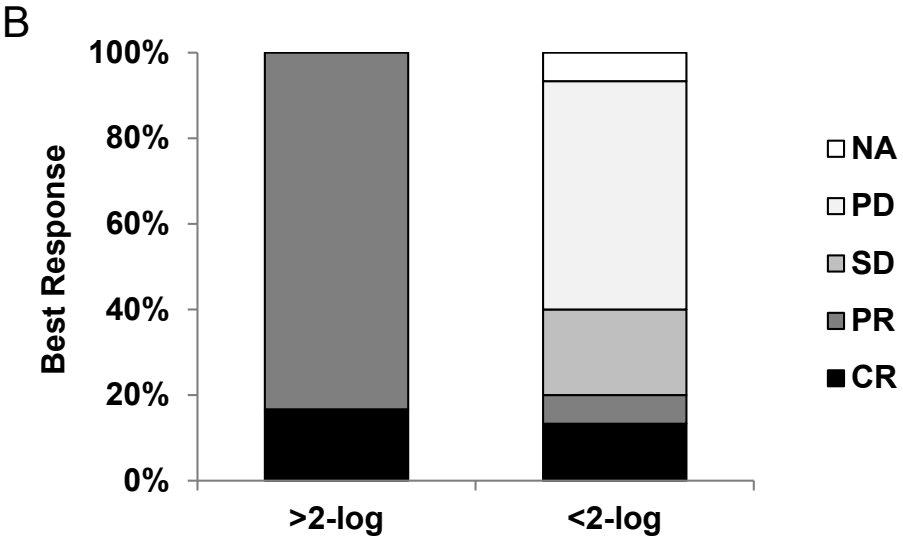




ctDNA dynamics in relapse DLBCL predict outcome under targeted therapy with ibrutinib plus nivolumab

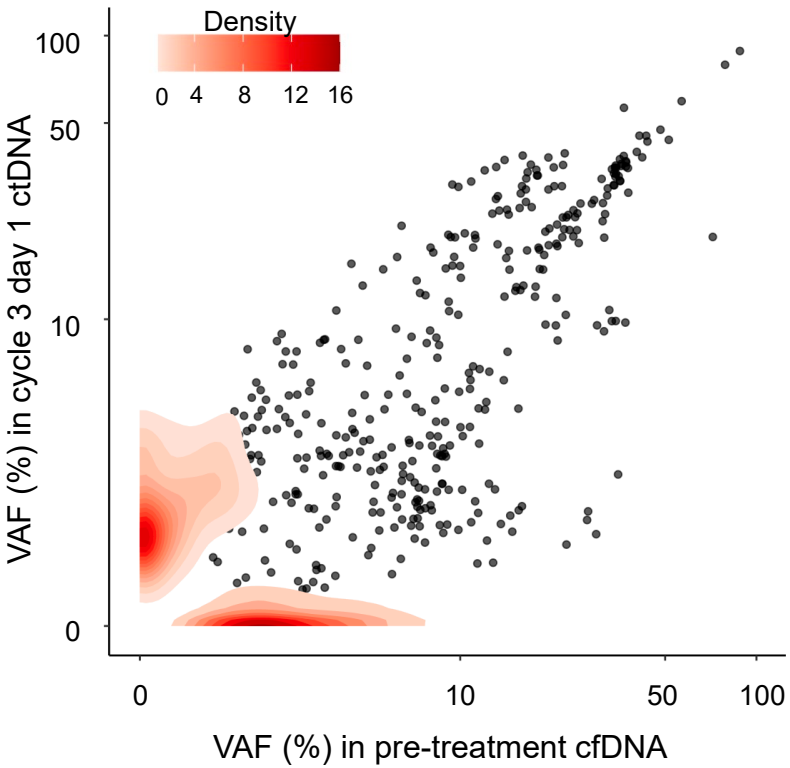






Clonal evolution in LYM1002 biomarker cohort

A



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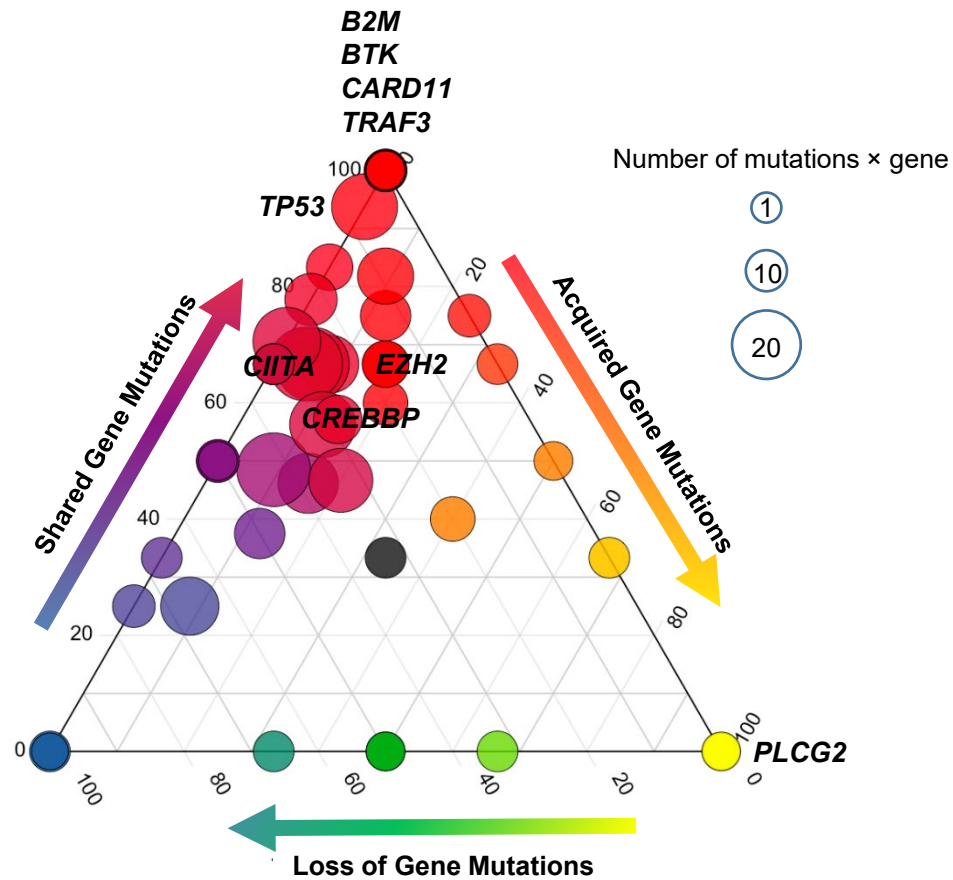
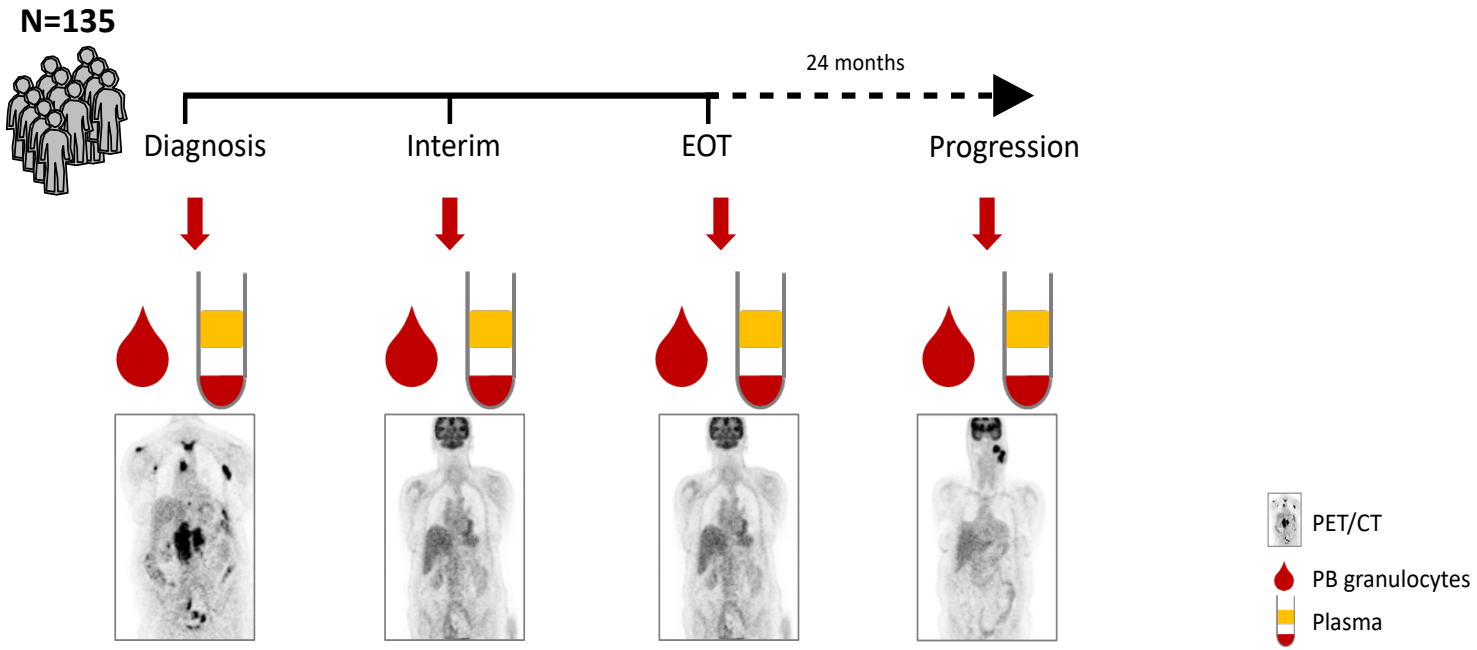
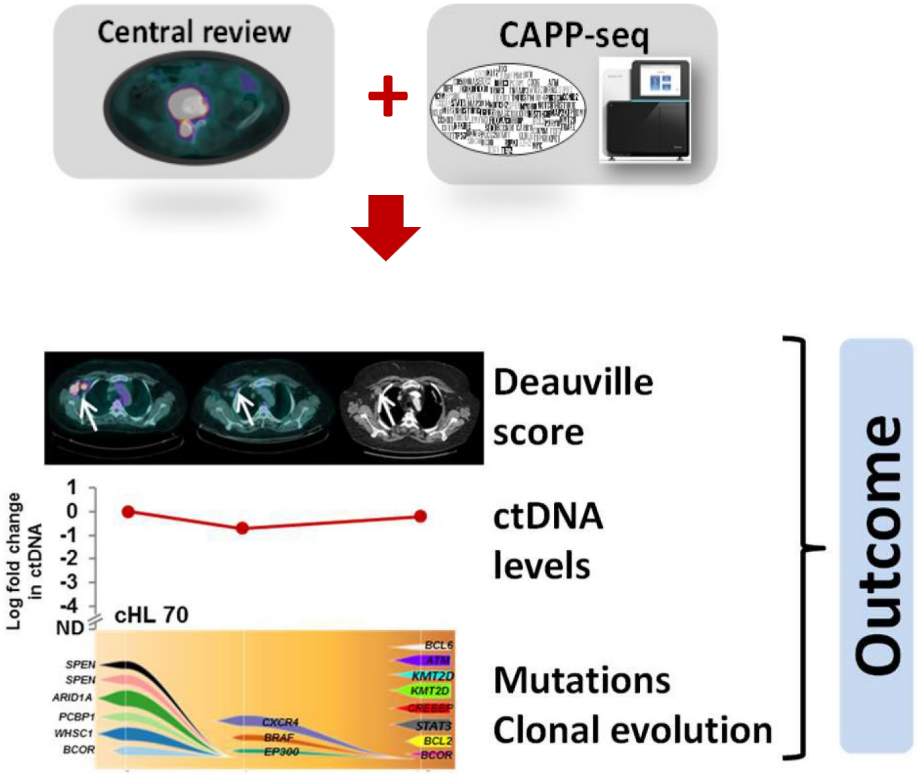


IOSI-EMA-003 study at a glance



Methodology



LyV4.0 ctDNA CAPP-seq assay

Target region

- ~ 340 kb
- 103 genes recurrently mutated in CLL and lymphomas
- 30 non-coding region targeted by aberrant somatic hypermutation

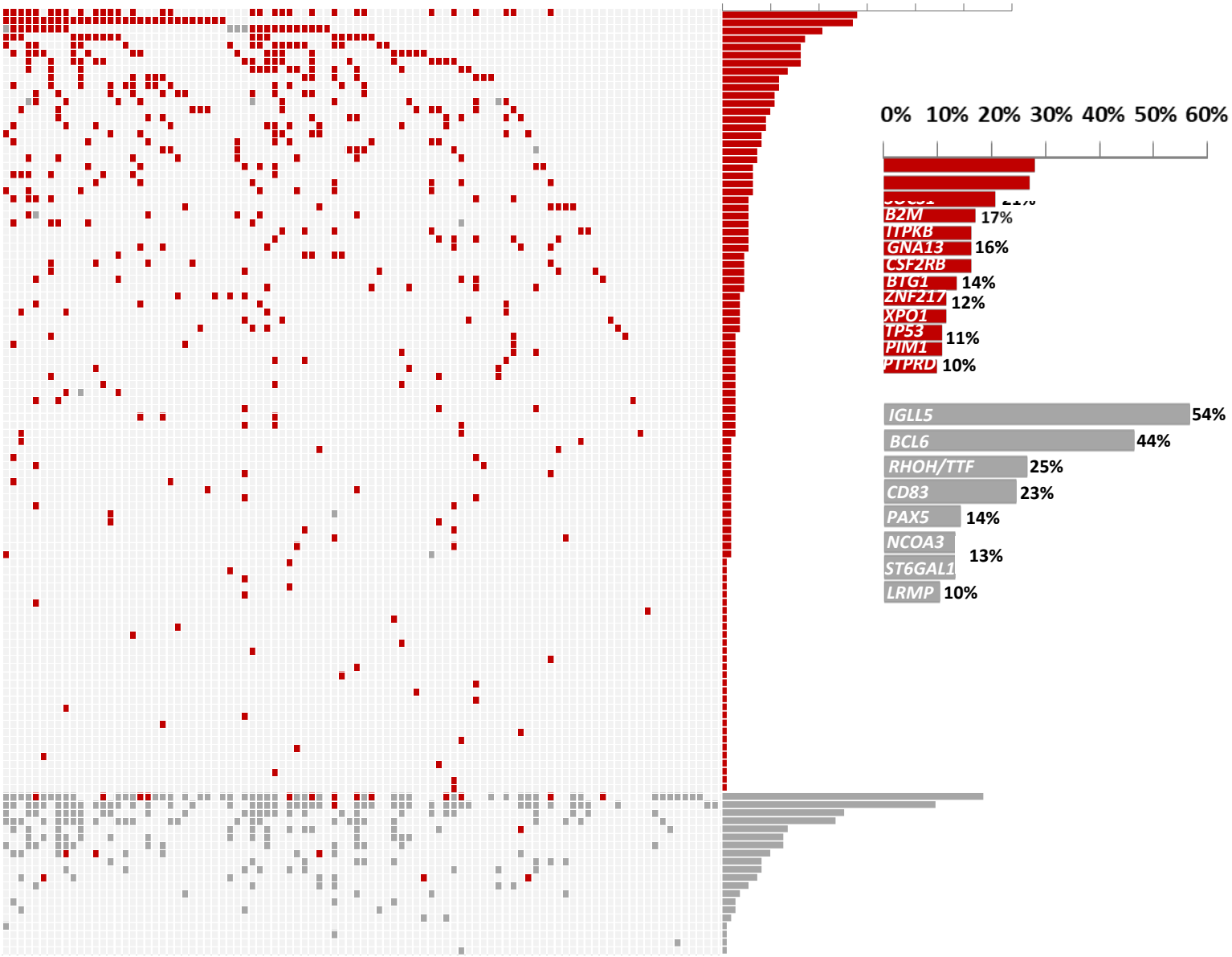
Ultra deep sequencing

- Paired cfDNA and gDNA from granulocytes
- Coverage ≥ 2000× in ≥ 80% of the target region

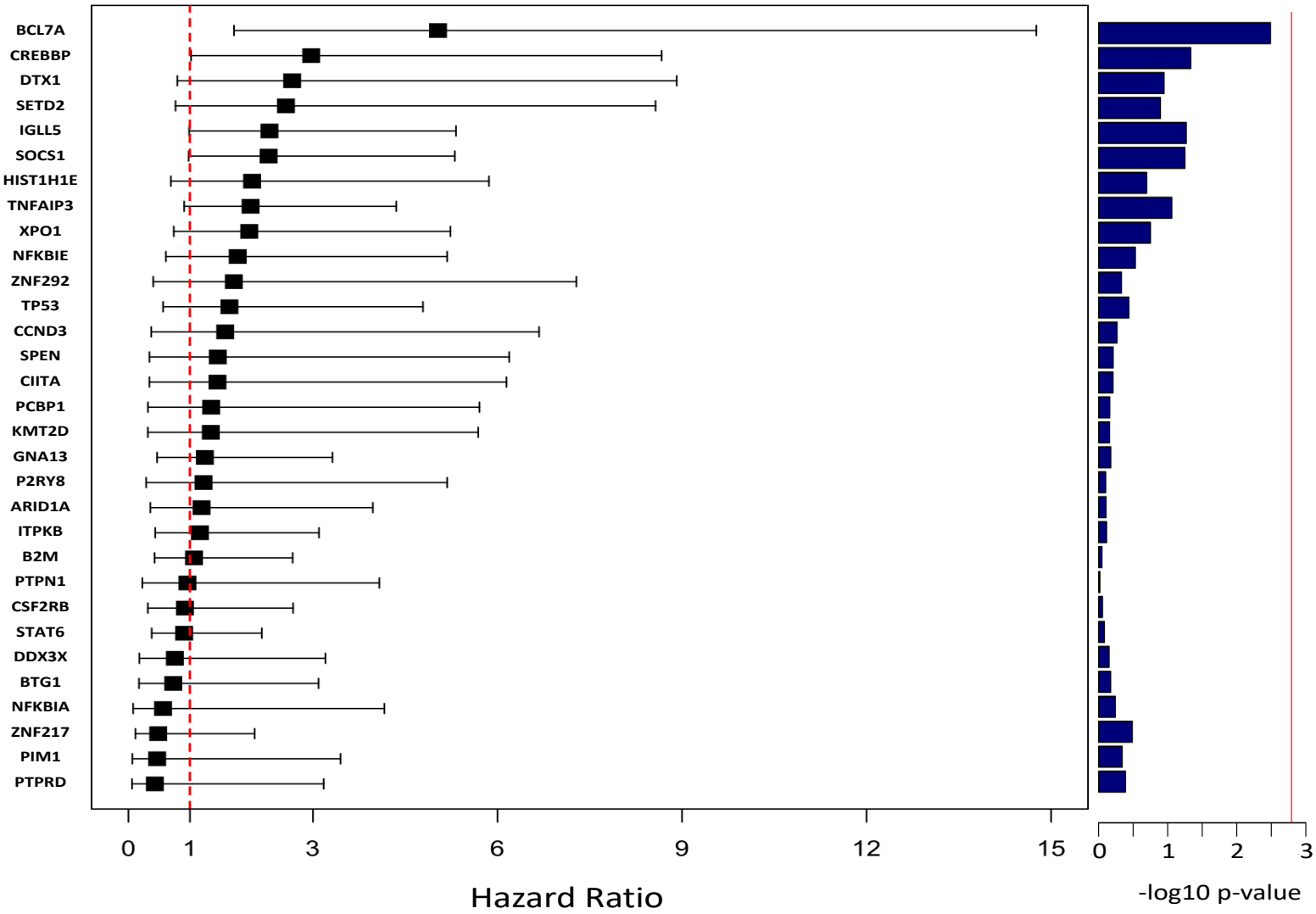
Bioinformatics

- Error suppression pipeline
- Analytical sensitivity: 10^{-3}

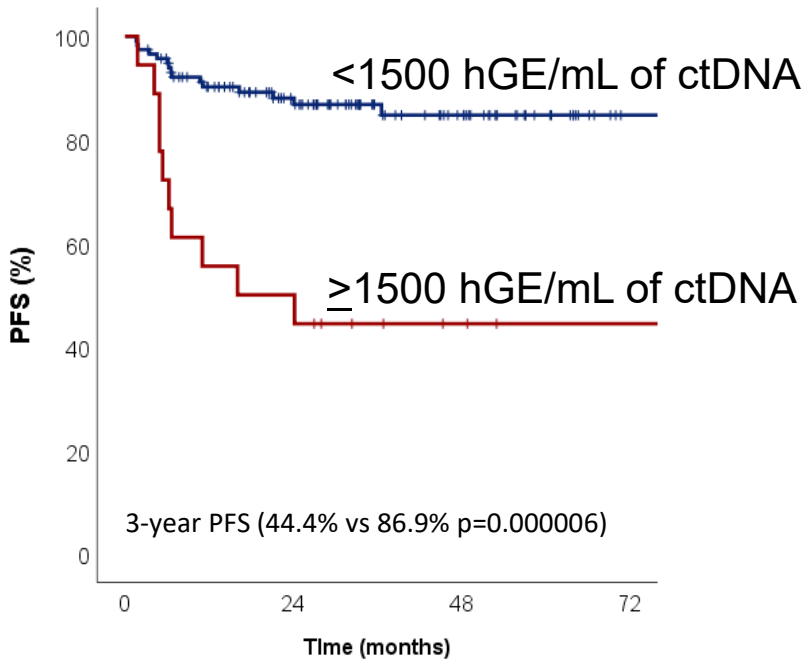
IOSI-EMA-003: baseline genotype

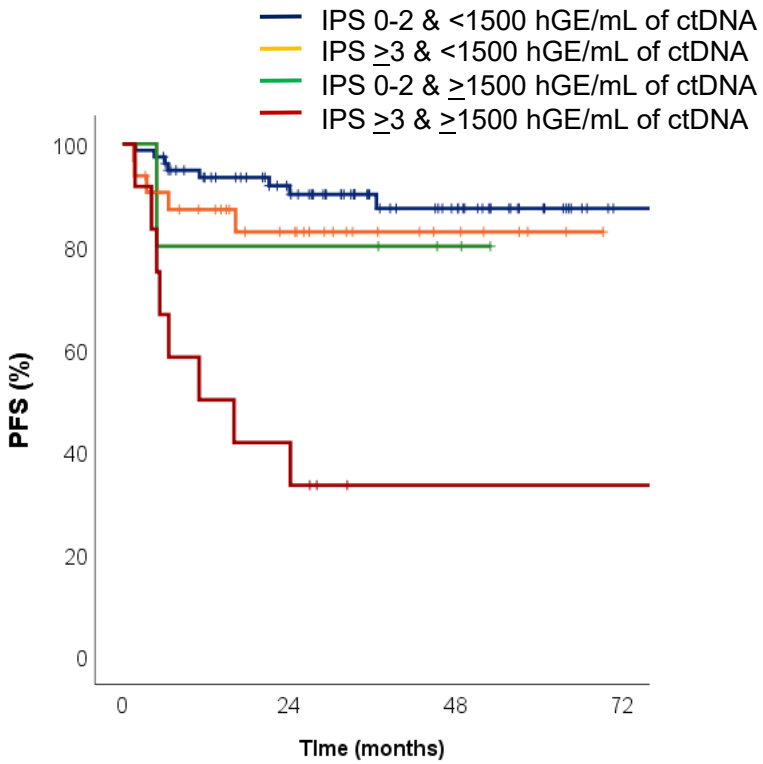


IOSI-EMA-003: baseline genotype and outcome



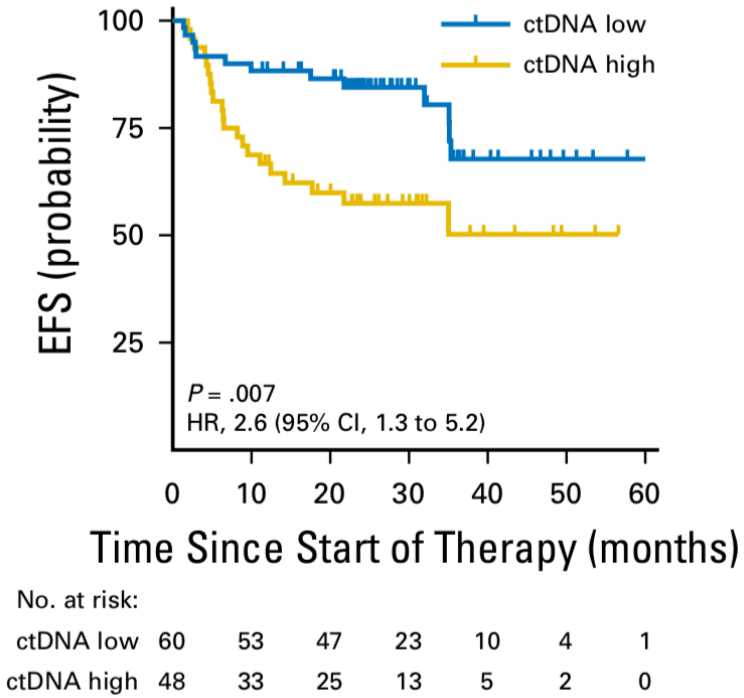
IOSI-EMA-003: baseline ctDNA load and outcome

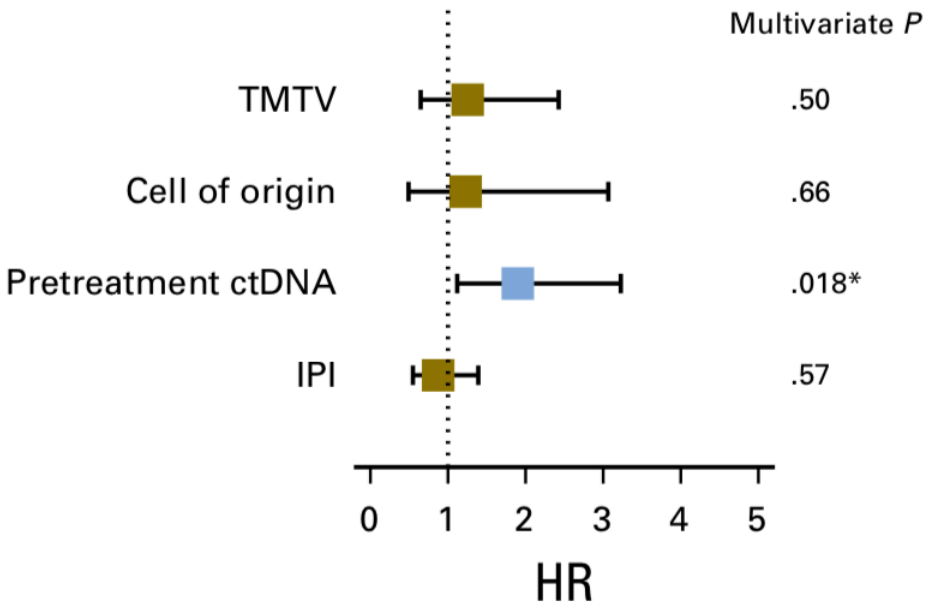




Strata	% of pts	3-year PFS
IPS 0-2 & <1500 hGE/mL of ctDNA	58%	90.1%
IPS ≥3 & <1500 hGE/mL of ctDNA	24%	82.8%
IPS 0-2 & ≥1500 hGE/mL of ctDNA	4%	80%
IPS ≥3 & ≥1500 hGE/mL of ctDNA	9%	33.3%

Quantitative levels of baseline ctDNA are prognostic in DLBCL

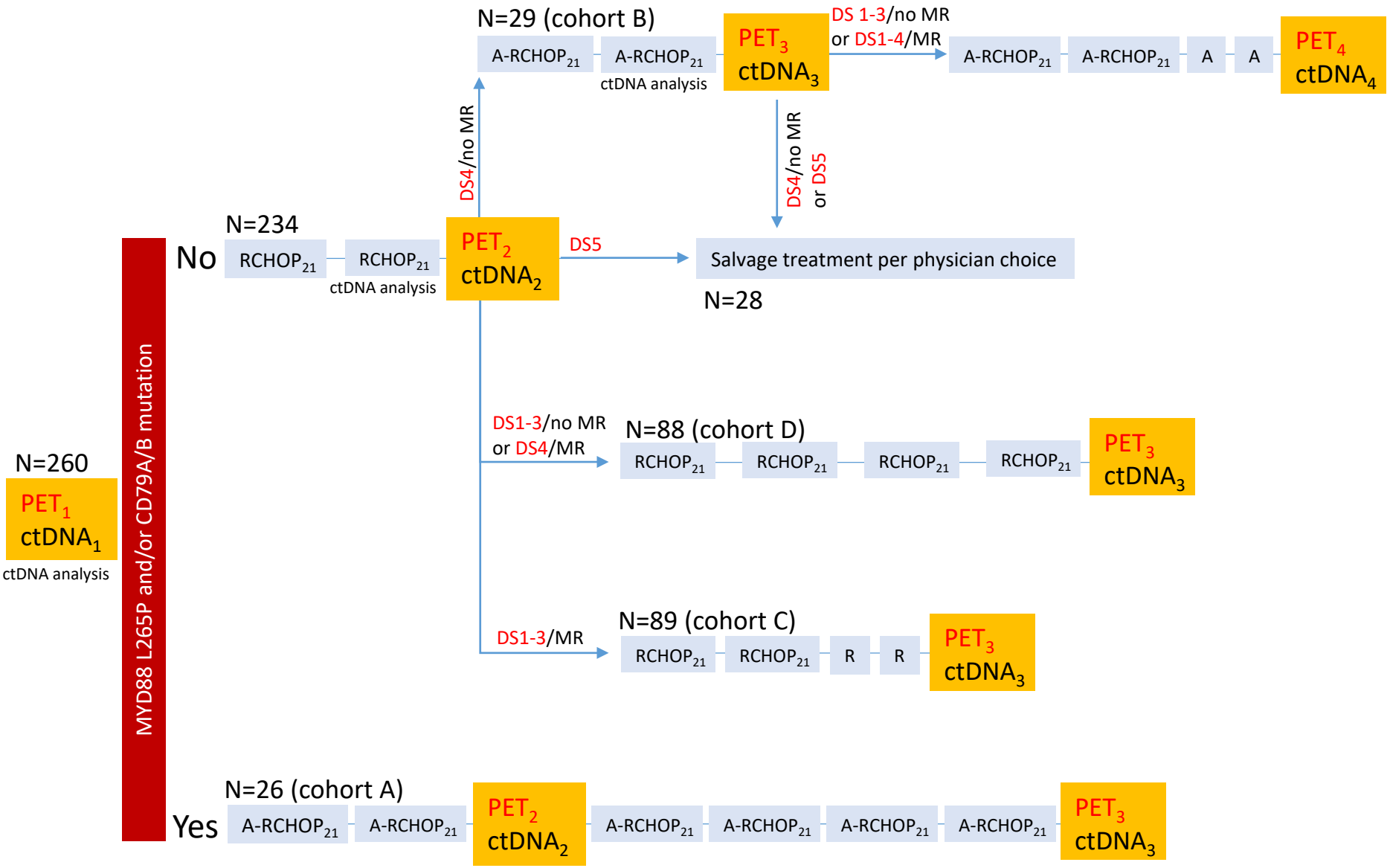




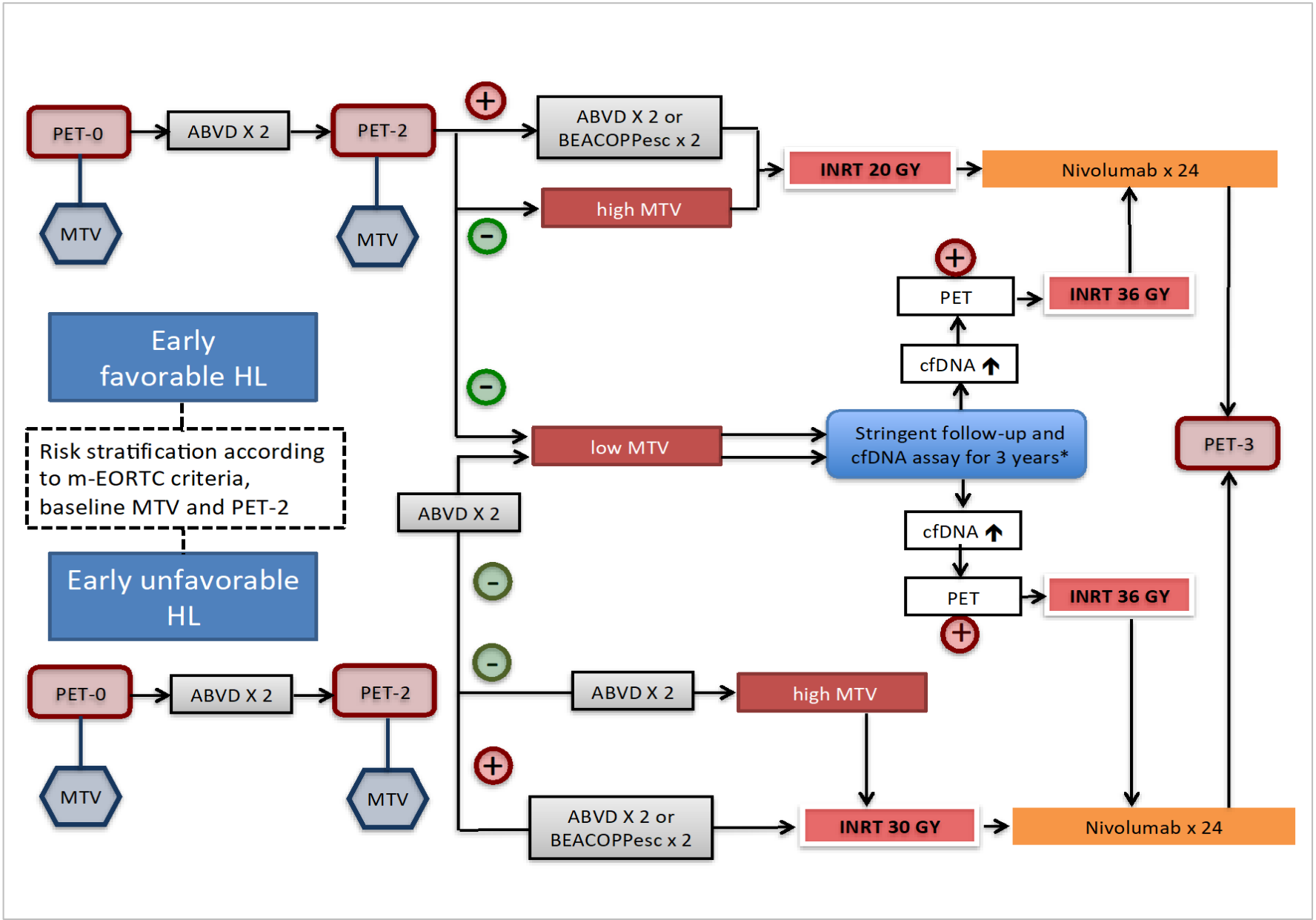
Kurtz et al. *J Clin Oncol.* 2018 Oct 1; 36(28):2845-2853

Perspectives

ctDNA-driven therapy of DLBCL: SAKK 38/19



ctDNA-driven therapy of cHL: RAFTING



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