FONDAZIONE ITALIANA LINFOMI

IMMUNOTERAPIA E FARMACI BIOLOGICI NEL TRATTAMENTO DEI LINFOMI E LLC: ATTUALITÀ E FUTURO

Il ruolo prognostico/predittivo della biopsia liquida nei linfomi

Ilaria Del Giudice

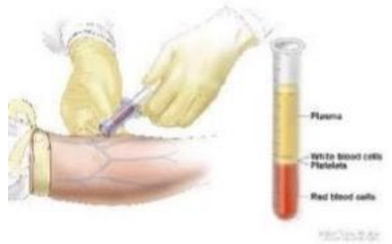
Sapienza Università di Roma

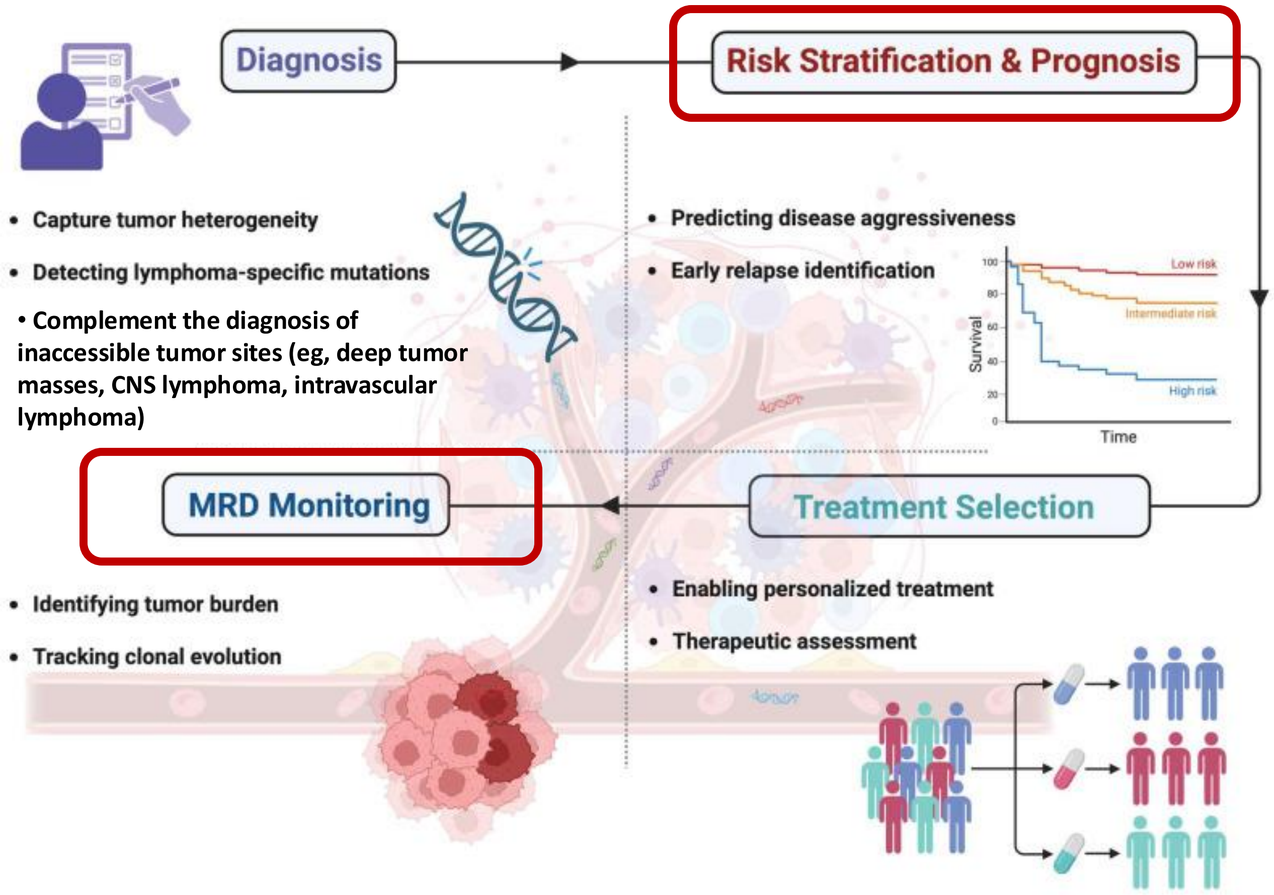
SAPIENZA UNIVERSITÀ DI ROMA

Disclosures of Ilaria Del Giudice

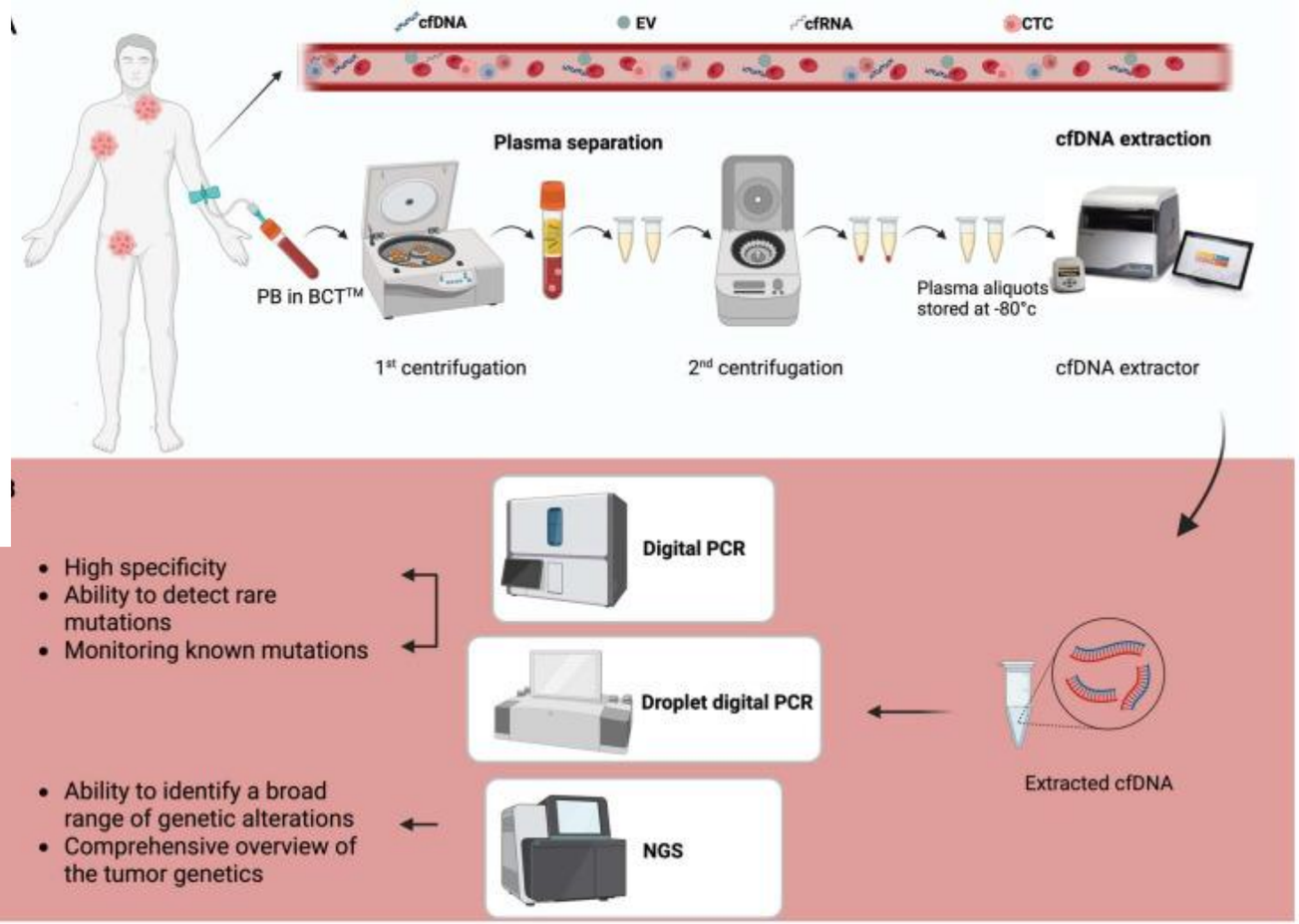
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Astra Zeneca							x
BeOne							x
Janssen						x	x
Roche						x	x
Takeda						x	x

Liquid biopsy: relevant clinical applications





Technical challenges



Liquid biopsy is the analysis of tumor biomarkers isolated from biological fluids of cancer patients

Almasri et al, Int J Mol Sci 2025

Reference no.	Technique	Focus	No of patients	Key findings
Diffuse Large B Cell Lymphoma				
Kurtz et al. [20]	IgHTS	Baseline evaluation	75	• Prognostic value of baseline ctDNA levels. • Correlation of ctDNA with TMTV
Roschewski et al. [59]	IgHTS	Baseline and interim evaluation	126	• Detectable interim ctDNA associated with risk of recurrence
Rivas-Delgado et al. [24]	Targeted NGS	Baseline evaluation	79	• Higher ctDNA correlated with tumor burden. • ctDNA determines tumor mutational profile and genetic classification.
Kurtz et al. [21]	CAPP-Seq	Baseline and dynamic assessment	217	• Pretreatment ctDNA levels are associated with EFS and OS following front-line treatment • A 2-log or 2.5-log fold decrease in ctDNA levels from baseline after 1 or 2 cycles of chemotherapy respectively is an independent biomarker of disease response.
Kurtz et al. [28]	PhasED-Seq	Dynamic assessment	213	• ctDNA negativity by ultrasensitive PhasED-seq after 2 cycles is associated with a significantly lower risk of relapse
Alig S et al. [25]	CAPP-Seq	Baseline evaluation	267	• Higher diagnostic ctDNA levels are associated with shorter EFS independent of IPI and diagnosis to treatment interval.
Meriranta et al. [22]	Targeted NGS (all); WGS (8 samples)	Baseline and dynamic assessment Fragmentation patterns	101	• High pretreatment baseline ctDNA levels were associated with inferior OS. • End of treatment ctDNA positivity (MRD) was associated with inferior OS
Roschewski et al. [29]	PhasED-Seq	Dynamic assessment	112	• Detectable ctDNA at the end of treatment (MRD) is a strong predictor of relapse/refractory disease.
Frank et al. [32]	IgHTS	Treatment response prediction	72	• Detectable ctDNA at day 28 following CD19 CAR-T (Axi-cel) was associated with median OS of 3 months versus not reached if undetectable.
Sworder et al. [34]	CAPP-Seq	Treatment response prediction	138	• Both tumor effector T-cell cfDNA can be isolated from a single plasma sample to enable the evaluation of lymphoma and CAR-T cells

Jamal E, et al. Leuk Lymphoma 2024

Lauer EM, et al. Leukemia 2022

Primary and secondary CNS lymphoma				
Hattori et al. [38]	ddPCR Targeted NGS	Baseline and dynamic assessment	14	• *MYD88* L265P is detectable by ddPCR in plasma cfDNA but only in just under 60% of the cohort.
Watanabe et al. [37]	ddPCR for *MYD88*	Baseline evaluation	26	• *MYD88* driver mutations can be detected by a combination of Sanger sequencing and ddPCR in the CSF derived cfDNA.
Bobillo S et al. [39]	ddPCR, WES, and targeted NGS	Baseline and dynamic assessment	19	• CSF ctDNA detection outperforms flow cytometry in the detection of MRD following treatment.
Mutter et al. [41]	CAPP Seq PhasED-Seq	Baseline and dynamic assessment	92	• CtDNA is detectable by PhasED-Seq at all disease time points and is a robust biomarker predicting clinical outcome.
Heger et al. [42]	Targeted NGS	Baseline and dynamic assessment	67	• Developed a risk stratification model for PCNSL using a combination of clinical and plasma-derived ctDNA features
Hodgkins Lymphoma				
Spina V et al. [43]	CAPP-Seq	Baseline and dynamic assessment	112	• Clonal evolution can be tracked during and after treatment in longitudinal ctDNA samples
Alig et al. [44]	PhasED-Seq	Baseline and dynamic assessment	366	• Pretreatment and on-treatment ctDNA levels are superior to conventional radiological assessment in MRD detection.
Follicular Lymphoma				
Sarkozy et al. [47]	IgHTS	Baseline evaluation	34	• Clonal heterogeneity is detectable in ctDNA and ctDNA concentration at diagnosis has prognostic significance.
Fernández-Miranda et al. [49]	Targeted NGS	Baseline and Dynamic assessment	36	• Failure to clear ctDNA during treatment is associated with poorer clinical outcomes including POD24
Jiménez-Ubieto et al. [50]	Targeted NGS	Dynamic assessment	84	• The integration of ctDNA with radiological measures of disease burden allowed for a better assessment of disease burden in comparison to PET/CT alone.

Jamal E, et al. Leuk Lymphoma 2024

Lauer EM, et al. Leukemia 2022

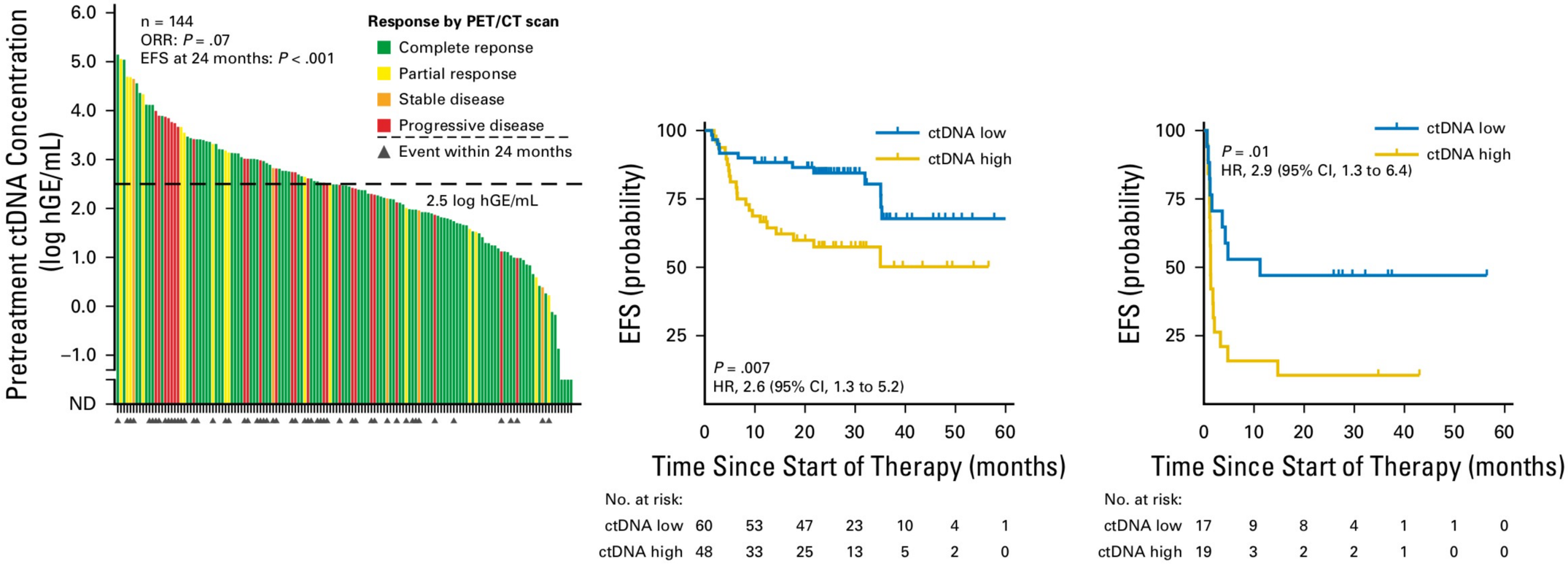
Liquid biopsy in DLBCL

The NCCN guidelines incorporate circulating ctDNA testing for MRD assessment in patients with PET-positive DLBCL at the completion of first-line therapy (2025)

IGH
Clono-seq

Baseline ctDNA is a robust biomarker in DLBCL

Pre-treatment levels of ctDNA correlate with EFS in both TN and in R/R DLBCL



Early molecular response (EMR), defined as a 2-log reduction in ctDNA levels after one cycle of therapy

Major molecular response (MMR), characterized by a 2.5-log reduction after two cycles, have been significantly correlated with better PFS and OS.

Kurtz DM, et al., JCO, 2018

Liquid biopsy reflects the molecular characteristics and clinical impact of molecular clusters identified on tissue biopsy

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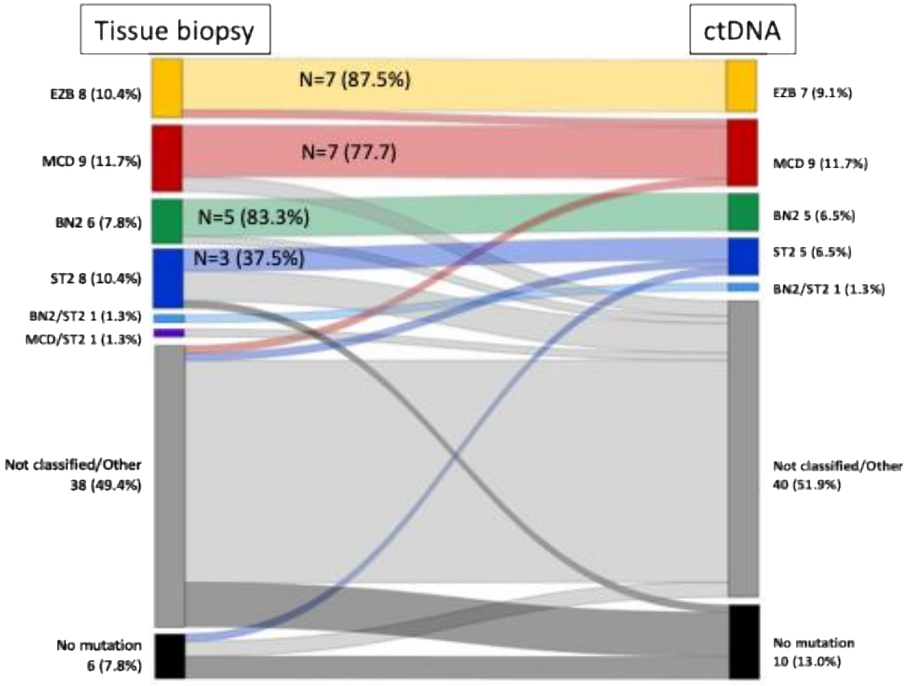
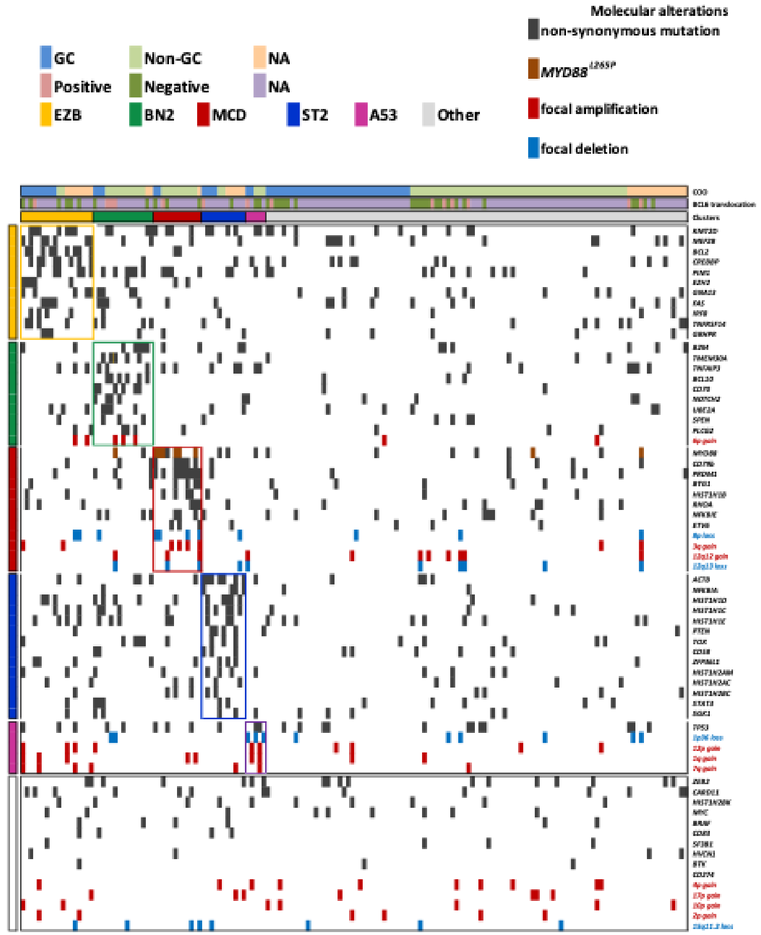
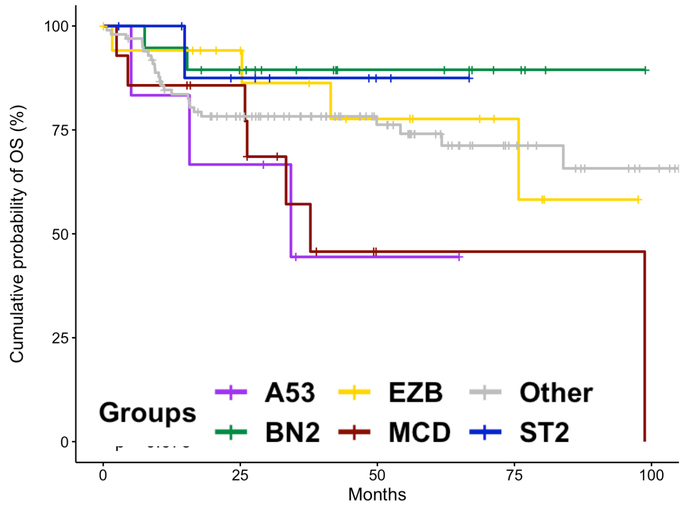
N=77 DLBCL



Capp-seq LymphGen tool
Classified 46.5% of LN and 40.3% ctDNA
95.8% concordance

N=166 DLBCL



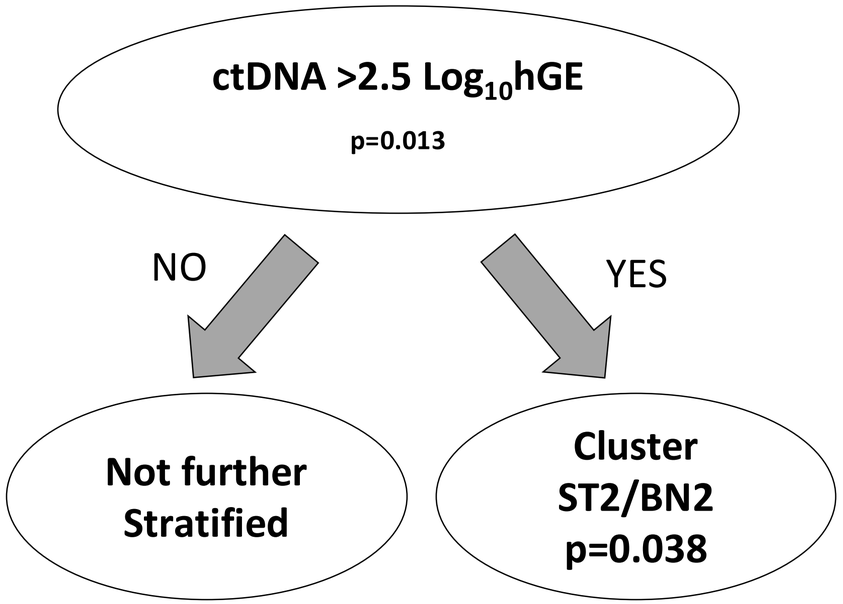


18 patients (29.5%) were classified as EZB, 15 (24.6%) as BN2, 12 (19.7%) as MCD, 11 (18.0%) as ST2, and 5 (8.2%) as A53.

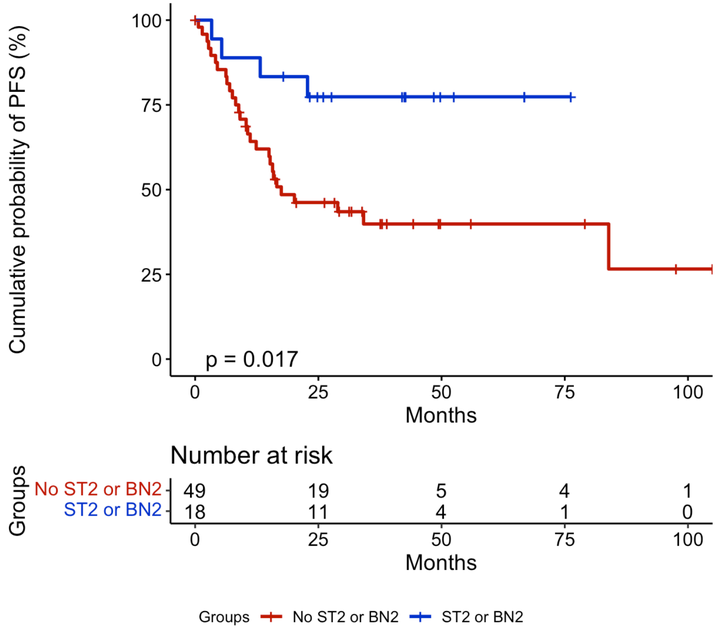
Moia et al., Blood Advances. 2025

After a median follow-up of 40.6 months, 40-m PFS 68.2% and OS 85.2%

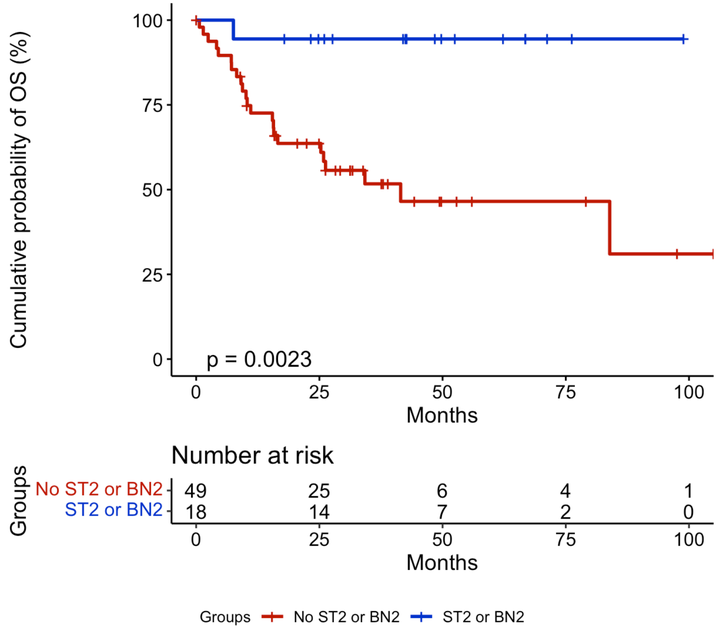
BN2/ST2 clusters predict outcome in patients with ctDNA levels >2.5 Log_{10} hGE



4-y PFS
75.3% vs 38%



4-y OS
87.8% vs 47.1%



ctDNA levels of <2.5 log10 hGE/mL and/or BN2/ST2 cluster **= Low-risk patients** (n= 51)
ctDNA levels of ≥2.5 log10 hGE/mL and no BN2/ST2 cluster = **High-risk patients** (n= 115)

Moia et al., Blood Advances. 2025

PET parameters, ctDNA levels and BN2/ST2 clusters independently predict PFS: A three-variable prognostic model

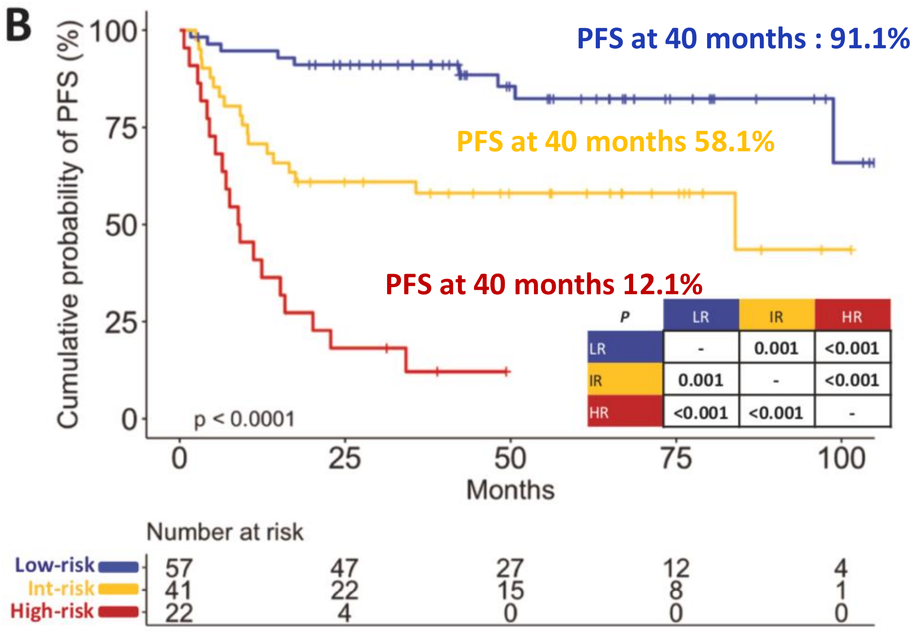
High risk PET: at least one PET/CT parameter among tMTV, tTLG and Dmax above the respective cut-off

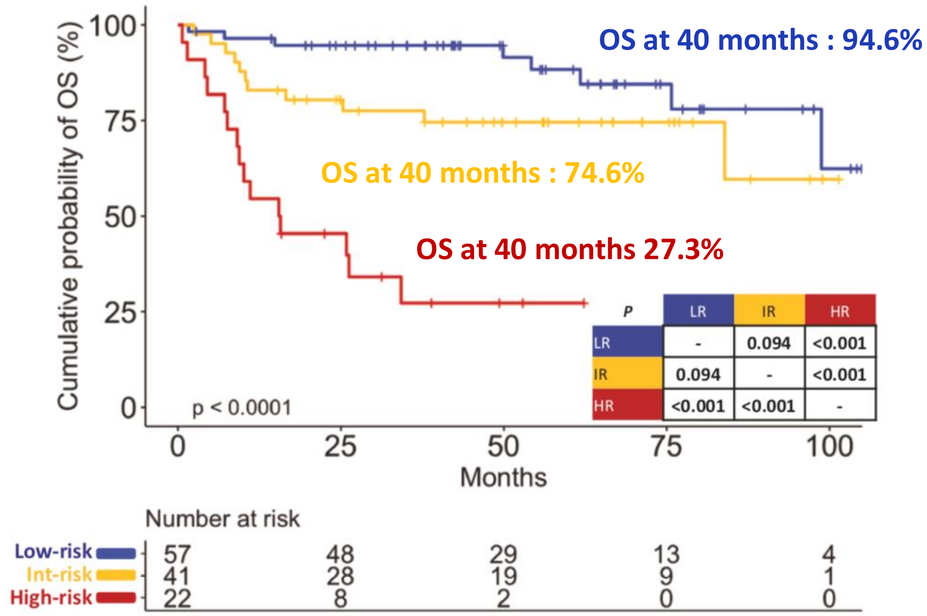
Multivariate analysis for PFS

Variable	Points	N	Hazard ratio	95% CI	β	p
ctDNA-high	+1	120		2.77 (1.45, 5.27)	1.018	0.002
High-risk PET	+1.5	120		3.90 (1.79, 8.48)	1.361	<0.001
BN2/EZB/ST2	-1.5	120		0.27 (0.12, 0.62)	-1.310	0.002

1.5 point PET+, 1 point high ctDNA, -1.5 point BN2/ST2

Low risk (n=57)	-1.5 to 0.5 points
Intermediate risk (n=41)	1 to 1.5 points
High risk (n=22)	2.5 points





Dondolin et al., Leukemia 2025

MRD by ctDNA IGH-IGk

N=73 DLBCL
LN 91% clonality IGH/IGk
ctDNA 93% clonal IGH/IGk

Tumor clonotype vs plasma clonotype:

1. Identical clone (69%)
2. Different clone (21%)
3. No clone on plasma (10%)

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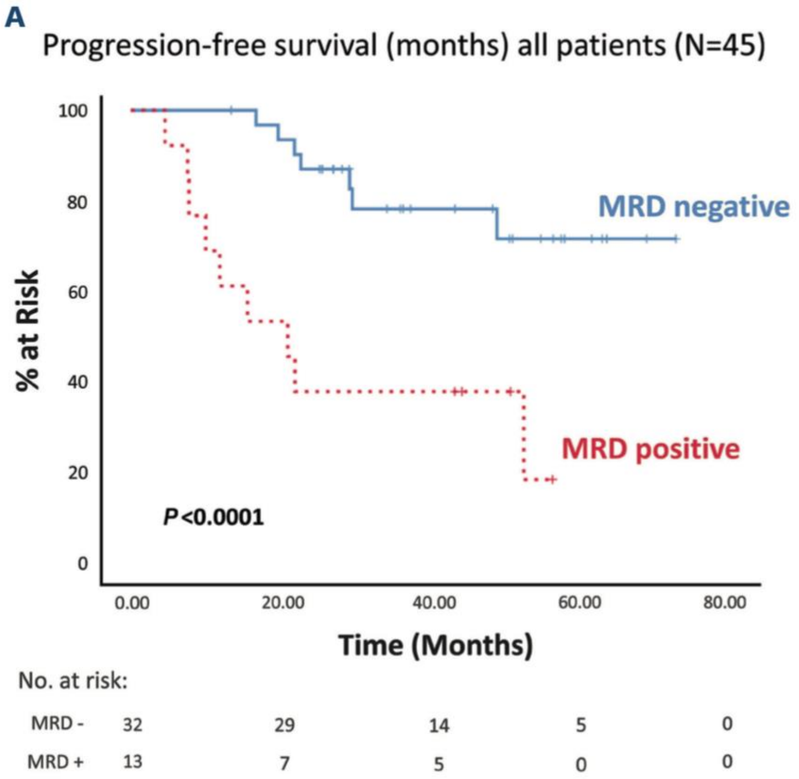
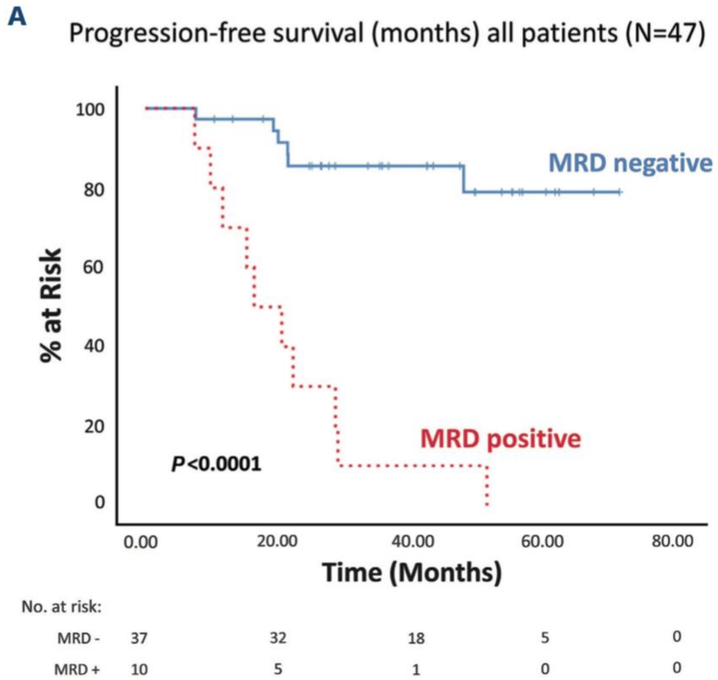
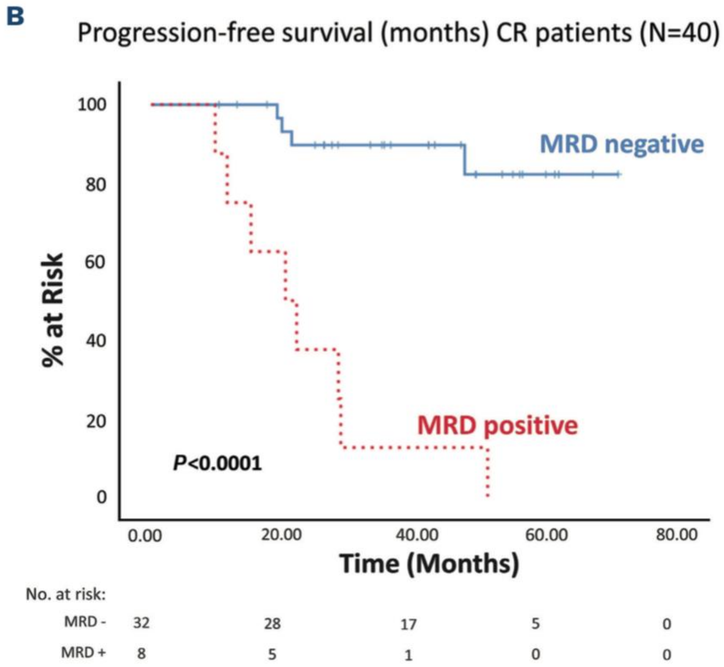
MRD on ctDNA during treatment



MRD on ctDNA at EOT



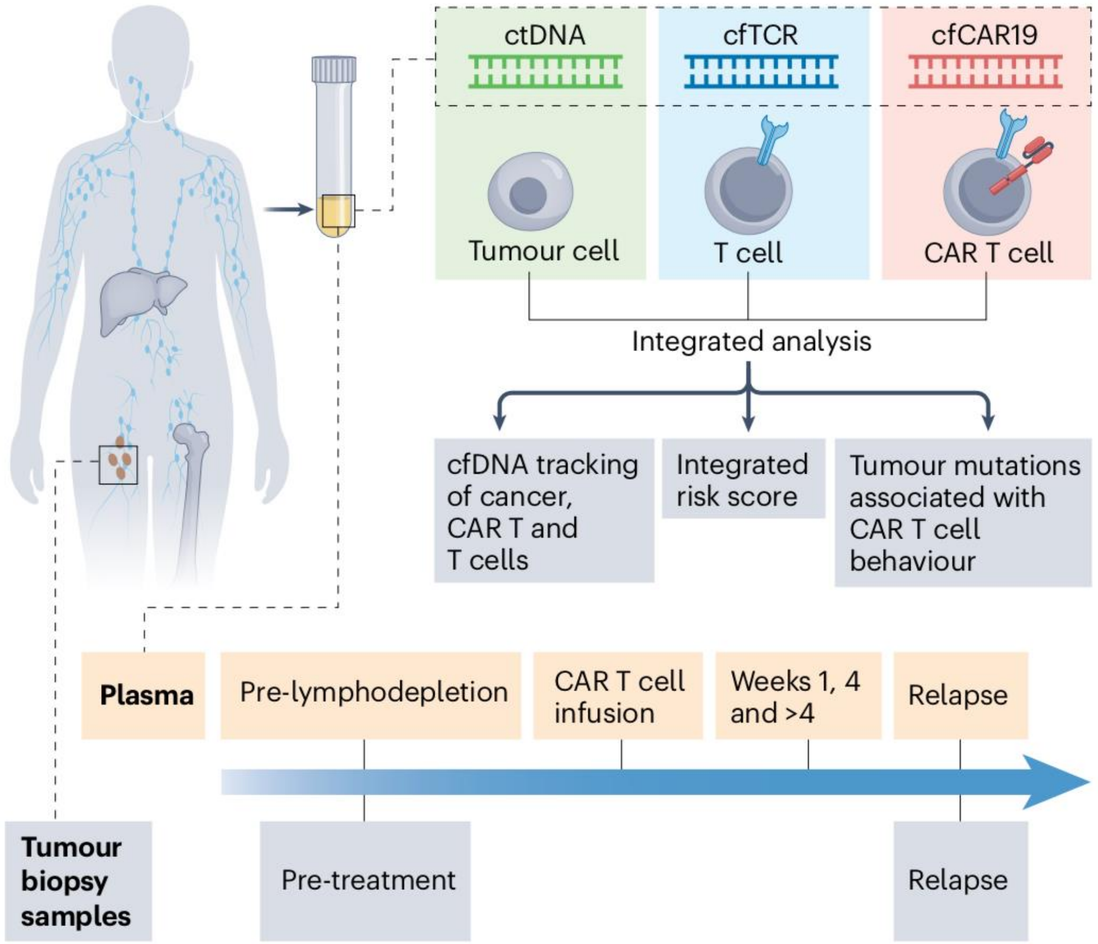
MRD on ctDNA in CMR PET/CT



Soscia et al. Haematologica 2025

After a median follow-up of 40 months (range, 1-72), OS was 76.6% and PFS 72.6%

Liquid biopsy after CAR-T



Simultaneous tumour and effector profiling (STEP)
an integrative targeted sequencing and analysis technique

cfDNA that comprised:
- ctDNA
- infused CAR19 DNA (cfCAR19)
- nonengineered T cell receptor DNA (cfTCR).

Mutations associate to inferior PFS
TMEM3OA (increased in TAM)
IRF8 (increased Tregs)
CD19
PPM1D
TP53
Gains CD274

The use of **short cfCAR19 DNA** as a surrogate metric for difficult-to-measure intratumoural CAR T cells

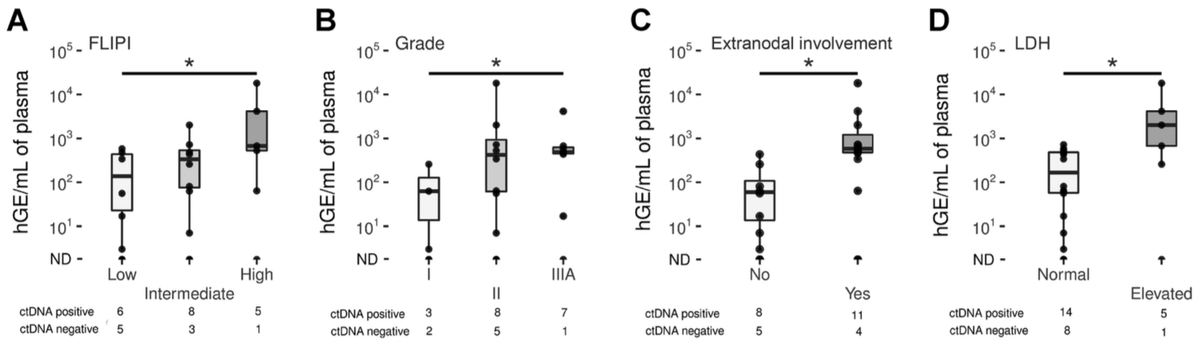
Sworder et al, Cancer Cell 2023

Liquid biopsy in follicular lymphoma

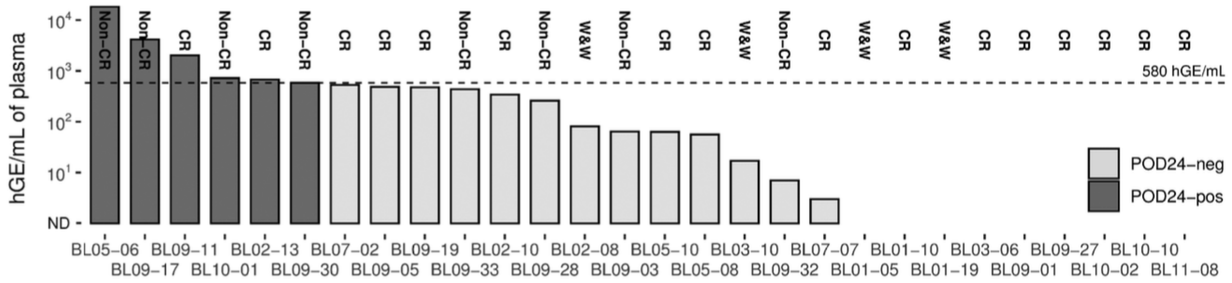
Targeted-seq

Baseline ctDNA levels and patients' outcome

73% of mutations detected in LN were identified in basal ctDNA

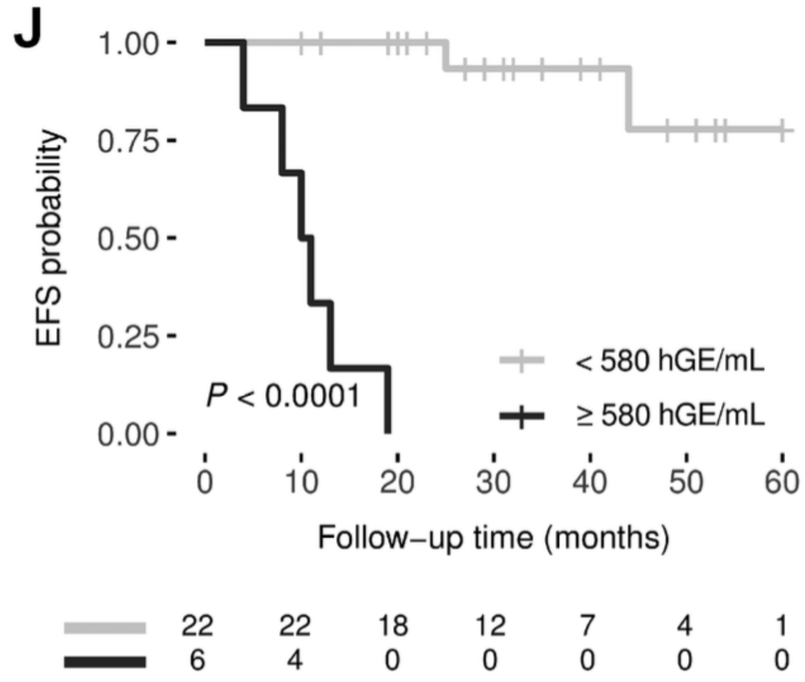


Higher levels of ctDNA correlates with FLIPI, Grade, extranodal involmente and LDH



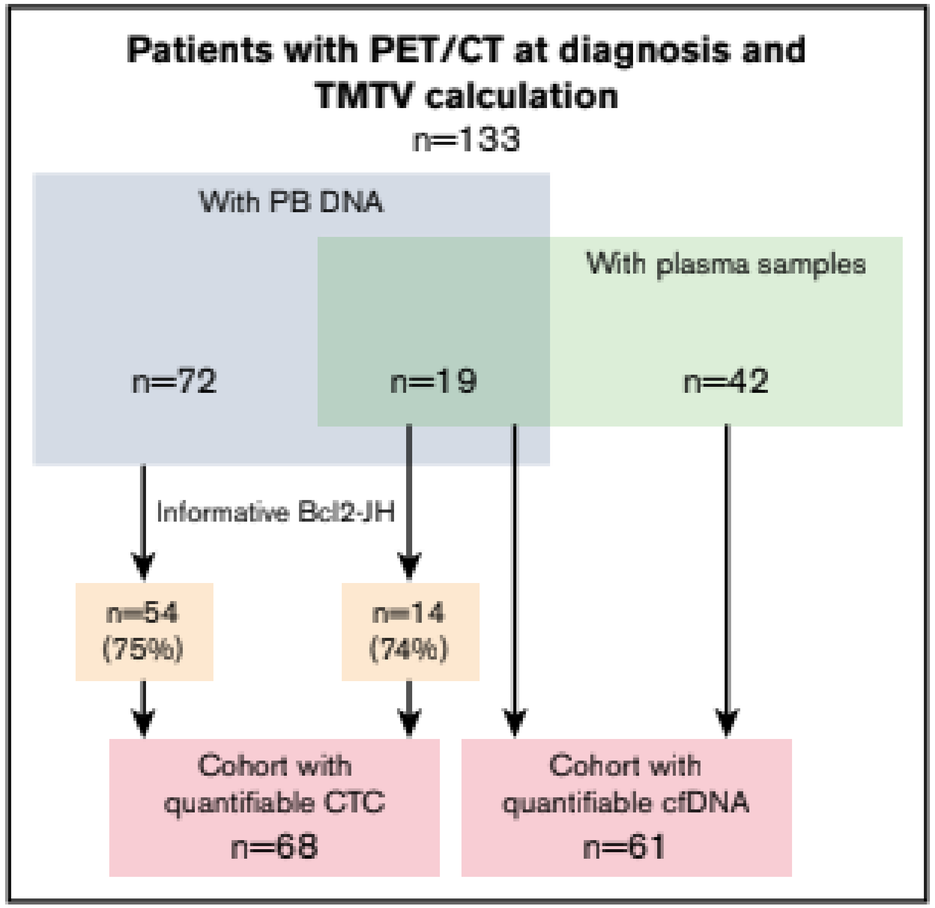
Pre-treatment 580 hGE/mL was established as the optimal cut-off for non-CR or POD24 prediction

N=36 patients (R-CHT)
Median f-up 3.4 y



Fernandez-Miranda et al., Clin Canc Res. 2023

Baseline ctDNA levels and PET



Plasma quantification of BCL2::IGH copies

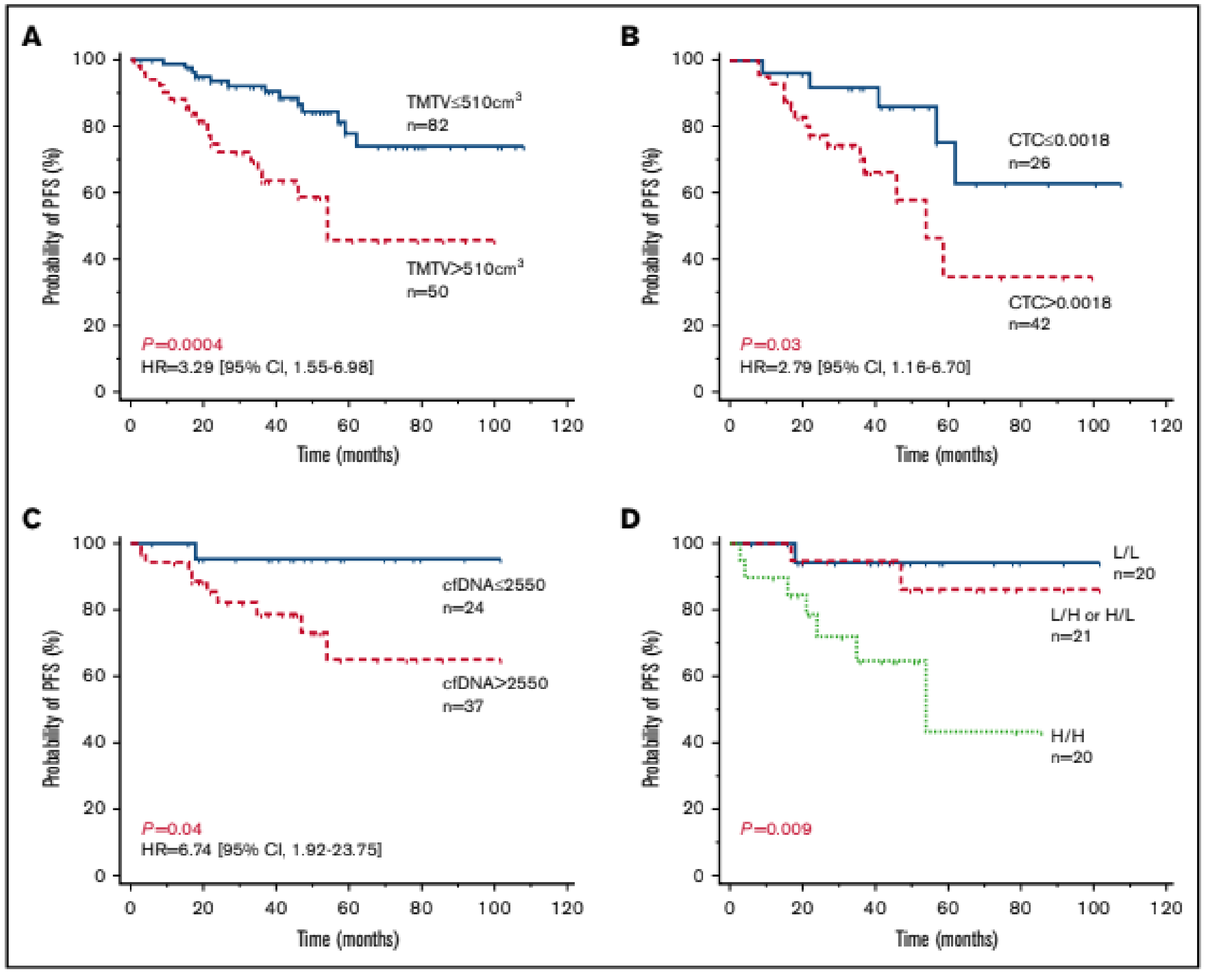
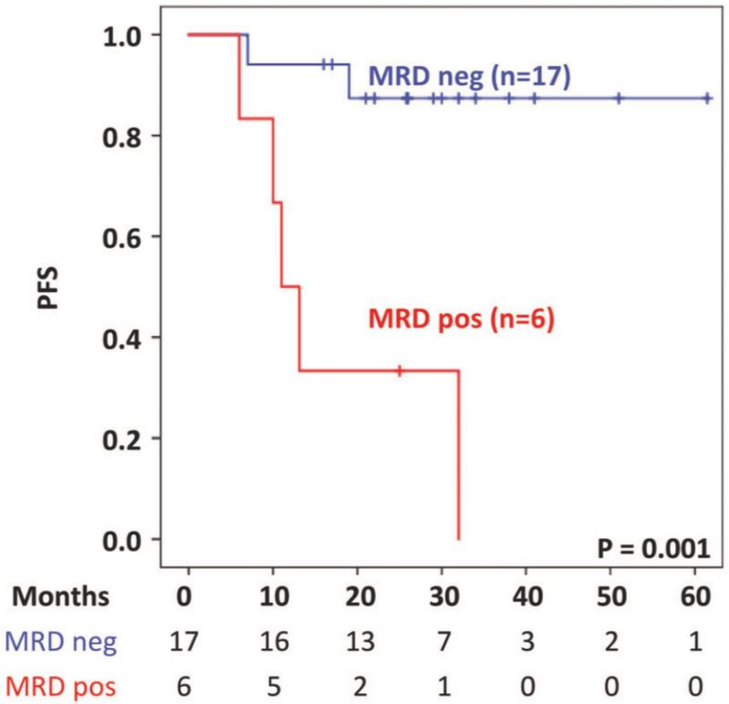


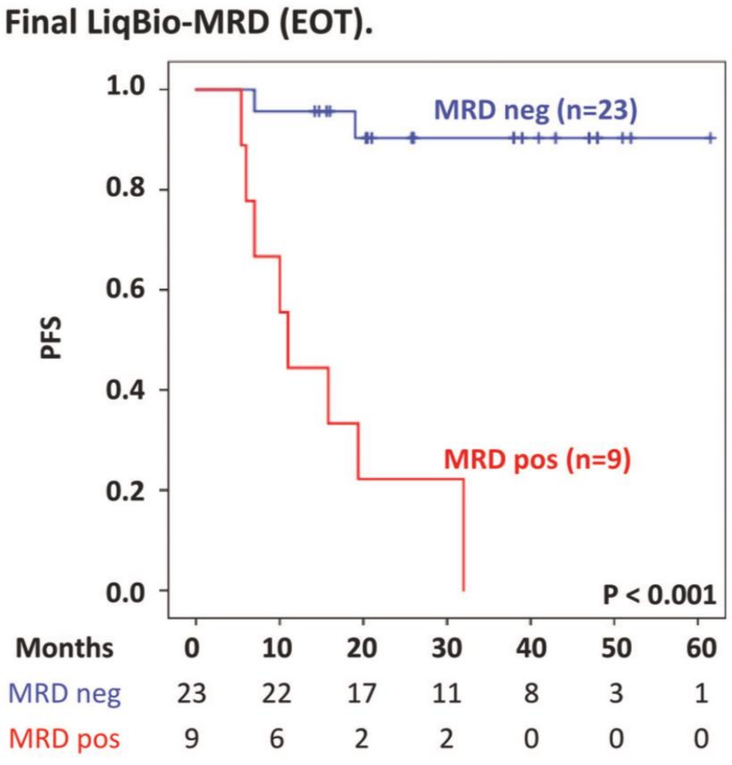
Figure 4. Progression-free survival according to tissue or circulating tumor burden biomarkers. Kaplan-Meier estimates of PFS based on TMTV (A), CTC (B), cfDNA (C), and TMTV and cfDNA combined (D). Of the 21 patients with combined high or low levels, 17 had high cfDNA levels and a low TMTV, and 4 had low cfDNA levels and a high TMTV. H, high level; HR, hazard ratio; L, low level.

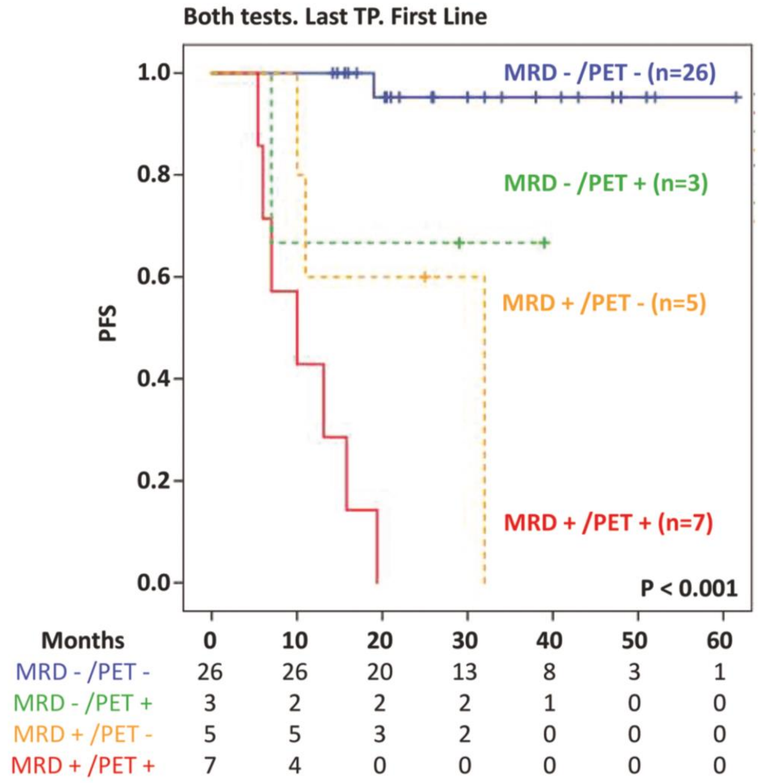
Delfau-Larue, M. et al. Blood adv. 2018

Targeted-seq

EOI PET and ctDNA to predict POD24?







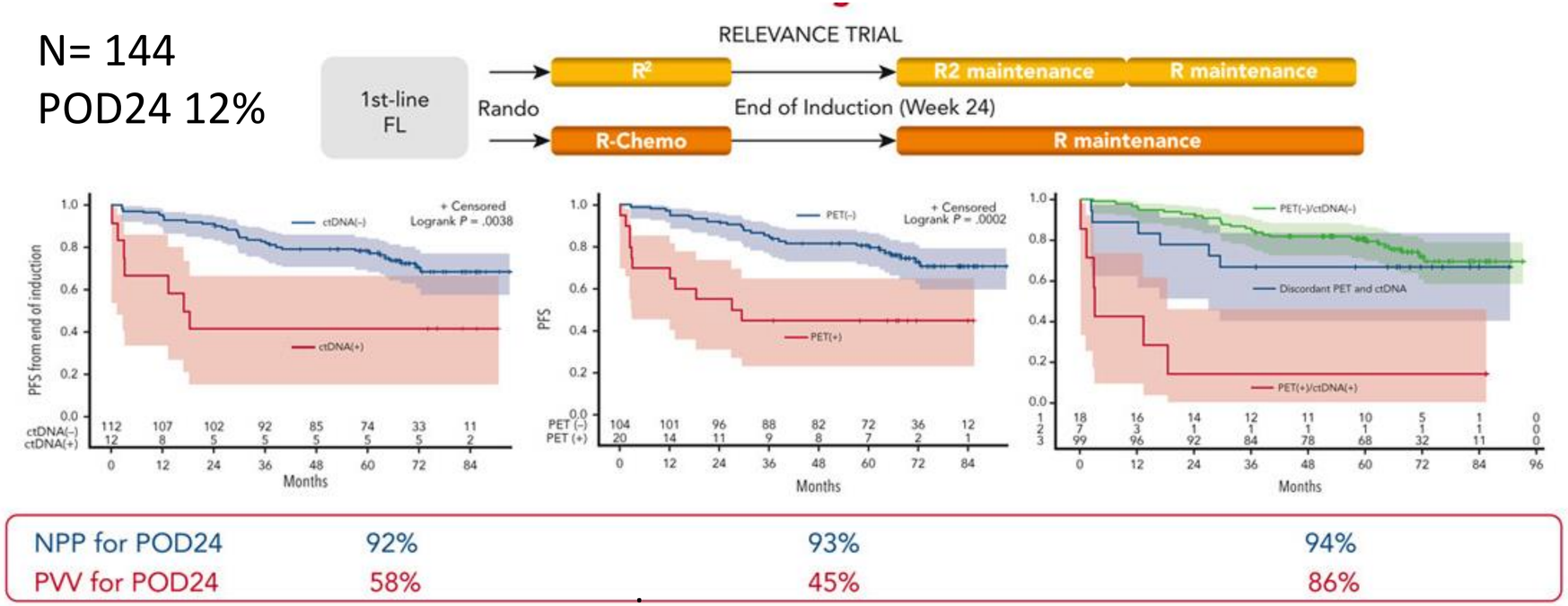
N= 58 FL in first line,
41 with PET and MRD at EOI

POD24 prediction sensitivity 88%, specificity of 100%
PPV 100% NPV 96%

Jiménez-Ubieto et al. Leukemia 2023

EOI PET and ctDNA to predict POD24? Phase III Relevance Lysa trial

N= 144
POD24 12%



NPP for POD24	92%	93%	94%
PVV for POD24	58%	45%	86%

- A **mutational profile** could be identified from cfDNA in **99%** (140/141) of patients.
- A significant **correlation** was found between **ctDNA load** (median 2.39 log hGE/mL (range 1.01 to 4.20) and **TMTV** (median 298 mL, range 5 to 3100) (p=0.023).
- **Phased variants** were detected in 124 patients (87.9%) at diagnosis and were used for MRD analyses.

EOI
PET+ 20/124 **(16%)**
ctDNA+ n= 12/124 **(9.7%)**

Median follow-up 6.4 years
Median PFS
NR PET- vs 28.3 m PET+ **(16%)**
NR MRD- vs 17.7 m MRD+

At EOI PET+/MRD+ identified **POD24** patients with a **NPV** and **PPV** of **94%** and **85.7%** respectively.

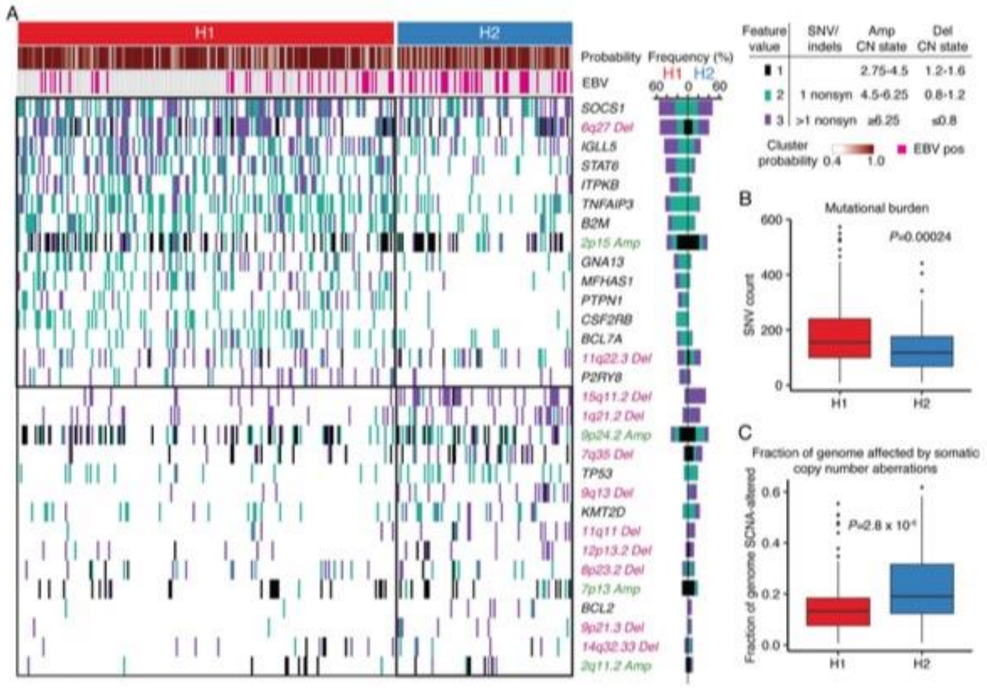
Claudel et al, Blood 2025

Liquid biopsy in Hodgkin lymphoma

Velasco-Suelto J, et al. J Exp Clin Cancer Res. 2024

Distinct Hodgkin lymphoma subtypes identified on liquid biopsy (i)

N=366 pediatric and adult cHL
293 patients (80%) with sufficient ctDNA burden
WES/TGS and bulk RNA-seq

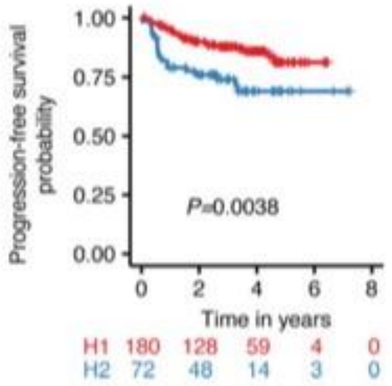


SOCS1 (60%), *TNFAIP3* (50%), *B2M* (39%), *STAT6* (34%), *CSF2RB* (24%), *GNA13* (23%), *PTPN1* (18%) *ARID1A* (17%)

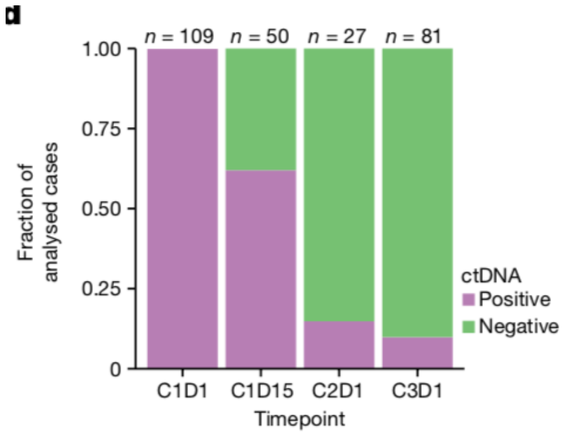
Amplification at 2p15 (*REL*), 9p24.1–9p24.2 (*CD274-PDL1*), 5p15.33 (*TERT*), 17q21.31 (*MAP3K14-NIK*)
Deletions at 6q27 (*TNFAIP3*), 17p13.1 (*TP53*), 9p21.3 (*CDKN2A/B*), 11q22.3 (*BIRC3*), 6p21–22 (*H1- 5,HLA-A,HLA-C)*

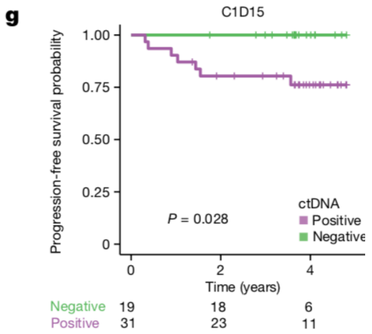
Cluster H1 (68%)
High mutation burden,
in NF-κB, JAK–STAT and PI3K signalling pathways.
Bimodal peak of age
Cytokine response signature
MHC class I loss

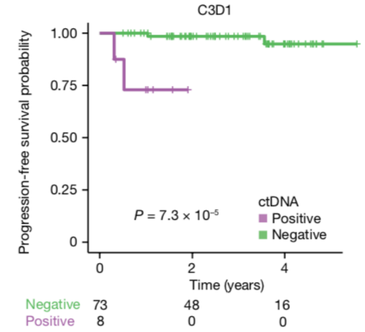
Cluster H2 (32%)
various CNA events,
TP53 and *KMT2D mut*
Younger patients
EBV enriched
Worse outcome
PDL1 amp & T-cell exhaustion



109 patients for ctDNA MRD by phased variant enrichment and detection sequencing (PhasED-seq)

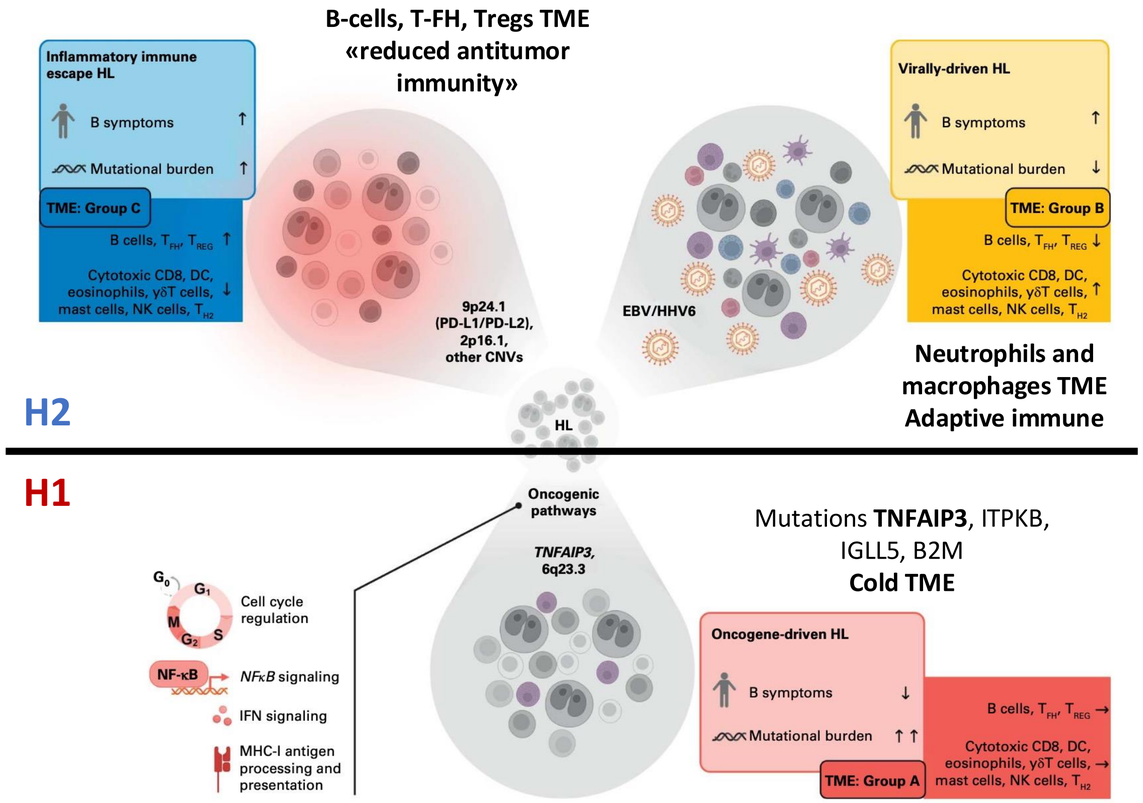




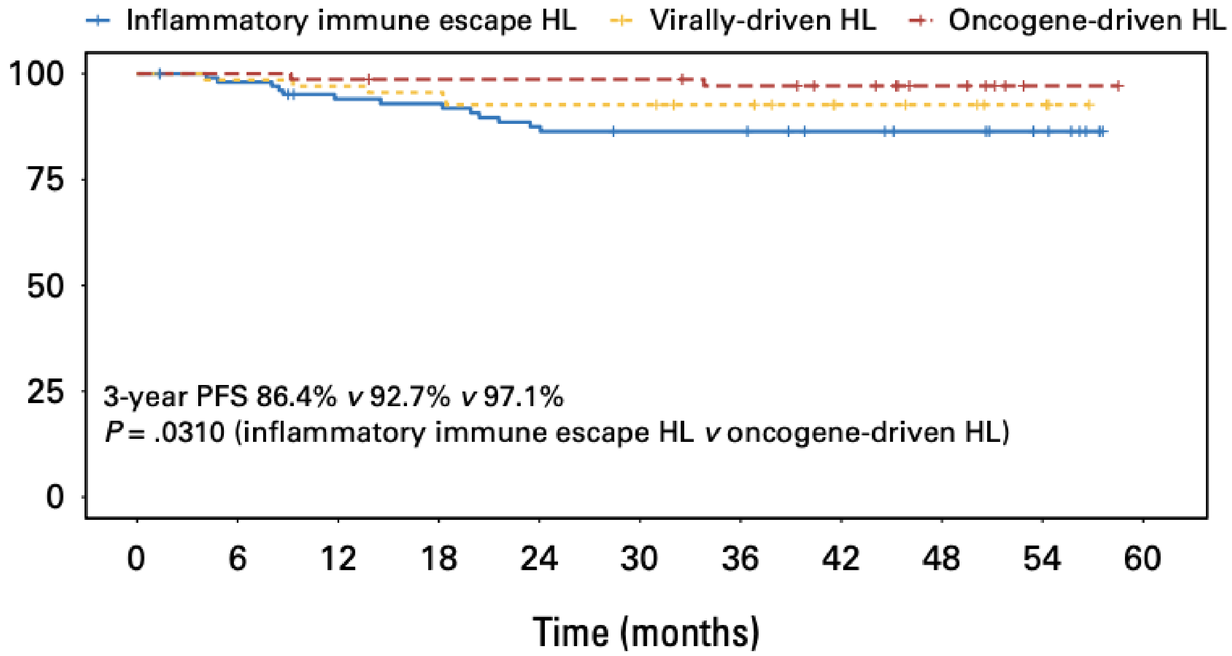


Alig et al., Nature. 2024

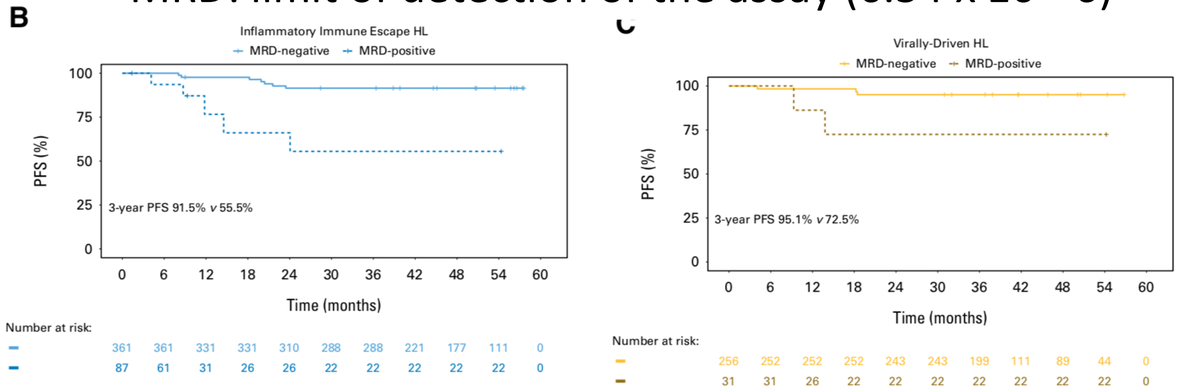
Distinct Hodgkin lymphoma subtypes identified on liquid biopsy (ii)



243 patients from GHSG trials
(HD21, NIVHAL, Euronet PHL C2)
WES/TGS and bulk RNA-seq



MRD: limit of detection of the assay (6.54 x 10^-6)

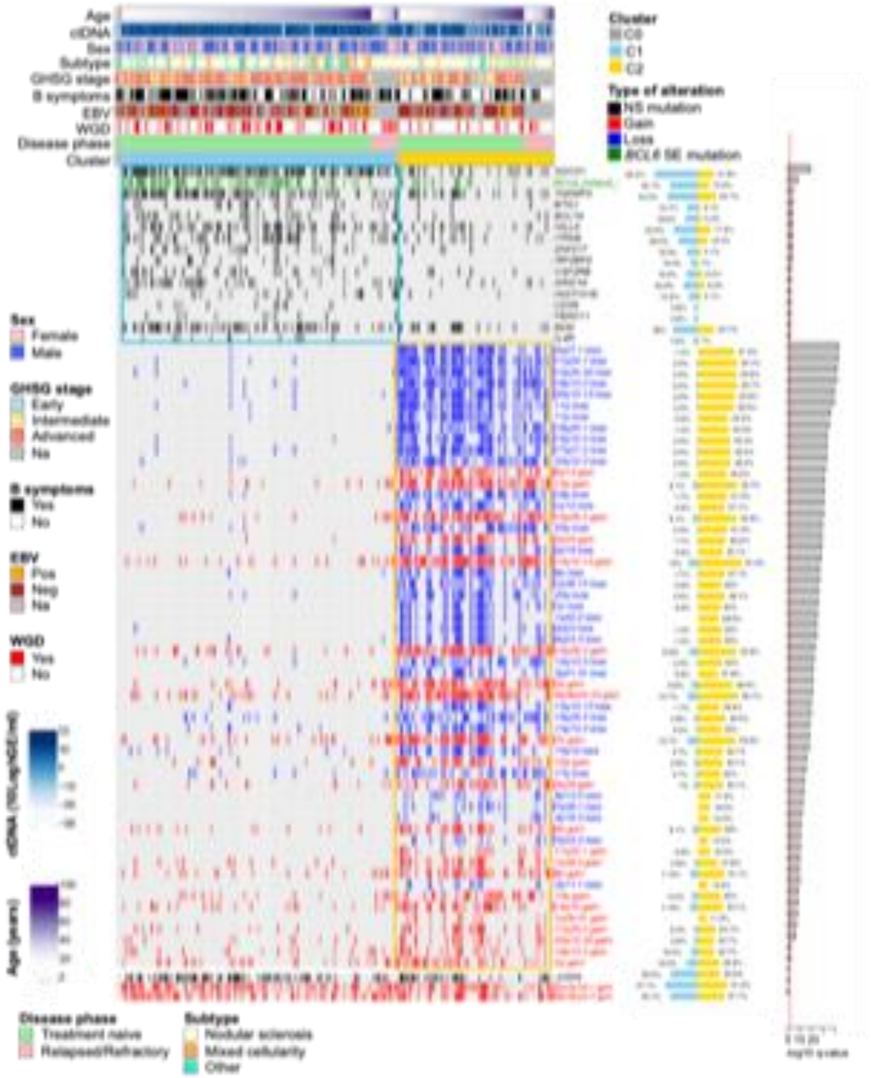


Different outcomes of biologic subgroups that harbor specific vulnerabilities?

Immune escape phenotype can be sensitive to CPI?

Heger et al., JCO. 2024

Distinct Hodgkin lymphoma subtypes identified on liquid biopsy (iii)



Genetic subtypes of cHL are driven by genetic instability rather than mutation clustering

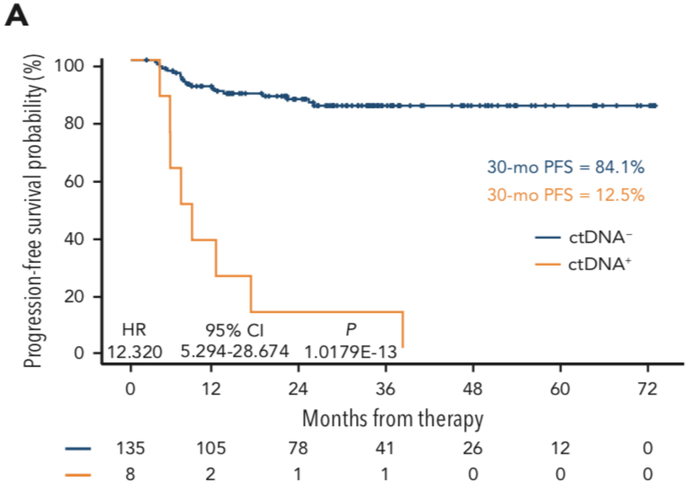
C1 (64%)
High mutation burden, nonsynonymous mutations in coding genes targeted by AID hypermutation and by noncoding SHM in the BCL6 intragenic superenhancers

C2 (36%)
higher SCNA burden, chromosomal instability (lower mutation density)

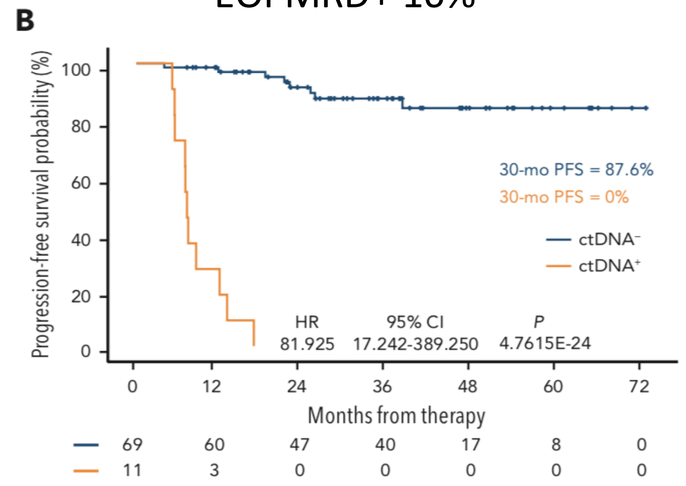
	HR	95% CI	p
MTV (continuous)	1.001	1.000-1.002	0.061887
GHSG stage	1.505	0.696-3.256	0.298710
IPS >2	2.787	1.340-5.769	0.006092
WGD+	2.411	1.125-5.165	0.023602

Whole-genome duplication (24% of cases) is the sole genetic factor significantly linked to adverse prognosis (+ in 28% C1; 13.5% C2)

MRD Capp-Seq
Interim MRD+ 6%



EOI MRD+ 16%



Pirosa et al., Blood 2025

Capp-seq

MRD by ctDNA in S1826 trial: Nivo-AVD vs Bv-AVD

388 advanced HL

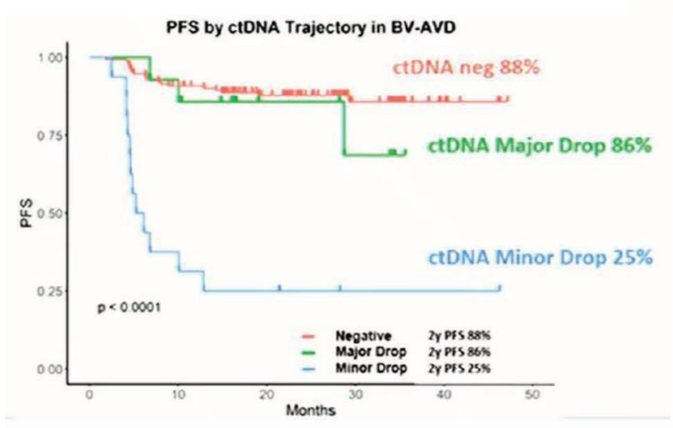
Molecular tumor burden, MTB
log-fold changes in haploid genome equivalents per milliliter of plasma (hGE/mL)

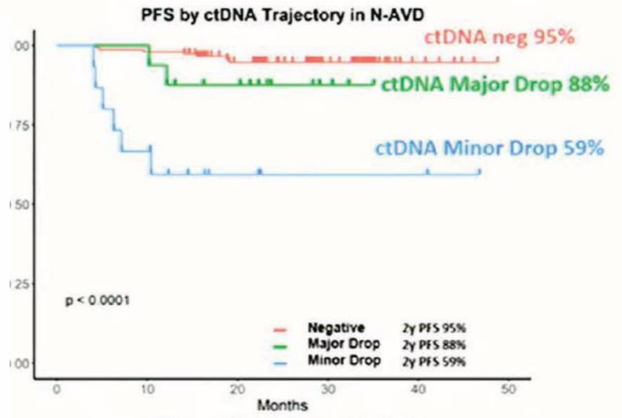
Baseline MTB correlated with clinical features:
IPS score (0–3 vs. 4–7, $p < 0.0001$)
B symptoms ($p < 0.0001$)

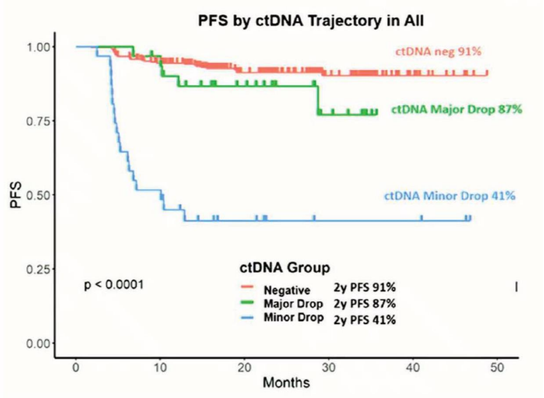
undetectable ctDNA at C3D1:
2 years PFS 91% versus 2 years PFS 64% in the ctDNA+ group, $p < 0.0001$.

C3D1 (N-AVD: ctDNA PFS 95% versus ctDNAþ, 74%; BV-AVD: ctDNA− 2 years PFS 88% versus ctDNAþ 53%, both p < 0.0001).
pts with a > median (3.64) log fold drop in MTB at C3D1 had slightly less favorable outcomes.
In contrast, pts with **< median log-fold drop at C3D1** had significantly inferior outcomes: 2 years PFS in all, N-AVD and BV-AVD pts of 41%, 59% and 25%, all p < 0.0001.

EOT presence versus absence of detectable ctDNA and PFS
All = ctDNA−, 2 years PFS 92% vs. ctDNAþ 42%
N-AVD = ctDNA−, 2 years PFS 93% vs. ctDNAþ 47%
BV-AVD = ctDNA− 2 years PFS 91% vs. ctDNAþ 39%, all $p < 0.0001$.



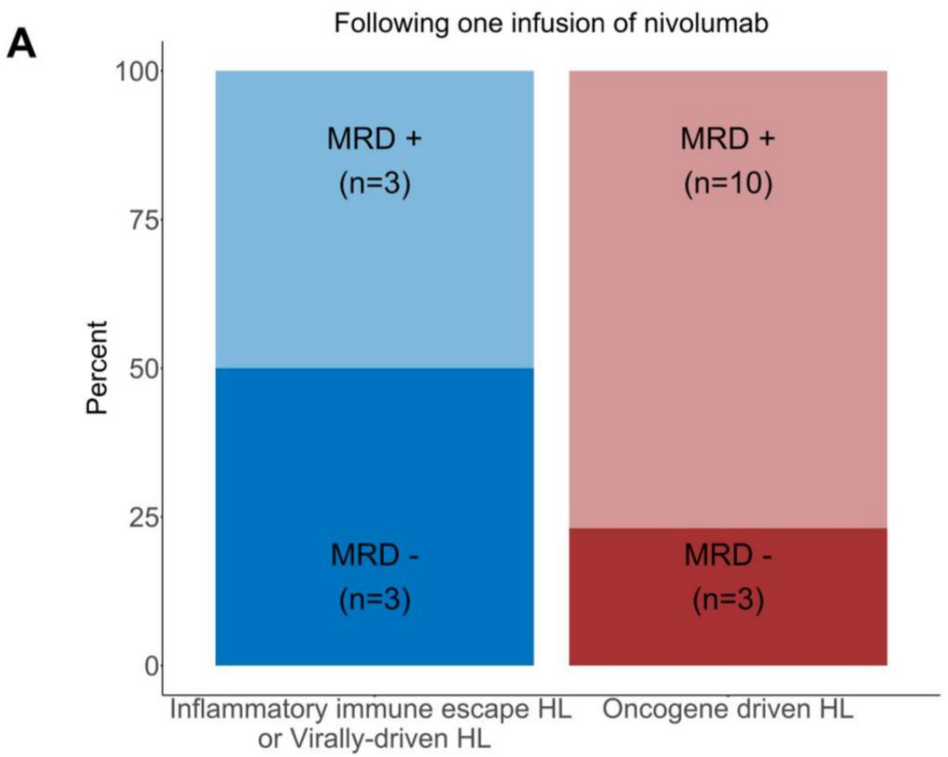




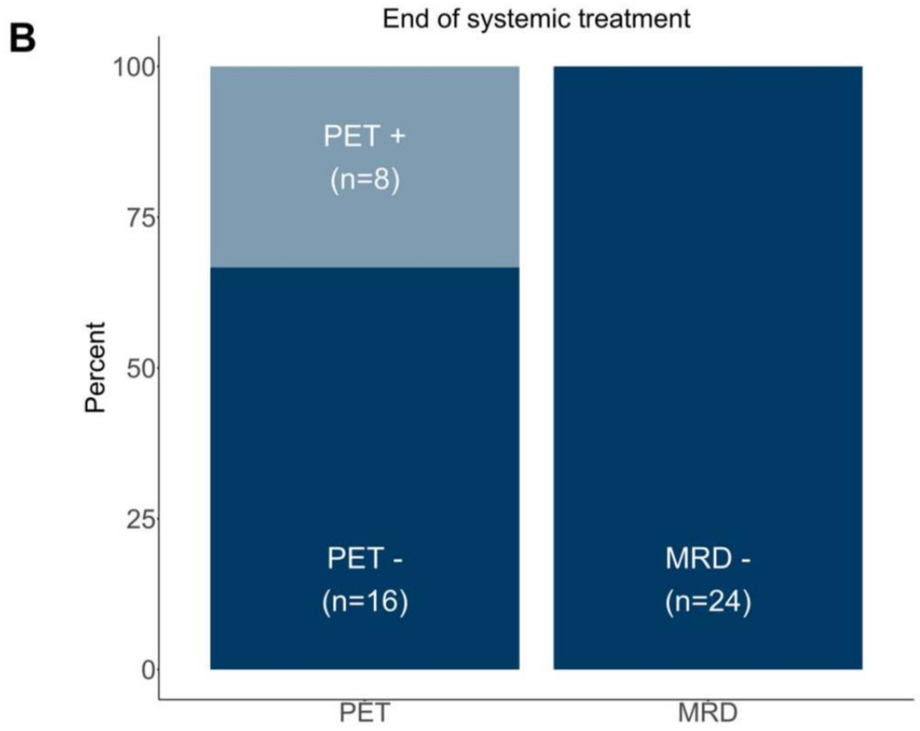
Paczkowska J et al. ICML #21, presented by Herrera A

MRD by ctDNA in GHSG phase II NIVAHL trial

Early unfavorable HL



6/19 (31.6%) MRD negativity after just one infusion of nivolumab.
Inflammatory immune escape HL or *Virally-driven HL* had higher rates of MRD negativity compared with *Oncogene-driven HL* (50% versus 23.1%)



At EOI, 8/ 24 (33.3%) patients were PET positive, while MRD indicated complete molecular remission in 24/24 (100%) of patients

Heger et al. ICML 2025

….but ct-DNA analysis in clinical practice is still…

clinical reviews

Potential of Circulating Tumor DNA for the Management of Patients With Lymphoma

Sarah Huet, PharmD, PhD[1,2,3] and Gilles Salles, MD, PhD[2,3,4]

- References: 51

«**Technical standardization** and careful prospective evaluation of the role of ctDNA monitoring in clinical studies **represent current important challenges to allow its application in routine practice**».

Huet & Salles. JCO Oncol Pract 2020

Reviews | Special Series: Molecular Testing and ctDNA in Oncology Practice

Cell-Free DNA in Hematologic Malignancies

Joseph G. Schroers-Martin, MD[1] and Ash A. Alizadeh, MD, PhD[1,2,3]

- References: 105

«In lymphomas, ctDNA is well characterized, increasingly integrated into clinical trial designs, and **may serve to inform future response-adapted treatment strategies**»

Schroers-Martin & Alizadeh, JCO Oncol Pract 2024

Technologies for ctDNA analysis: multiple assays and methods are available in DLBCL

PENDING

Assay type	Basic methodology	Advantages	Disadvantages	Relative cost
clonoSEQ	Sequencing of VDJ rearrangement	• Commercially available	• Single target limits sensitivity • Unable to genotype	$$
Targeted hybrid capture	Hybrid capture enrichment of lymphoma-specific targets	• Genotyping • High sensitivity	• Duplex sequencing required • Complex workflow	$$
Amplicon	PCR-based enrichment of lymphoma-specific targets	• Genotyping • Simpler workflow	• Low sensitivity	$
PhasED-seq	Hybrid capture enrichment of phased variants	• Highest sensitivity • Concurrent genotyping (hybrid capture) possible	• Complex workflow	$$$
lpWGS	Low coverage sequencing to detect copy number variants	• Simplest workflow	• Low sensitivity	$
Tumor-informed	Bespoke panel for monitoring based on baseline sample	• Commercially available	• Patient-specific panel design	$$

PCR polymerase chain reaction, *PhasED-seq* phased variant enrichment and detection sequencing, *lpWGS* low-pass whole genome sequencing.

- **ClonoSeQ (Adaptive Biotech)** relies on the use of multiplex primers targeting the IGH and Igk to identify and quantify tumor-specific clonotypic rearrangements.
- **cAPP-seq** detects tumor-specific mutations in ctDNA using a selector probe set designed for the specific tumor type of interest and suppresses technical errors through the use of a unique barcoding strategy together with a downstream bioinformatic algorithm that eliminates sequencing errors and stereotypic background noise
- **Phased variant enrichment and detection sequencing (PhaseD-seq)** tracks two or more phased variants on the same strand of DNA molecule. It lowers both the technical and background signal-to- noise ratio enabling ctDNA monitoring down to a detection limit of ~0.00005%

PENDING

. **Barriers to adoption of ctDNA sequencing in DLBCL and potential solutions**

Barrier	Summary	Potential solution
Feasibility of real-time sequencing unvalidated	Quick turnaround (< 14 days) of ctDNA analysis result is needed to guide treatment	Troubleshooting workflow by central laboratory or dissemination of ctDNA assay kits and bioinformatics software
Multiple assays potentially available	ctDNA assays in development have different performance (sensitivity and specificity)	Prospective study comparing assays performed on same timepoints/samples, harmonization across laboratories
Lack of standardization	No consensus for appropriate assessment schedule or definition of molecular response	ctDNA working groups and workshops to help establish recommendations
Sample quality dependent	Sensitivity of assays dependent on amount of input cfDNA	Recommend larger volume blood draws and collection into cfDNA-stabilizing tubes
Pre-treatment sample dependent	Even for assays with tumor-agnostic targets, baseline plasma or tissue sample needed to calibrate for tumor-specific reporters	Adequate tissue sampling and or standardized banking of plasma at diagnosis
Cost and availability	ctDNA sequencing can be expensive and research assays unavailable outside of academic centers	Cost-effectiveness studies, economies of scale, and commercialization of additional assays
Best applications remain undefined	Unknown whether MRD-guided treatment (escalation, de-escalation, consolidation, etc.) superior to standard of care	ctDNA-adapted clinical trials to answer well-defined research questions for specific scenarios
Complex trial design	ctDNA-adapted designs vulnerable to screen fails and high proportion ineligible patients	Robust workflows, inclusion of "non-matched" patients to answer other research questions or serve as comparators

ctDNA circulating tumor DNA, *cfDNA* cell-free DNA, *MRD* measurable residual disease.

Cherng & Herrera. et al, Current Treatment Options in Oncology 2024

Conclusions

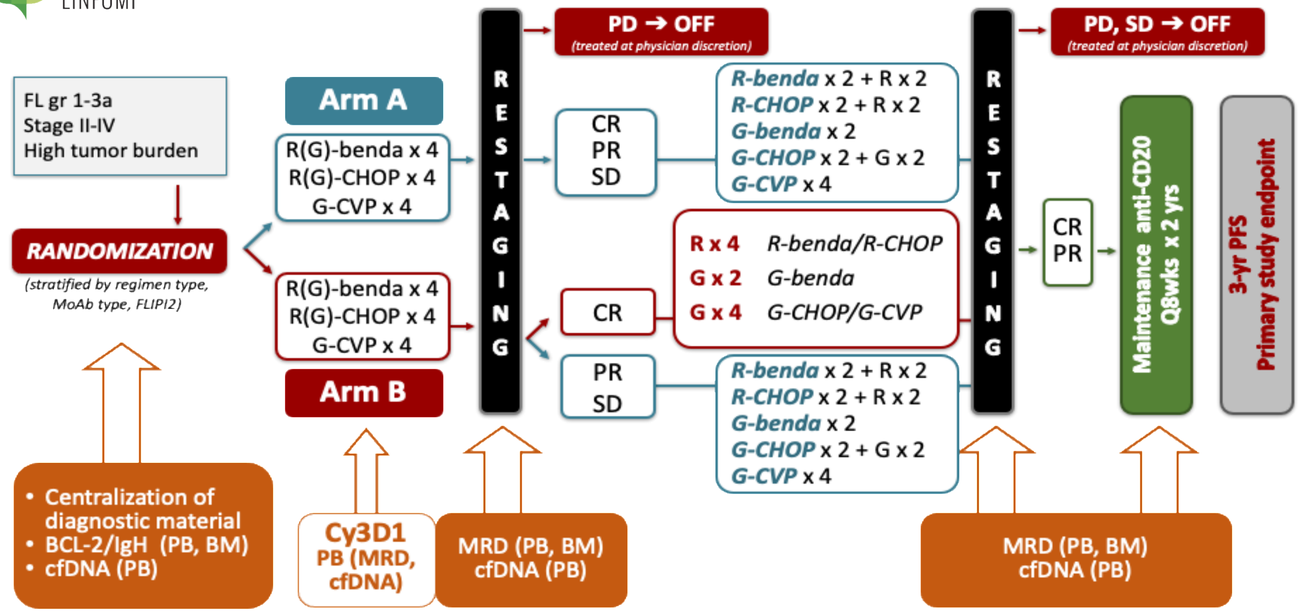
- **Before treatment** and with a non-invasive approach, liquid biopsy allows to identify molecular subgroups with prognostic relevance and possibly predictive impact (DLBCL, HL)

- **After treatment,** liquid biopsy allows a non-invasive MRD evaluation with prognostic impact and refines the metabolic response of PET/CT (DLBCL, HL, FL)

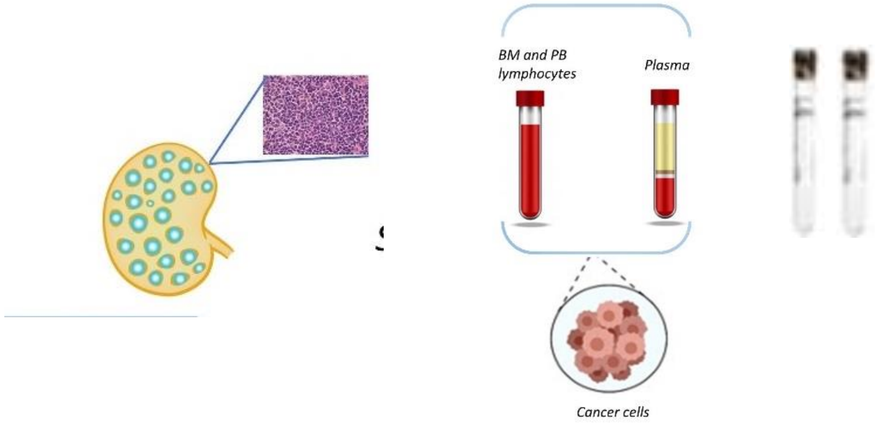
- The integration of liquid biopsy and PET parameters and other clinical and histopathological features should be used to design clinical trials for high-risk patients

- The applicability in clinical practice remains an issue

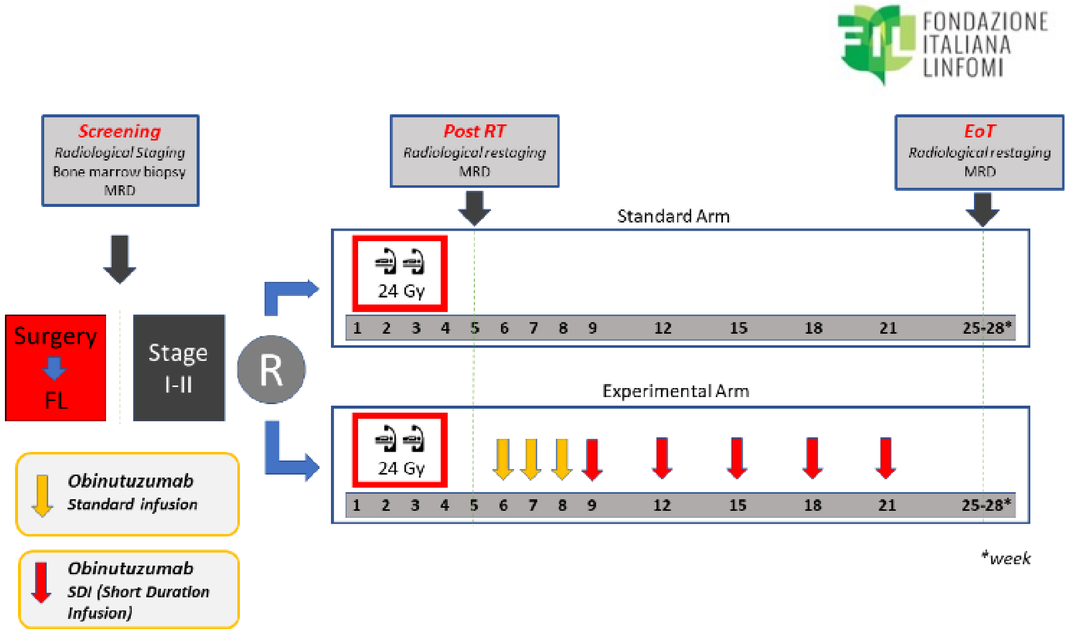
FOLL19 TRIAL



CHOP or Bendamustine combined with R (rituximab, original or biosimilar; D1 of each cycle) or combined with G (obinutuzumab, according to approved label, i.e., D1,8,15 in C1, D1 in subsequent cycles) or CVP combined with G (D1,8,15 in C1, D1 in subsequent cycles); *Response assessment per International Lugano 2014 criteria.*



GAZEBO TRIAL



- Space for :

1) Tumor burden at baseline (cfDNA + PET TMTV)
2) Mutations (liquid biopsy) at baseline
3) Esplorative marker screening at baseline and MRD on plasma
4) Microbioma
5) Other….

Networks are the key of success

Acknowledgments

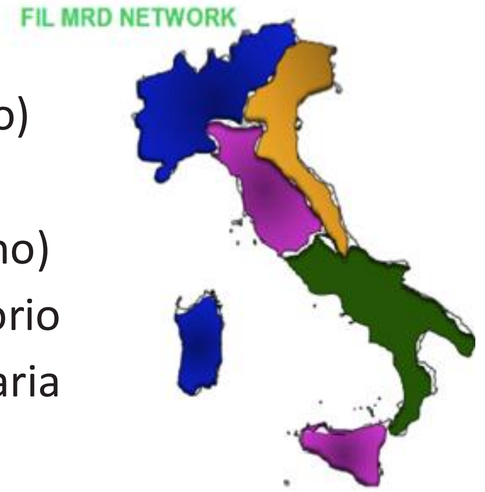
The FIL MRD network (Italy)

Simone Ferrero, Daniela Drandi, Elisa Genuardi (Torino)

Sara Galimberti, Clara Bono (Pisa)

Valter Gattei, Riccardo Bomben, Tamara Bittolo (Aviano)

Ilaria Del Giudice, Irene Della Starza, Vittorio Bellomarino, Giovanni Assanto, Roberta Soscia, Ilaria D'Antuono (Roma)



UNIVERSITÀ DEL PIEMONTE ORIENTALE

Università del Piemonte Orientale (Novara)

Riccardo Moia

Gianluca Gaidano

Commissione Studi Biologici FIL

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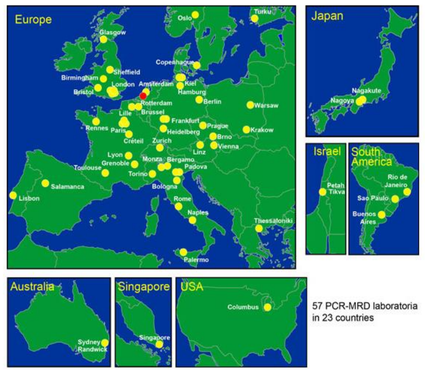

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Grazie!