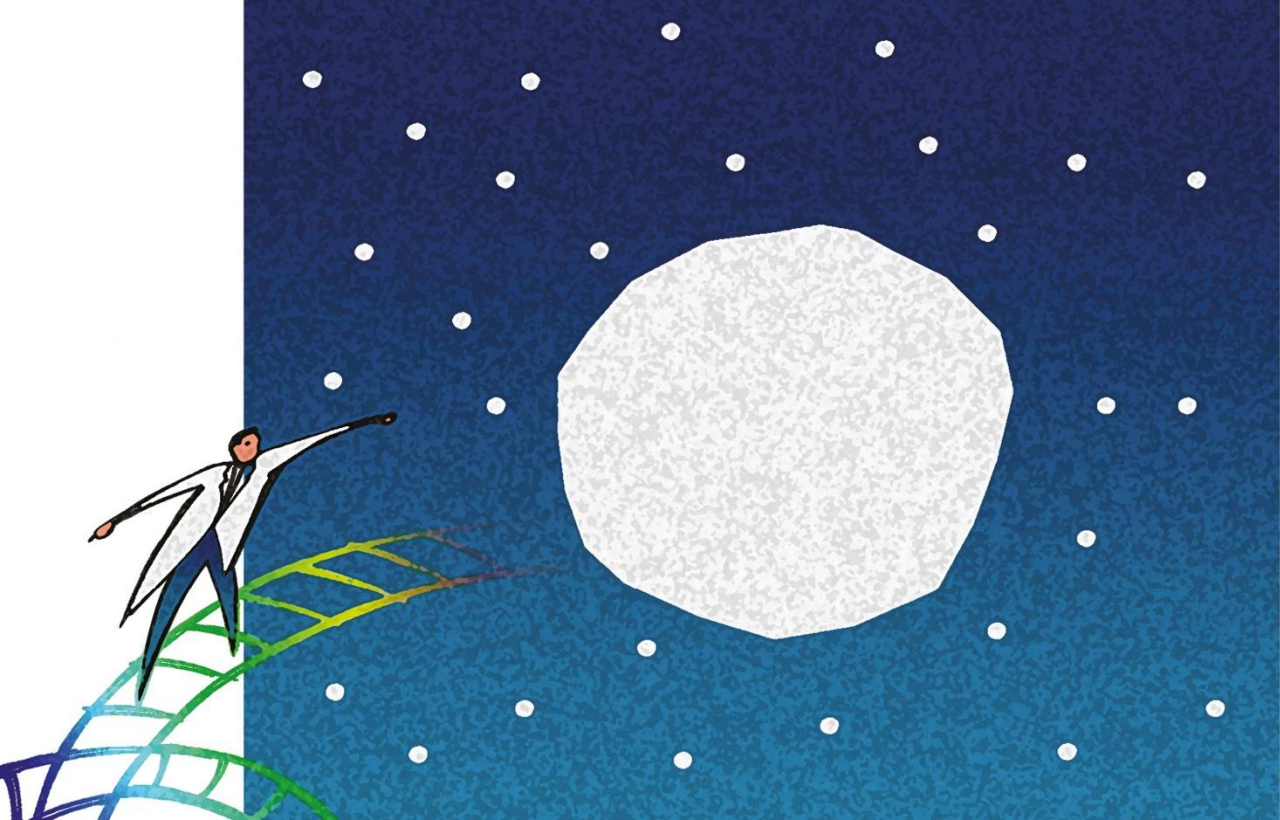




The young side of **LYMPHOMA**

gli under 40 a confronto

Reggio Emilia, Hotel Mercure Astoria
2-3 ottobre 2026

Valutazione dell'MRD nel trattamento dei Linfomi

**ctDNA in Lymphoma:
The Liquid Revolution**

Giovanni Manfredi Assanto, MD, PhD

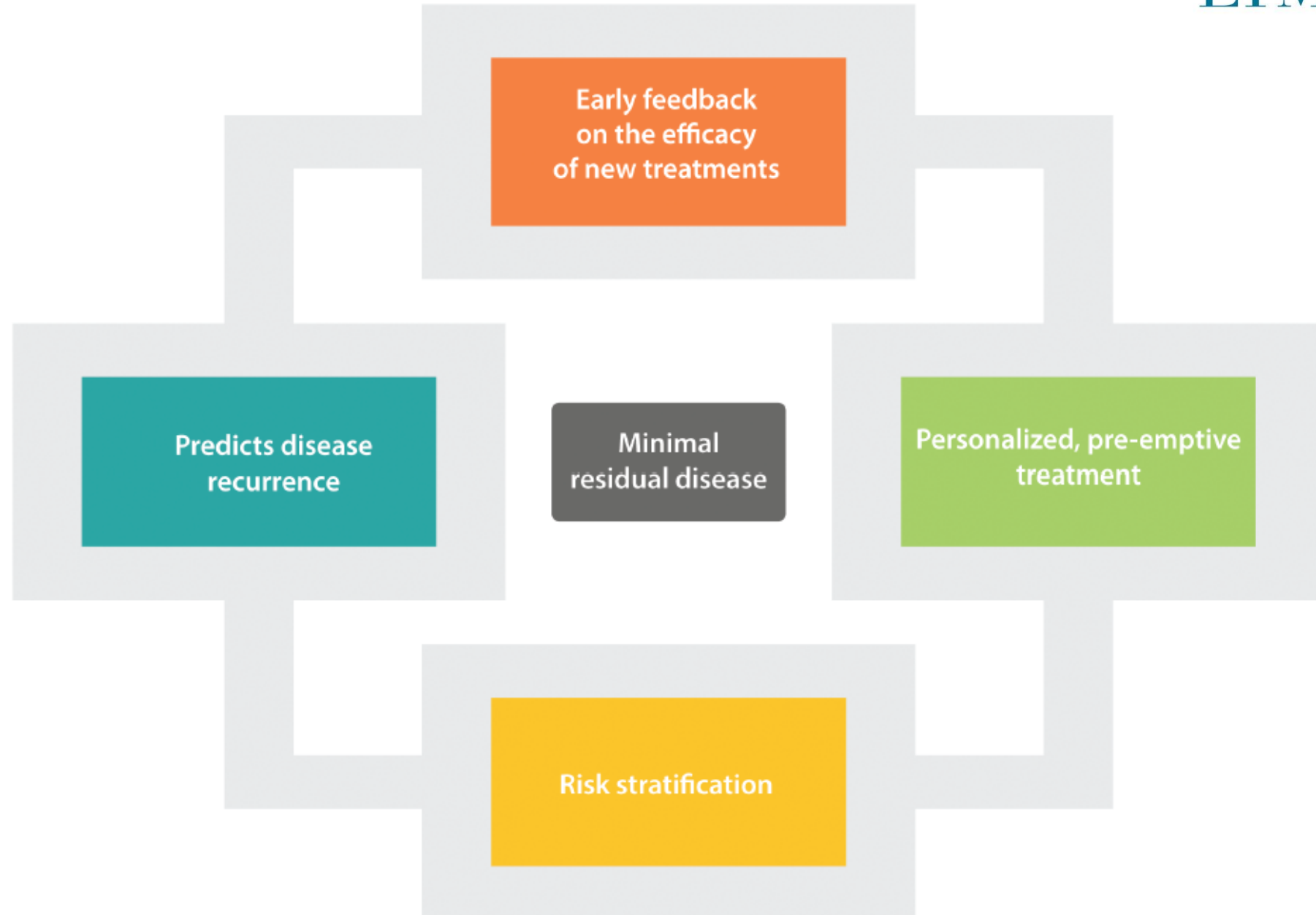
Hematology · Department of Translational and Precision Medicine · Sapienza University of Rome



Disclosures — Giovanni Manfredi Assanto, MD

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Travel Grant
Takeda						X	
BeOne	X						X
Gilead	X						
Astrazeneca							X

The clinical role of MRD in Lymphoma



one liquid biopsy at a time

02

The PET-CT problem

Why imaging alone leaves hematologists guessing

02

The toolbox of Liquid biopsy

From IG rearrangements to phased variants — sensitivity race

03

Baseline & interim ctDNA

Prognosis before you've even started treatment

04

End-of-treatment MRD

The single strongest predictor we have right now

05

Others settings

CNS lymphoma, Hodgkin, PMBCL, CAR-T & transplant

06

What's next

Trials that will change how you treat in 2027

THE SETUP

PET/CT tells you "maybe"

Metabolic remission is not molecular remission — and residual FDG uptake is often just inflammation.

25–45%

false-positive PET lesions
at interim/ EOT in lymphoma

~50%

positive predictive value
of a positive EOT PET-CT

~20%

PET-negative LBCL patients
who still relapse

The clinical risk

Unnecessary confirmatory biopsies · Repeat imaging and radiation exposure · Delayed access to salvage / CAR-T · Over-treatment of patients who are actually cured · Under-treatment of patients with truly residual disease

Quale errore temete di più nella vostra pratica clinica?

- A** Trattare troppo un paziente di fatto guarito (falso positivo)
- B** Trattare troppo poco un paziente con malattia residua (falso negativo)
- C** Dipende dal sottotipo di linfoma e dal contesto
- D** Preferisco sempre un esame di conferma in più, qualunque sia il rischio

THE CONCEPT

A tumor fingerprint, floating in plasma

Apoptotic and necrotic lymphoma cells shed fragments of DNA into the bloodstream (ctDNA). The tumor-derived fraction — circulating tumor DNA (ctDNA) — carries the lymphoma's own genetic signature.

1

Find the signature

Sequence tumor tissue and/or baseline plasma to identify clonal IG rearrangements, somatic mutations, or phased variants unique to the lymphoma.

2

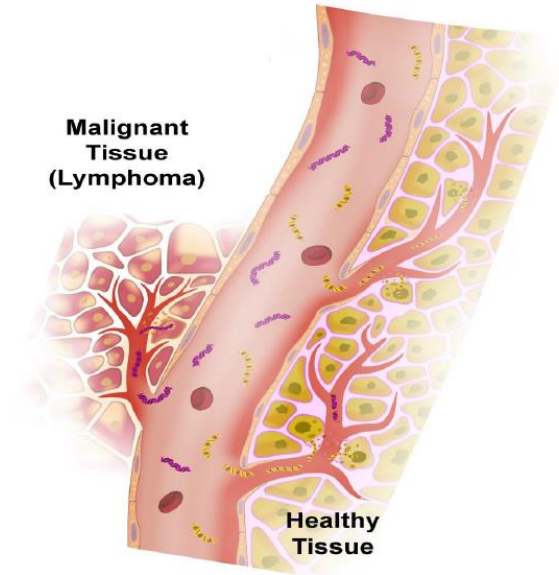
Track it in blood

Design a **personalized (or panel-based) assay to hunt** for that exact signature in serial plasma draws — before, during, and after therapy.

3

Read the trend

Detectable ctDNA = molecular disease.
Undetectable ctDNA = deep molecular response — often before imaging can tell the difference.



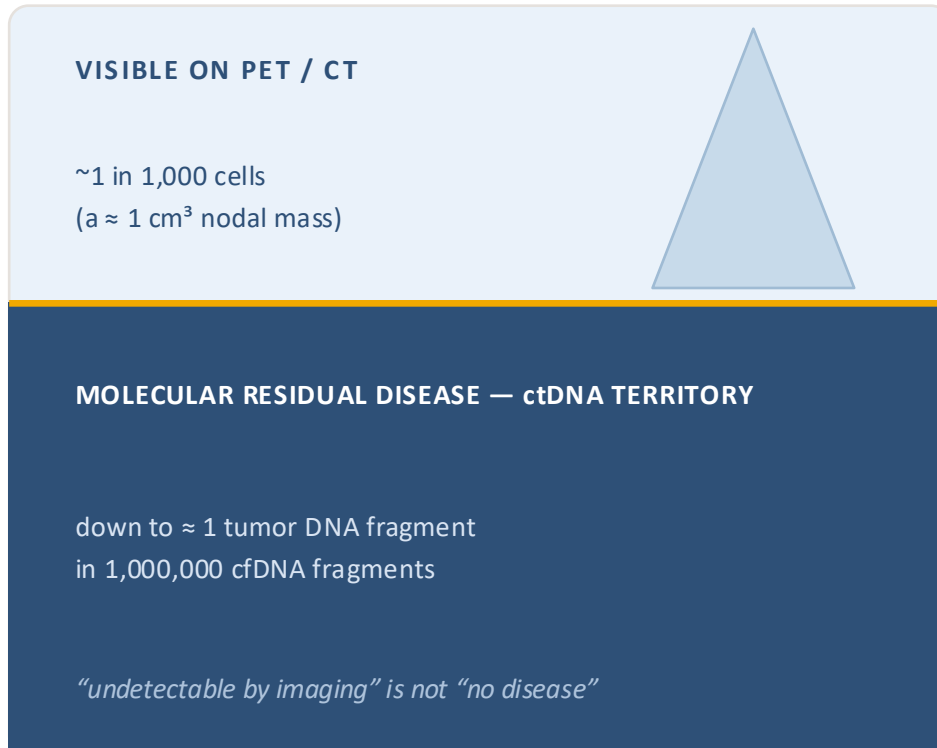
Why lymphoma is a perfect candidate:

clonal B-cell origin (trackable IG rearrangements) + high mutation/hypermutation burden (BCL6, SOCS1, IGLL5...) = abundant, patient-specific targets.

CONCEPT CHECK

MRD: the iceberg beneath the scan

Measurable/Minimal Residual Disease = tumor burden that persists below the resolution of clinical exam, CT, or even PET —is still detectable by ultrasensitive molecular methods.



A paradigm borrowed — and reinvented

MRD already changed practice in ALL, CLL and myeloma, where malignant cells circulate and flow cytometry / PCR can find them directly. Lymphoma has no reliable circulating tumor cells — so ctDNA became the surrogate: not counting cells, but reading the tumor's DNA signature in plasma.

Three questions, three timepoints

- **Baseline** Is there a trackable signature — and how much disease is there?
- **Interim / EOT** Is the tumor clone gone — before, or in addition to, what the scan shows?
- **Surveillance** Is molecular relapse emerging before it becomes clinical relapse?

MRD doesn't ask “is there a mass?” — it asks “is there tumor DNA in this tube of blood?”

Se aveste oggi un test ctDNA-MRD affidabile, dove lo usereste per primo?

A Per confermare una remissione completa ed evitare l'overtreatment

B Per confermare un PET dubbio prima di una biopsia

C Per intercettare una recidiva molecolare in sorveglianza

D Non modificherebbe le mie decisioni cliniche attuali

The sensitivity arms race

Every generation of assay pushed the limit of detection further — from 1 in 10,000 to 1 in a million.

Other methods:

dPCR $\sim 10^{-4}$

WGS $\sim 10^{-2}$

1

IG-NGS

IGH/IGK rearrangements

$\sim 10^{-4}$

LymphoTrack, donoSEQ — DLBCL, HL, PMBCL MRD

2

CAPP-Seq

Multiplex PCR + hybrid capture of recurrent SNVs

$\sim 10^{-3}$

PMBCL (CAMIL/LYSA), early DLBCL series

3

EuroClonality-NDC

IG/TCR + SNV + CNA + translocations, one assay

$\sim 10^{-4-5}$

Pan-lymphoma genomic + MRD capture panel

4

PhasED-Seq

Phased variants = 2+ mutations on one DNA fragment

$\sim 10^{-6}$ (ppm)

DIRECT, Roschewski pooled, HOVON-902, TRANSFORM

PROOF OF PRINCIPLE

Same tube of blood, 1,000x more sensitive

Conventional NGS, CAPP-Seq and PhasED-Seq all count mutations in cfDNA — the difference is HOW they tell true signal from sequencing noise.

Conventional / IG-NGS



Tracks ONE clonal marker (the IGH/IGK VDJ junction) per read.

Noise floor: ~1 in 1,000–1 in 10,000 reads

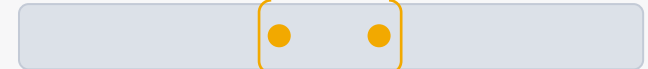
CAPP-Seq



Hybrid-captures dozens–hundreds of recurrent SNVs, but calls each one independently, on separate reads.

Noise floor: ~1 in 1,000

PhasED-Seq

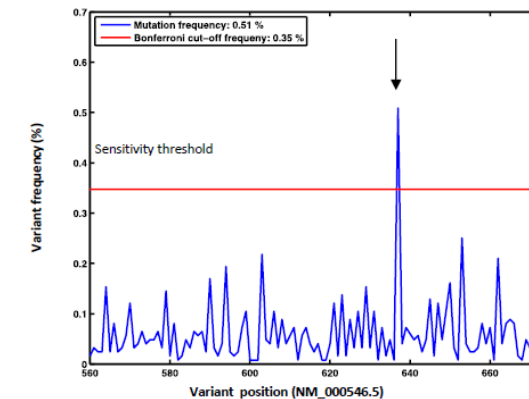


Requires 2–3 mutations to co-occur on the SAME cfDNA fragment (“phased”) — an AID-hypermutation signature.

Noise floor: <1 in 10,000,000

The dilution-series proof

Spiking known tumor fractions into normal plasma (DIRECT study): copy-number signal plateaus at a tumor fraction of $\sim 10^{-2}$, single-nucleotide variants plateau at $\sim 10^{-4}$ — but phased-variant (doublet/triplet) signal stays perfectly linear all the way down to 10^{-6} . The math: the odds of two independent sequencing errors landing on the exact same fragment, at the exact right loci, are the product of two tiny probabilities — background noise collapses by orders of magnitude.



True mutations

Background noise

ROUND 1 — BASELINE

Even before treatment starts, the plasma talks

DLBCL

cfDNA ≥ 0.7 ng/ μ L at baseline

63.8 vs 88.5 mo

median PFS (P=.022)

PMBCL

ctDNA detectable in

98%

of newly diagnosed patients (82/84)

cHL

Baseline biology alone

3 subtypes

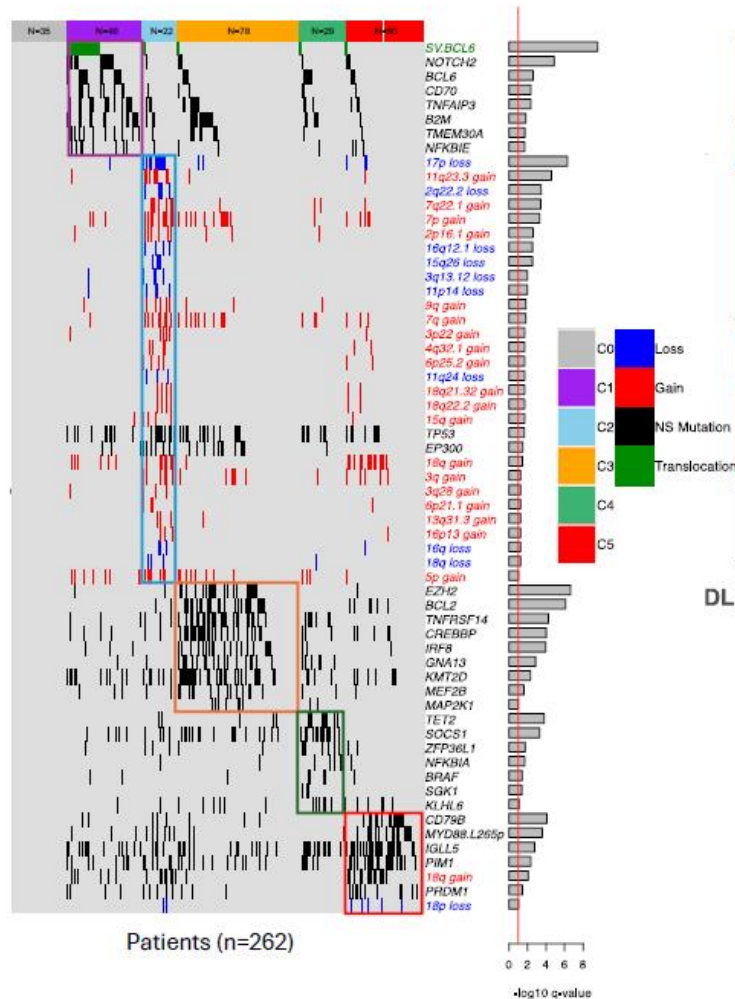
inflammatory / viral / oncogene-driven — each with distinct PFS

Bottom line

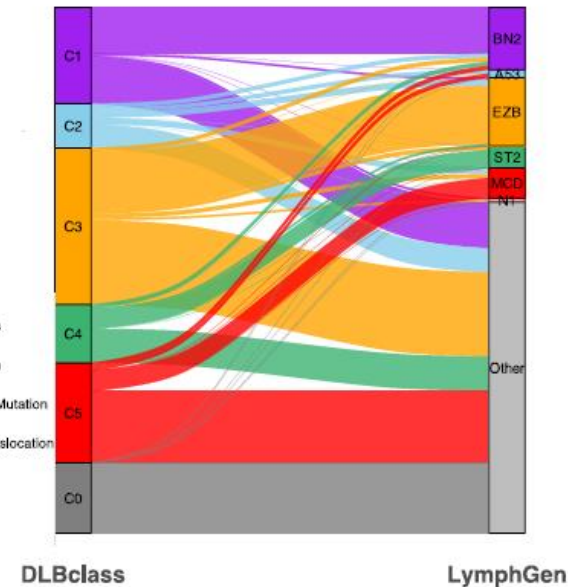
Baseline ctDNA correlates with tumor burden (LDH, stage, IPI, TMTV) across DLBCL, PMBCL and Hodgkin lymphoma. It's a liquid surrogate for disease bulk — and a launchpad for everything that follows: interim response and MRD.

An alternative to tissue based subtyping

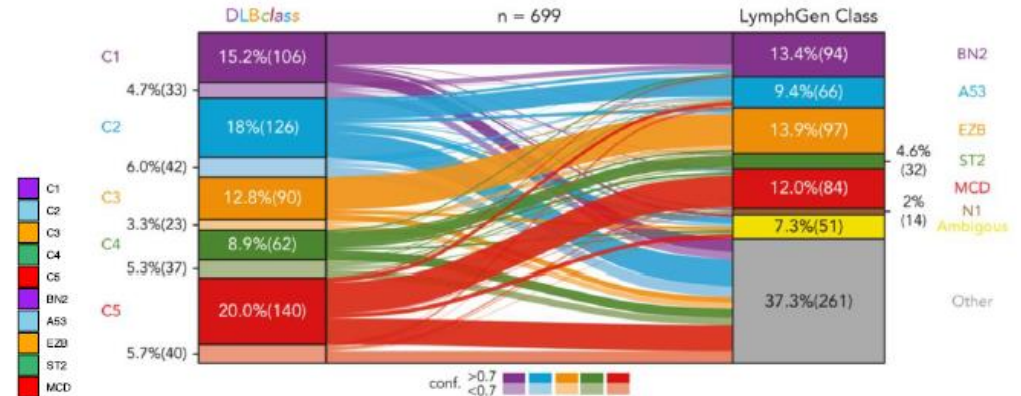
NMF Clustering



DLBclass vs LymphGen 2.0
ctDNA – Ly4.0



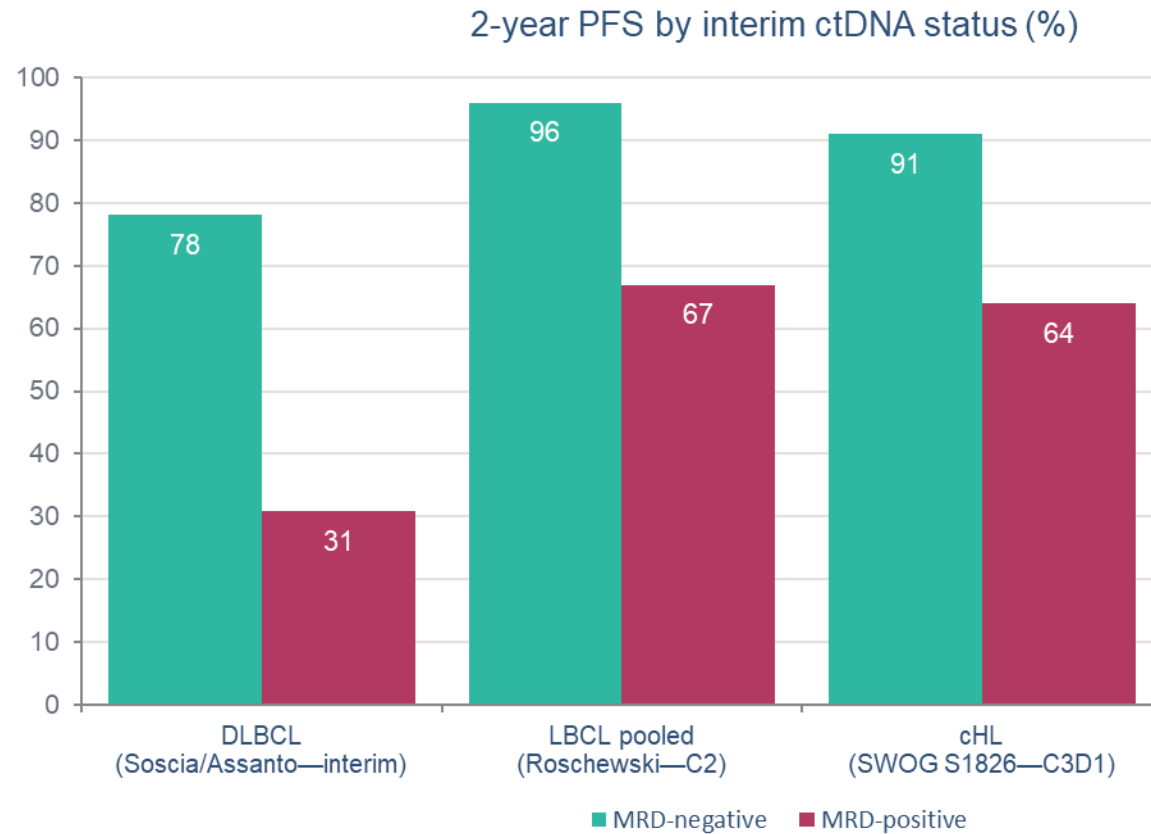
DLBclass vs LymphGen 2.0
gDNA - WES



Chapuy B, et al. Blood 2025

ROUND 2 — INTERIM

Two cycles in, interim ctDNA



Italian experience

Interim MRD stratifies even partial responders:
78.9% vs 37.5% PFS in PR-by-CT patients (P=.018).

LBCL (pooled)

5 trials, n=137. Undetectable ctDNA after 2 cycles:
HR 6.9 for PFS vs detectable.

Classic HL

SWOG S1826: molecular tumor burden (MTB) drop
at C3D1 outperforms a simple
detectable/undetectable call.

Changes in tumor cfDNA complement iPET

ROUND 3 — END OF TREATMENT

The number that should change your practice

End-of-treatment ctDNA-MRD is the single strongest, most reproducible prognostic signal across every recent lymphoma cohort.

Study (n)	Setting	PFS/TTP: MRD– vs MRD+	HR
Soscia/Assanto 2025 (73)	DLBCL, R-CHOP	83.8% vs 0%	24.5
Roschewski 2025 (137)	LBCL, pooled 5 trials	97% vs 29%	28.7
Wang / HOVON-902 2025 (134)	LBCL, real-world	85% vs 17%	9.8
Krupka / DIRECT 2025 (155)	LBCL, prospective UK	95% vs 42%	13.7
Camus / CAMIL 2026 (84)	PMBCL	98.4% vs 33.3%	78.1

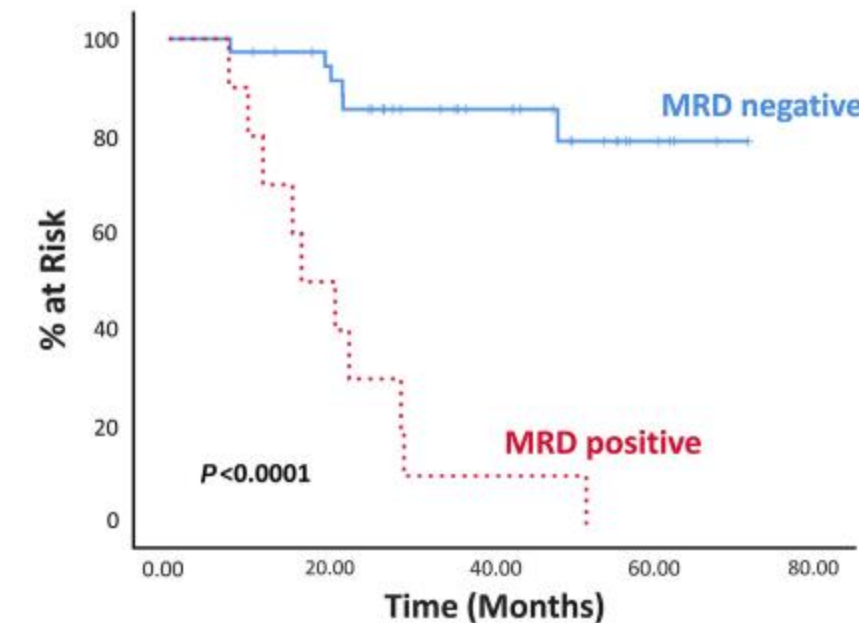
All five cohorts, five countries, five assays — same conclusion: undetectable EOT ctDNA = durable remission.

See references on final slide. HR = hazard ratio for progression/relapse in MRD-positive vs MRD-negative patients.

IG rearrangement driven MRD monitoring

Predictive power of ctDNA at EOT

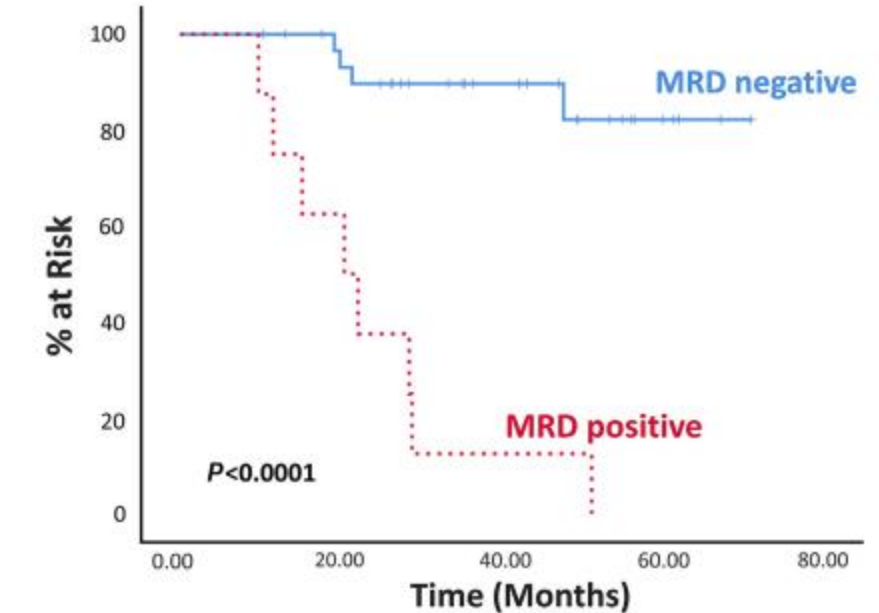
A Progression-free survival (months) all patients (N=47)



No. at risk:

MRD -	37	32	18	5	0
MRD +	10	5	1	0	0

B Progression-free survival (months) CR patients (N=40)

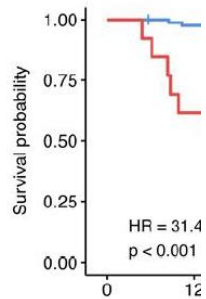


No. at risk:

MRD -	32	28	17	5	0
MRD +	8	5	1	0	0

PhasED ctDNA shows improved predictive value

NCCN Guidelines B-Cell Lymphomas Version 2.2025

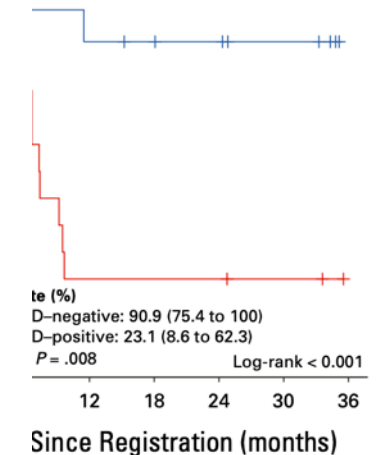


DLBCL category 2B recommendation

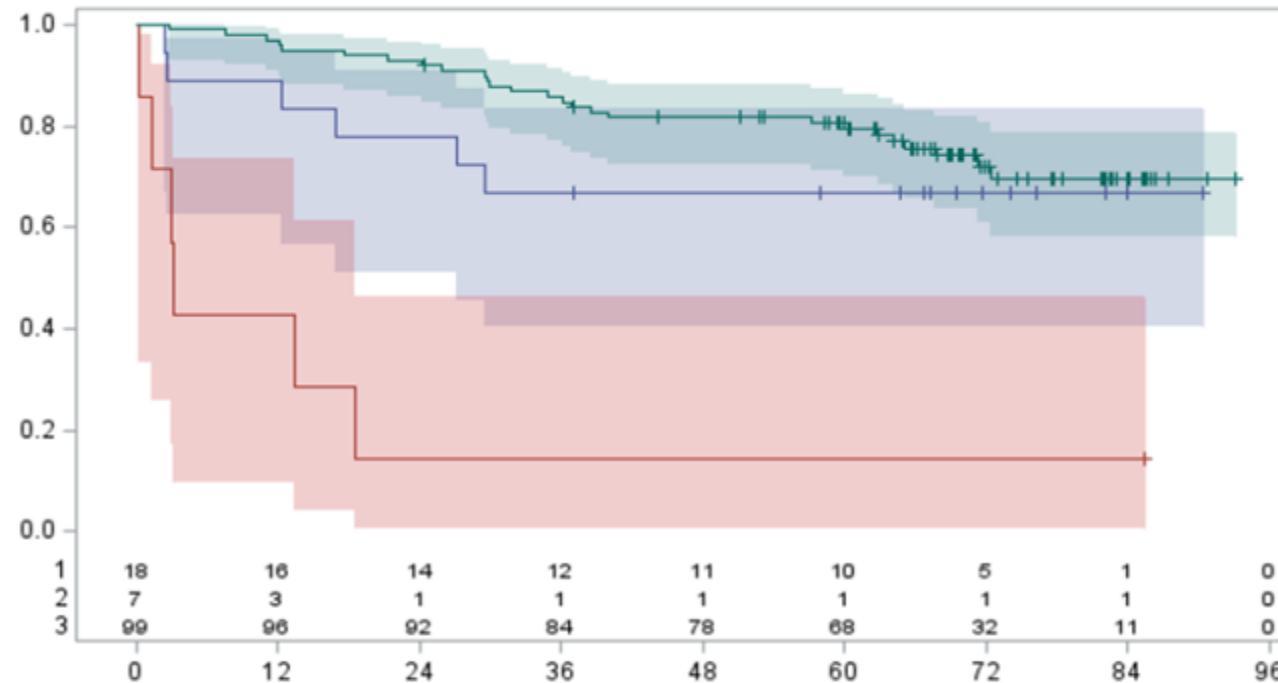
- If end-of treatment PET is positive
 - consider repeat biopsy
 - if biopsy not feasible, consider ctDNA-MRD assessment
- If biopsy and/or ctDNA-MRD–negative, follow PET-negative pathway

NCCN categories* of evidence and consensus	Description
1	Based upon high-level evidence ,** there is uniform NCCN consensus that the intervention is appropriate
2A	Based upon lower-level evidence , there is uniform NCCN consensus that the intervention is appropriate
2B	Based upon lower-level evidence , there is NCCN consensus that the intervention is appropriate
3	Based upon any level of evidence , there is major NCCN disagreement that the intervention is appropriate

ET positive EOT



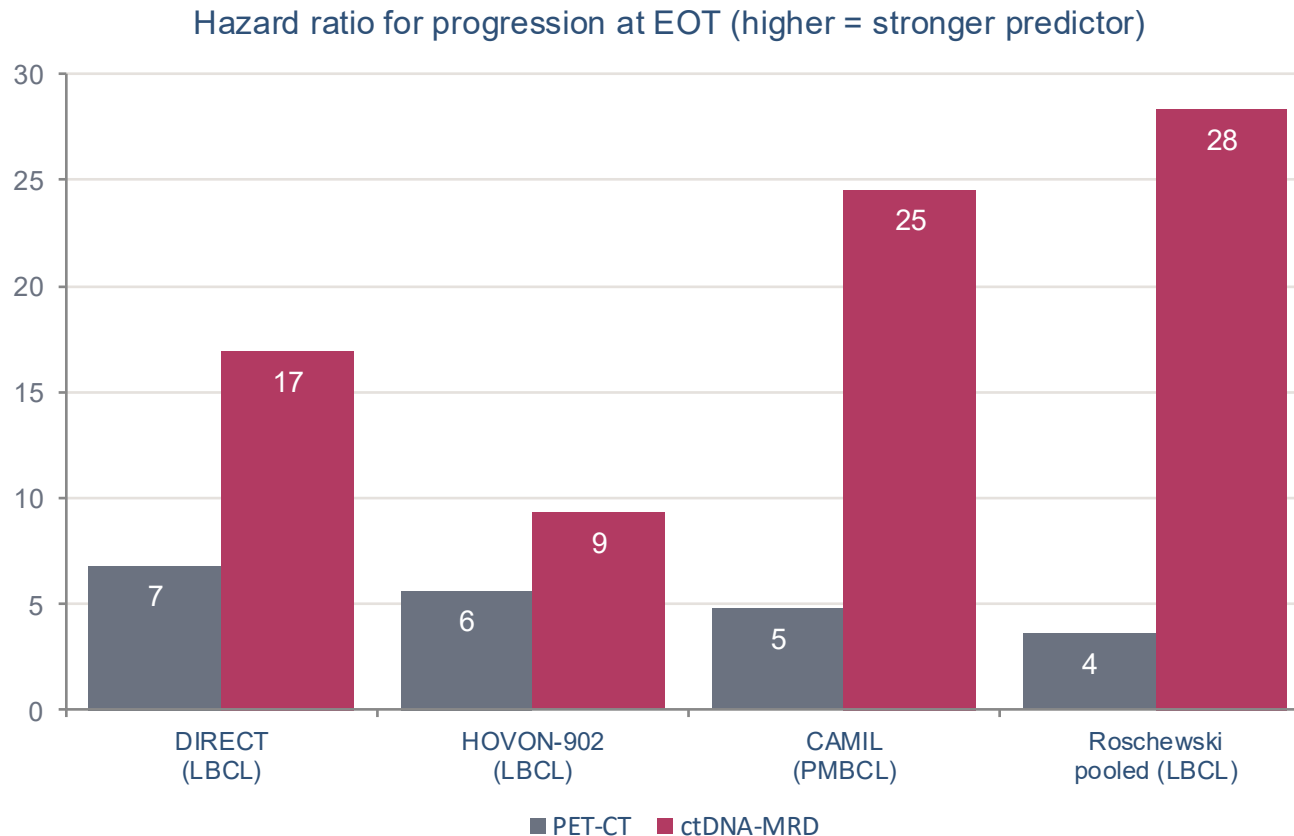
Higher specificity and PPV of ctDNA-MRD than PET/CT in FL



	No. of Subjects	Event	Censored	Median Survival (95% CL)
Discordant PET and ctDNA	18	33.3% (6)	66.7% (12)	Not reached (27.1; NA)
PET + / ctDNA +	7	85.7% (6)	14.3% (1)	3.1 (0.3; 18.5)
PET - / ctDNA -	99	26.3% (26)	73.7% (73)	Not reached

THE SHOWDOWN

ctDNA-MRD vs PET-CT: same NPV, much better PPV



PPV 68% vs 56%

ctDNA-MRD outperforms PET for positive predictive value
(HOVON-902, real-world LBCL)

NPV ≈89% both

Negative predictive value is comparable — both are excellent at
confirming true remission

PET+ / MRD- → no relapse

In PMBCL & LBCL, MRD-negative patients rarely progress even with a
positive PET — likely inflammation/fibrosis, not disease

PET-CT positivo a EOT ma ctDNA-MRD negativo. Cosa fareste?

- A** Procedo comunque con la biopsia di conferma
- B** Mi fido del ctDNA e osservo (watch and wait)
- C** Ripeto entrambi i test a breve distanza
- D** Dipende dal contesto clinico specifico (bulk, sede, IPI)

ctDNA doesn't just monitor Hodgkin — it classifies it

Inflammatory Immune-Escape HL

9p24.1/PD-L1 amplification, cold-ish TME, TREG-rich

86.4% 3-yr PFS

Virally-Driven HL

EBV/HHV6-associated, low TMB, adaptive immune
TME

92.7% 3-yr PFS

Oncogene-Driven HL

High TMB, TNFAIP3/ITPKB/SOCS1 mutated, cold
TME

97.1% 3-yr PFS — best prognosis

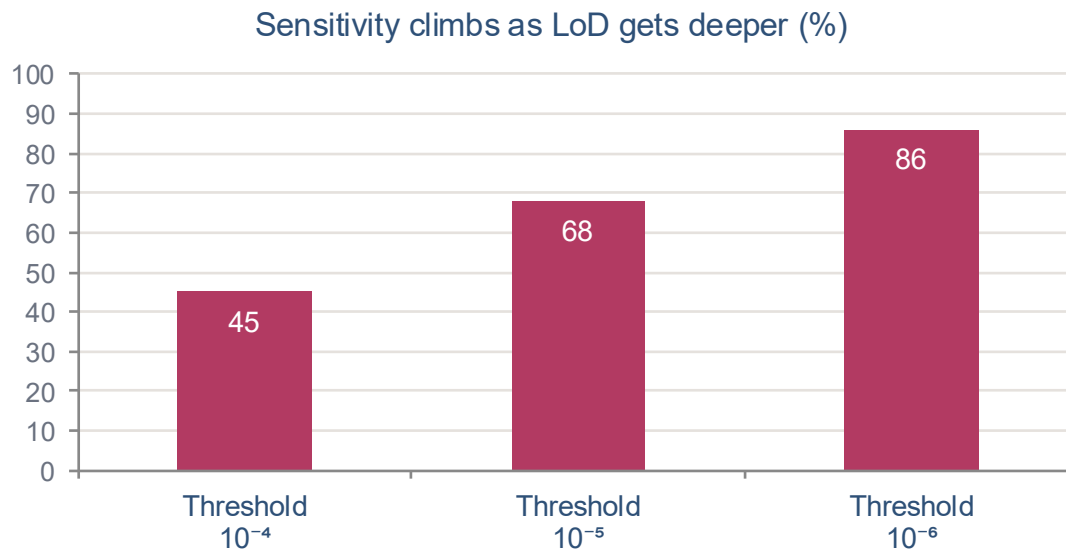
MRD sharpens it further

- HD21 (LymphoVista HL): MRD-2 positivity → 4-yr PFS 95.3% vs 72.2%, HR 6.9 — holds across BrECADD, eBEACOPP, PET2+ and PET2– subgroups
- MRD adds risk stratification WITHIN each biologic subtype — inflammatory-immune-escape MRD+ patients drop to 55.5% PFS
- SWOG S1826: dynamic molecular tumor burden change at C3D1 outperforms a simple binary MRD call

REALITY CHECK

Not all “undetectable” is created equal

Every assay has a limit of detection (LoD) — and it is patient-specific, not a fixed number on a brochure.



Patient-specific LoD95

DIRECT: LoD95 spans >2 orders of magnitude between patients (median 4.54 ppm) — driven by cfDNA yield & number of trackable variants.

Biopsy beats plasma-only genotyping

In 78% of DIRECT patients, tissue-informed variant selection gave a more sensitive assay than plasma-only baseline.

“Persistent MRD” ≠ always relapse

3 of 4 transformed follicular lymphoma patients in DIRECT stayed PV-MRD+ without relapse — a truly indolent clone can masquerade as residual disease.

Sareste disposti a de-escalare il trattamento in un paziente MRD-negativo, anche prima delle linee guida?

Una domanda sulla vostra disponibilità al cambiamento.

- A** Sì, se il paziente informato lo desidera
- B** Solo all'interno di un trial clinico
- C** No, aspetto le linee guida ufficiali
- D** Dipende dal rischio basale del paziente (bulky, IPI alto...)

The trials that will decide how we act on MRD

ALPHA3

NCT06500273

LBCL, EOT MRD+

Consolidation with allogeneic CAR-T vs observation in MRD-positive patients — the escalation question, finally tested.

SHORTEN-ctDNA

NCT06693830

DLBCL, early clearance

De-escalating chemotherapy cycles once early molecular clearance is achieved — the de-escalation question.

G043075

NCT04980222

1L LBCL, high-risk ctDNA

Glofitamab + R-CHOP guided by ctDNA dynamics for patients predicted to do poorly with chemo alone.

What it actually takes to use this tomorrow inside FIL

Step	What's needed	Where we are
Improve Baseline genotyping	Tumor FFPE and/or pretreatment plasma; IG, SNV or phased-variant panel	Feasible in 93–99% of patients across DLBCL, HL, PMBCL, CNSL
Collect Serial sampling	Standardized collection (interim + EOT $\pm$ relapse) across centers	Multicenter feasibility shown (HOVON-902, CAMIL/LYSA, DIRECT: 6 UK sites)
Provide Turnaround	Rapid, reproducible result to inform real decisions	ALPHA3 shows <14-day turnaround is achievable in the clinic
Guideline uptake	NCCN now allows ctDNA-MRD as an alternative to biopsy for PET-positive EOT in LBCL (category 2B)	Adoption starting — standardization across assays still needed

What we are proposing inside FIL: ITALYMPH

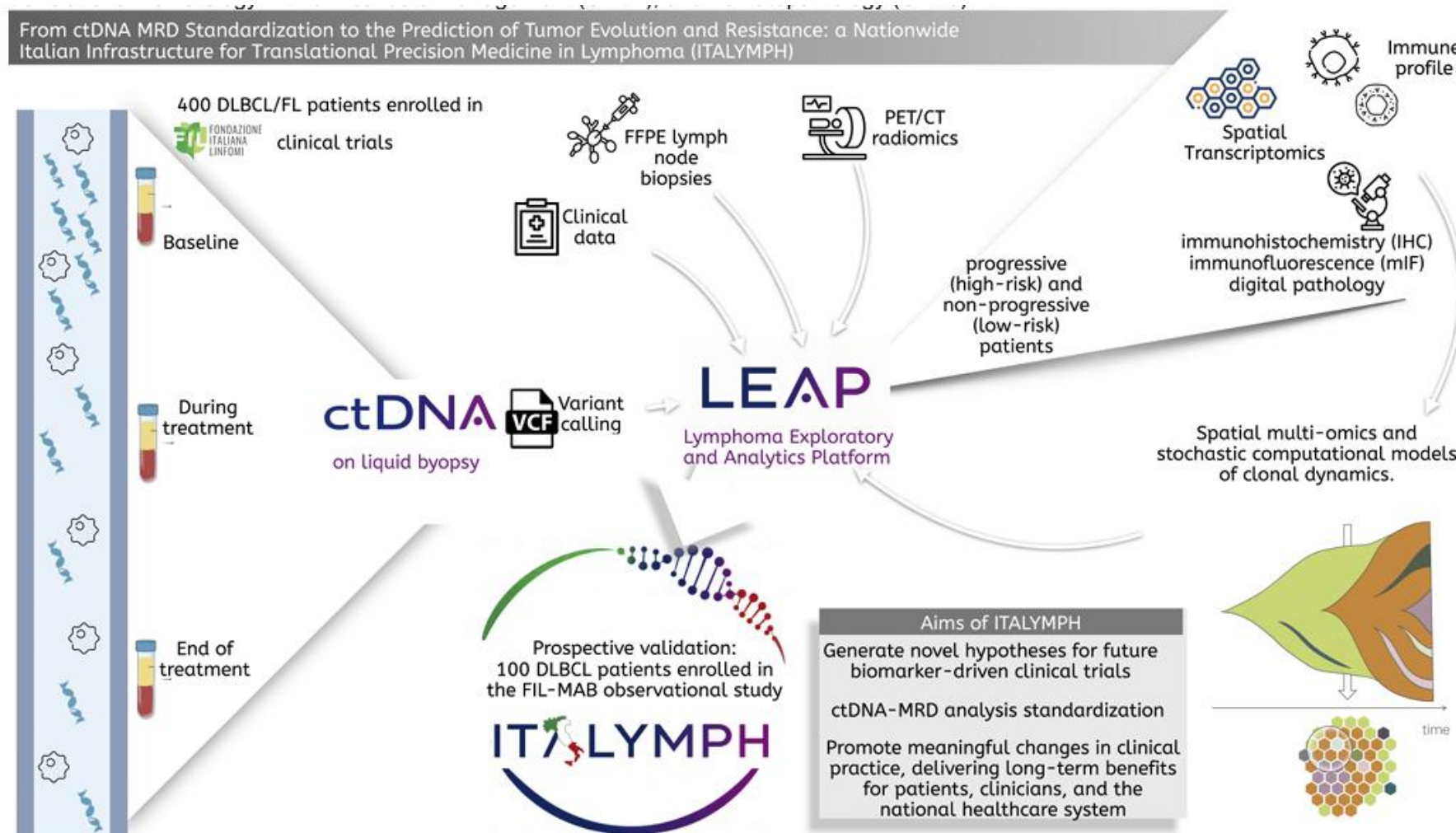
The young side of
LYMPHOMA

Principal Investigator

Riccardo Moia

Università del Piemonte Orientale

Application PRIN 2026



Aims of the project

The aims of *ITALYMPH* are closely interconnected and will be dynamically integrated throughout the study.

The standardization of MRD assessment using ctDNA across laboratories in Italy (MS1) is fully aligned with the objectives of the PRIN call. In a short timeframe, this assay is expected to complement PET imaging, improving risk stratification and enabling more accurate allocation of patients to different post-induction treatment strategies in Italy.

TAKE-HOME

Five things to remember on Monday morning

- 1 ctDNA-MRD at end of treatment is the strongest single prognostic factor we have in aggressive lymphoma — stronger than IPI, stronger than PET.
- 2 It doesn't replace PET — it disambiguates it. Use ctDNA to interrogate discordant, equivocal, or PET-positive results.
- 3 Undetectable ctDNA at EOT = very low relapse risk. Detectable ctDNA = talk about escalation before the scan lights up.
- 4 The technology now works beyond DLBCL: Hodgkin subtyping, CNS lymphoma risk models, PMBCL, and CAR-T vs transplant.
- 5 “Undetectable” is assay- and patient-specific — consider the LoD before you trust a negative result.

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Ilaria D'Antuono, Vittorio Bellomarino, Deborah Cardinali, Irene Della Starza, Roberta Soscia



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