XIX CONGRESSO NAZIONALE SIES 2026

Le nuove sfide tecnologiche della biopsia liquida: il panorama genomico e fragmentomico del cfDNA

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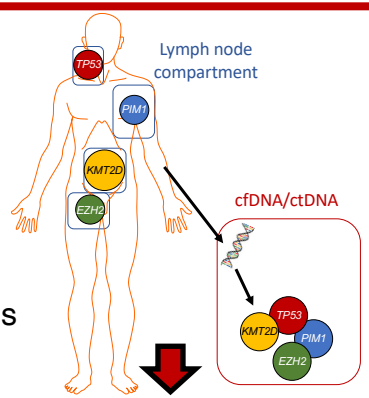
Firenze | 4-6 marzo 2026
Palazzo degli Affari

Disclosures of Riccardo Moia

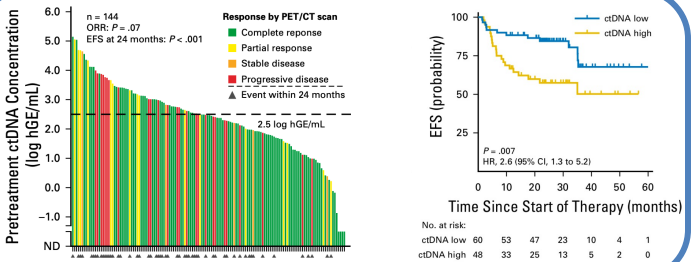
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Abbvie					X	X	
AstraZeneca					X		
BeOne					X	X	
Johnson & Johnson			X		X	X	
Lilly					X	X	

Clues potentially exploited from cfDNA/ctDNA

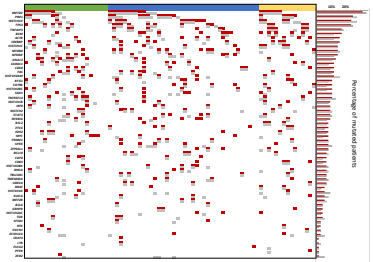


- Relies on the detection of somatic mutations
- Does not rely on the detection of somatic mutations

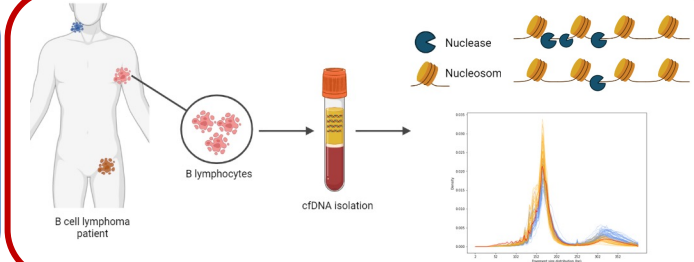
ctDNA levels



Molecular characterization



cfDNA fragmentation profile



Rossi *et al., Blood*. 2017; Kurtz *et al., JCO.* 2018; Chapuy *et al., Nat Med*. 2018; Moia *et al., Blood Adv*. 2025; Schmitz *et al., NEJM*. 2018; Lacy *et al., Blood*. 2020; Wright *et al., Cancer Cell*. 2020; Esfahani *et al., Nat. Biotechnol*. 2022; Mouliere *et al., Sci Transl Med*. 2018; Cristiano *et al., Nature*. 2019.

Agenda

- Exploiting cfDNA for lymphoma (and CH) genotyping
- cfDNA analysis integrates other lymphoma biomarkers
- The potential role of cfDNA fragmentomics

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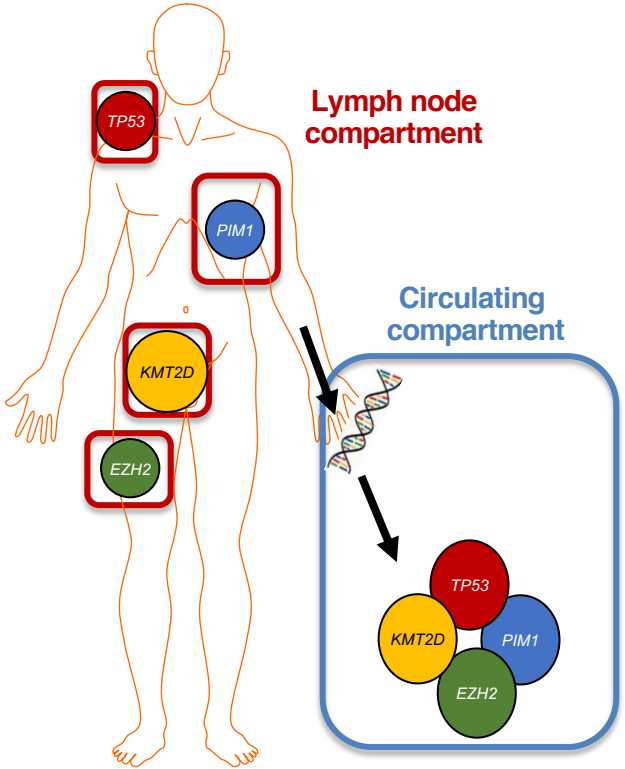
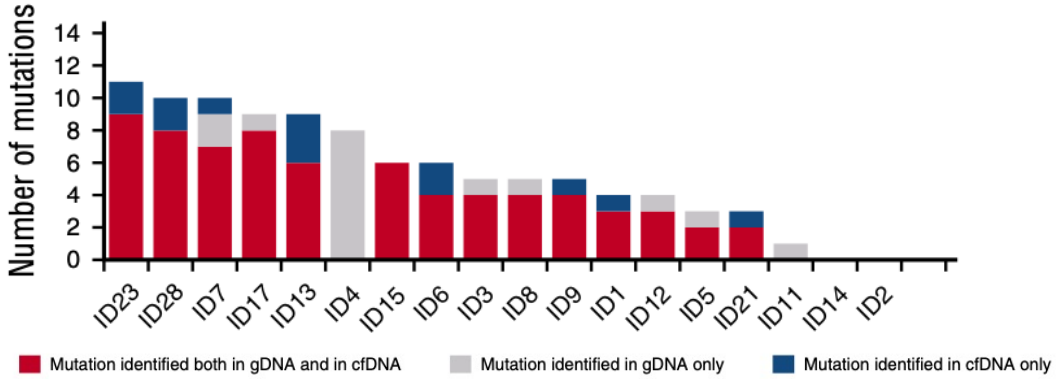
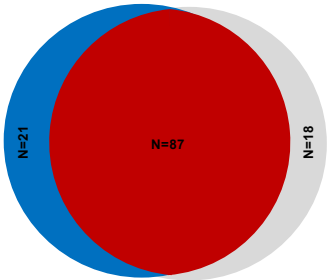
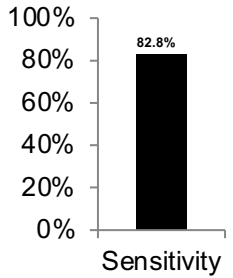
- **Exploiting cfDNA for lymphoma (and CH) genotyping**
- cfDNA analysis integrates other lymphoma biomarkers
- The potential role of cfDNA fragmentomics

ctDNA is a tool for DLBCL genotyping



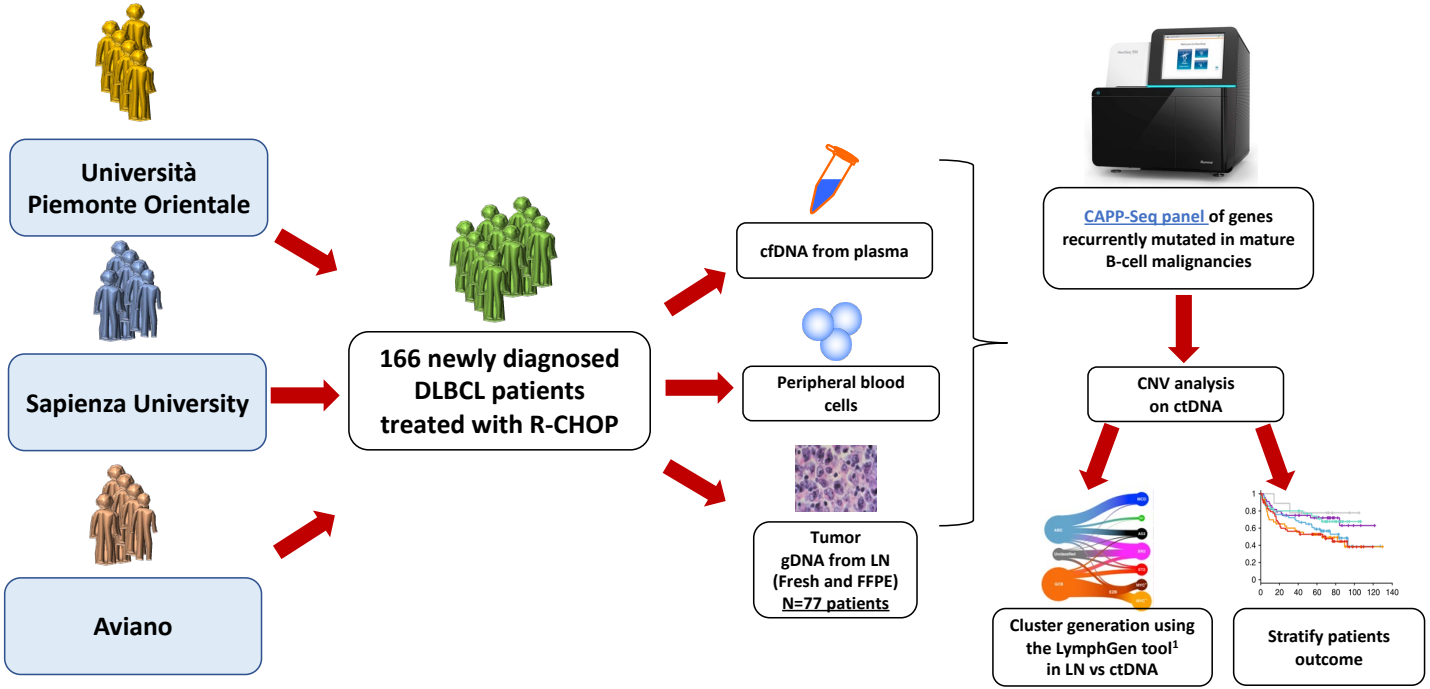






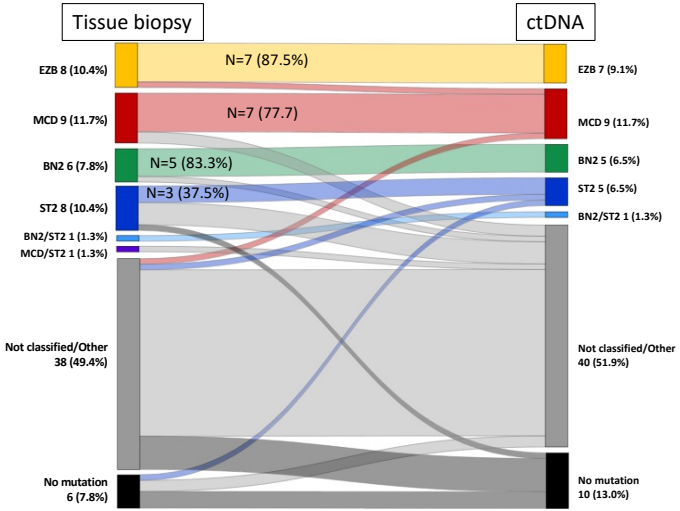
Rossi *et al., Blood*. 2017

DLBCL molecular cluster identification on liquid biopsy

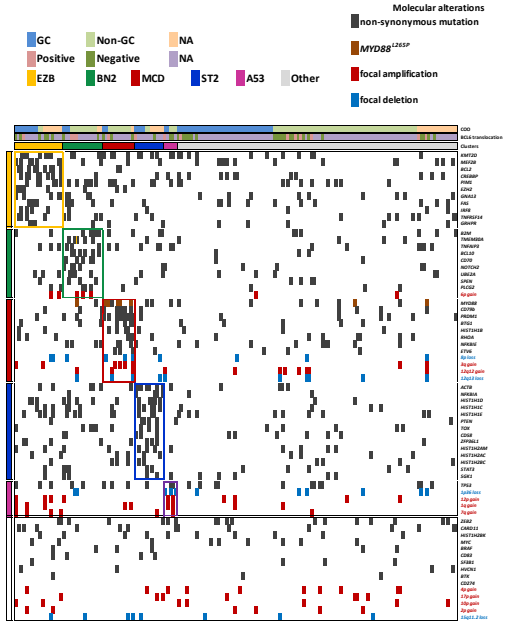


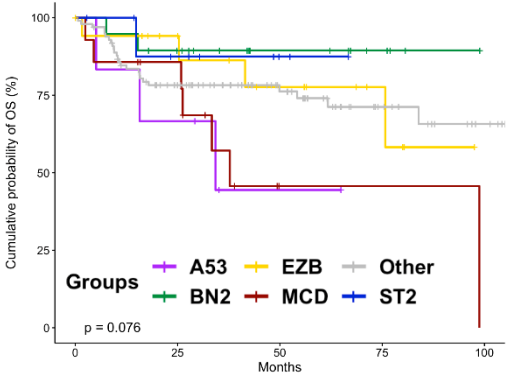
Liquid biopsy reflects the molecular characteristics and clinical impact of molecular clusters identified on tissue biopsy

N=77 DLBCL



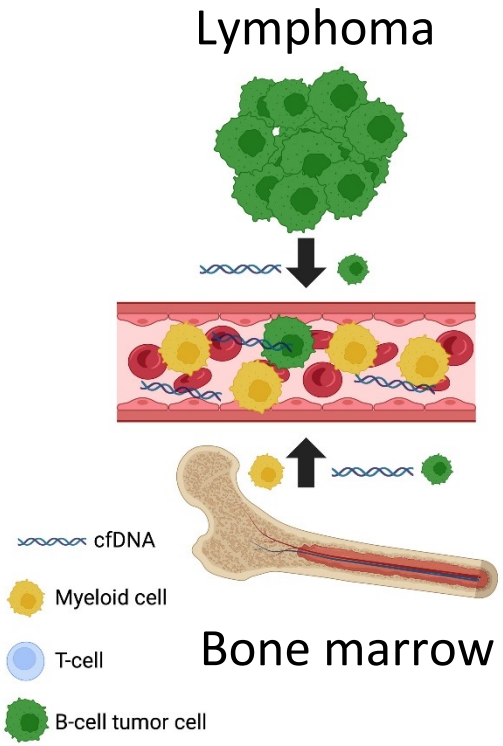
N=166 DLBCL





Moia *et al., Blood Advances*. 2025

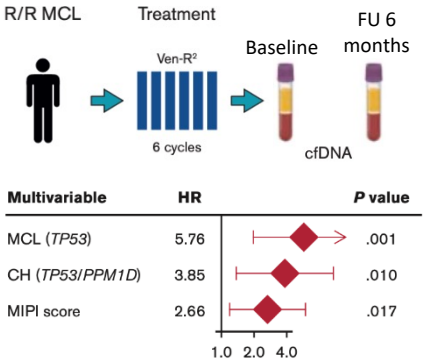
Clonal hematopoiesis (CH) is also detectable in cfDNA

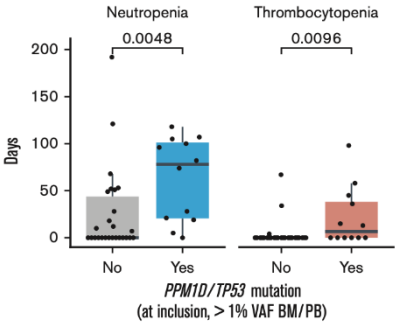


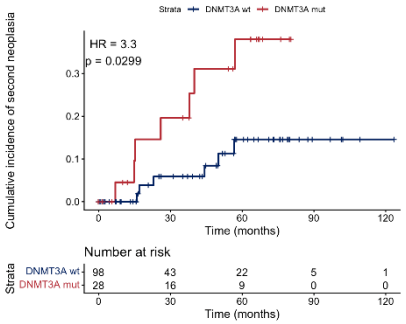
Granulocytes can be exploited as:

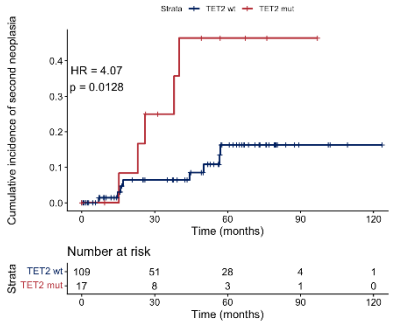
- a source of germline DNA to be used as blank for lymphoma genotyping
- a source of CH to clearly assessing the origin of mutations (lymphoma vs CH), especially for those potentially shared by both lymphoma and CH (i.e. *TP53*, *TET2*…)

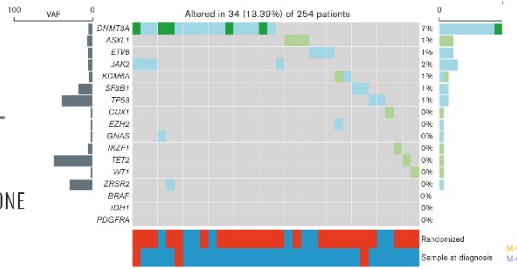
CH is not only a bystander phenomenon in lymphomas

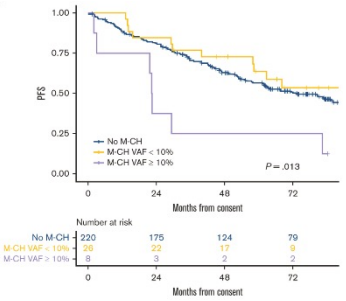


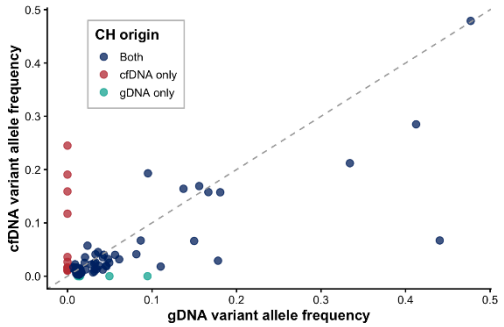












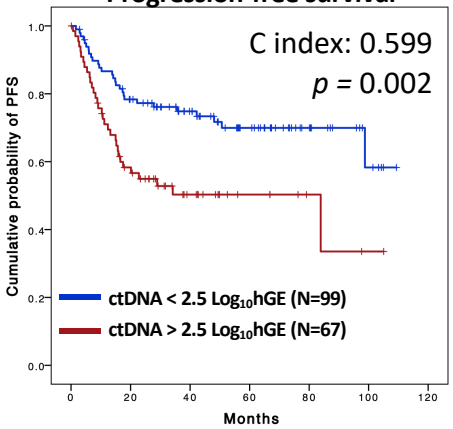
Maher *et al., SIES* 2026, oral presentation Friday March 6th 8.30-10am

Agenda

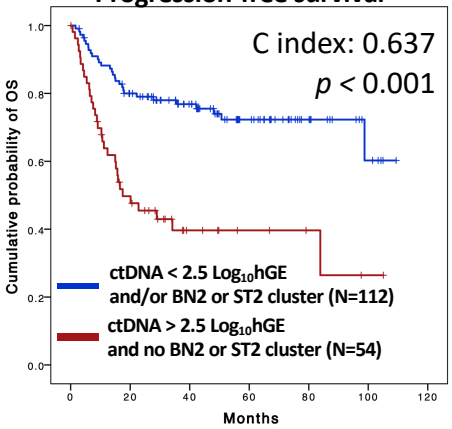
- Exploiting cfDNA for lymphoma (and CH) genotyping
- **cfDNA analysis integrates other lymphoma biomarkers**
- The potential role of cfDNA fragmentomics

ctDNA levels and molecular clusters improve outcome stratification

ctDNA level only



ctDNA level + BN2/ST2



Multivariate analysis for PFS

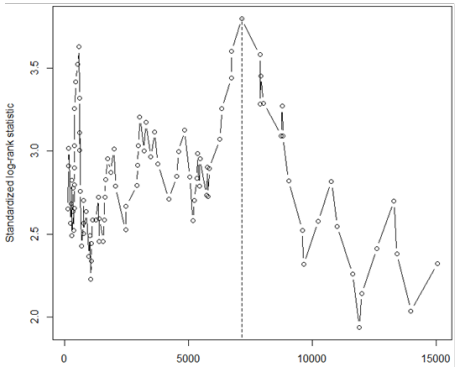
Variable	Hazard ratio	HR (95% C.I.)	*P*
Cluster BN2 or ST2		0.24 (0.07, 0.80)	.02
ctDNA≥Log_{10}hGE		3.64 (1.88, 7.07)	< .001
IPI>2		1.69 (0.84, 3.38)	.14
Non GC		1.58 (0.83, 3.02)	.17

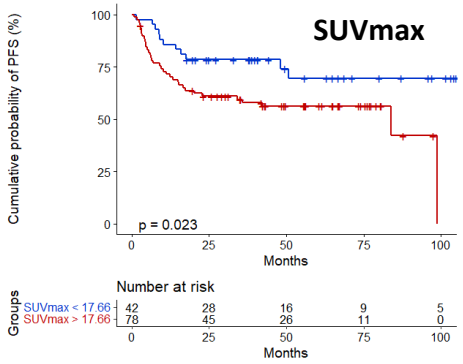
Moia *et al., Blood Advances*. 2025

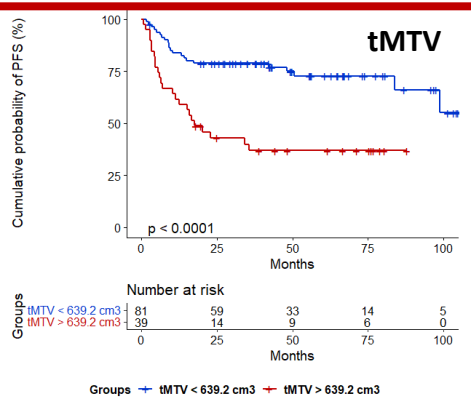
Prognostic impact of [^{18}F]FDG-PET/CT parameters (N=120)

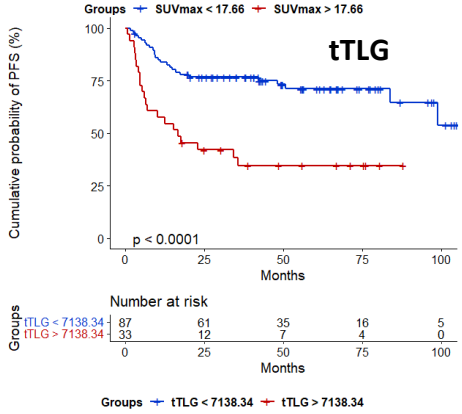
Maxstat test

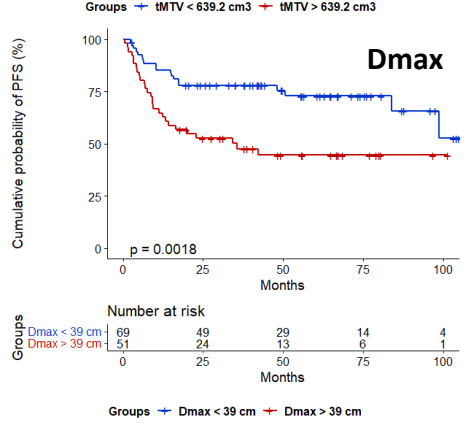
PET parameter	cutoff
SUVmax	17.66
MTV	639.2 cm^3
TLG	7138.34
Dmax	39 cm











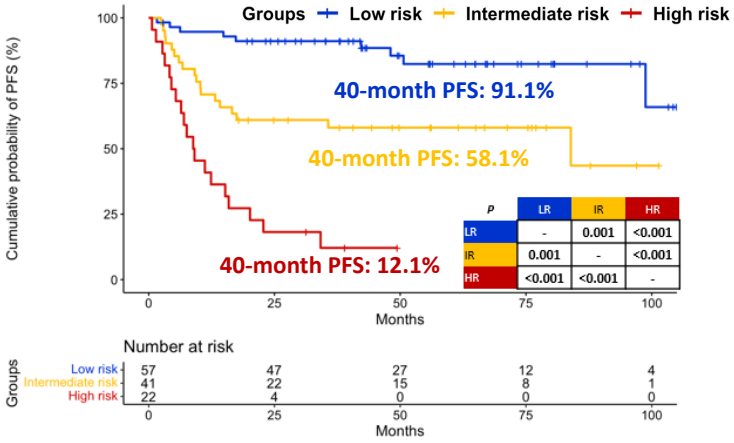
Dondolin *et al., Leukemia*. 2025

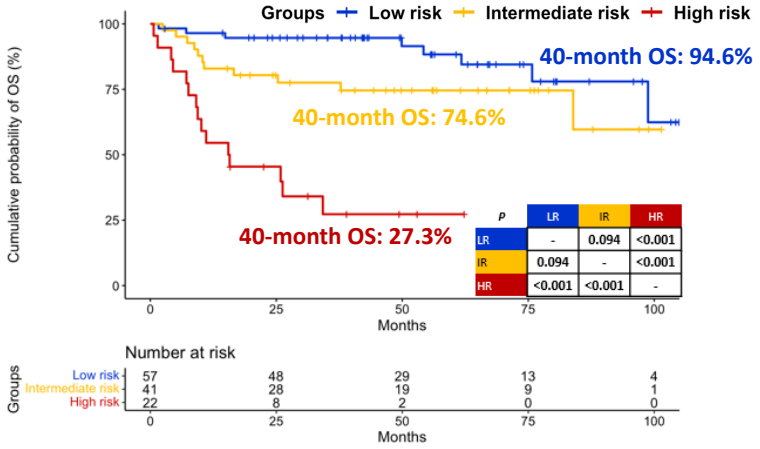
3-factor model: high-risk PET, ctDNA-high and molecular clusters

Bootstrapping validation

Variable	Points	N	Hazard ratio	95% CI	β	p	HR	LCI	UCI	Bootstrapping selection
ctDNA-high	+1	120		2.77 (1.45, 5.27)	1.018	0.002	2.77	1.46	6.09	85.0%
High-risk PET	+1.5	120		3.90 (1.79, 8.48)	1.361	<0.001	3.90	1.60	8.63	96.4%
BN2/ST2	-1.5	120		0.27 (0.12, 0.62)	-1.310	0.002	0.27	0.10	0.69	91.5%

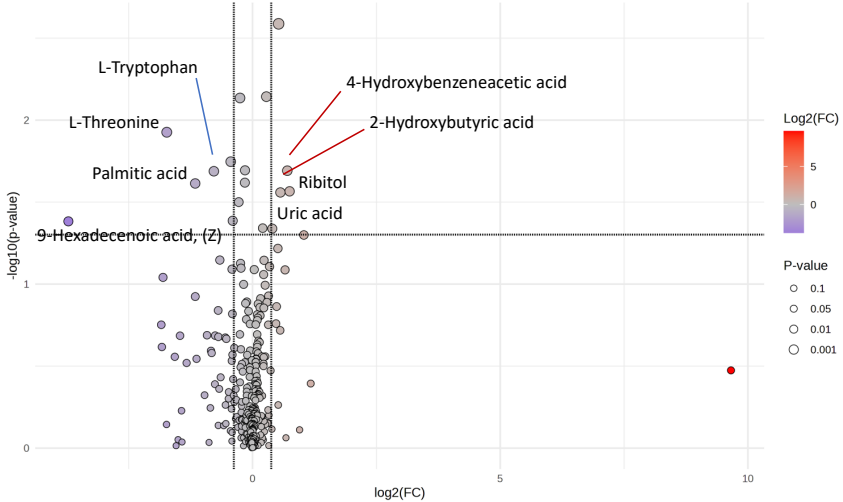
Low risk	-1.5 to 0.5 points
Intermediate risk	1 to 1.5 points
High risk	2.5 points





The metabolomics profile of newly diagnosed DLBCL

96 DLBCL patients → Blood plasma samples at baseline → Classification based on PD at 24months after chemotherapy → **62 non-progressive** patients / **36 progressive** patients → **5 upregulated** and **5 downregulated** **metabolites in progressive patients**

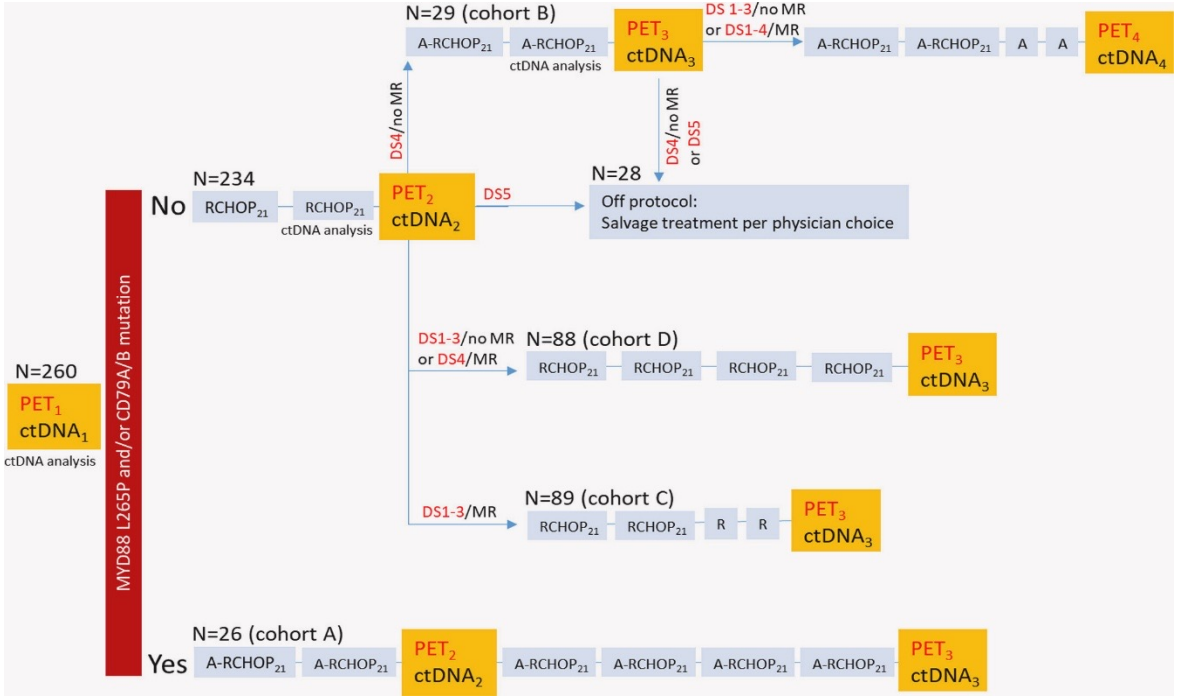


Modulated metabolite	FC	p-value
Trichloroacetic acid, pentadecyl ester	1.4406	0.002587
L-Threonine	0.30129	0.011853
Isopropyl stearate	0.73707	0.017925
4-Hydroxybenzeneacetic acid	1.6272	0.020335
L-Tryptophan	0.58224	0.020506
Palmitic Acid	0.44858	0.024333
Ribitol	1.6796	0.027174
2-Hydroxybutyric acid	1.4773	0.027604
9-Hexadecenoic acid, (Z)	0.076041	0.041421
Uric acid	1.3184	0.04588

Almasri *et al., SIES* 2026, oral presentation Friday March 6th 8.30-10am

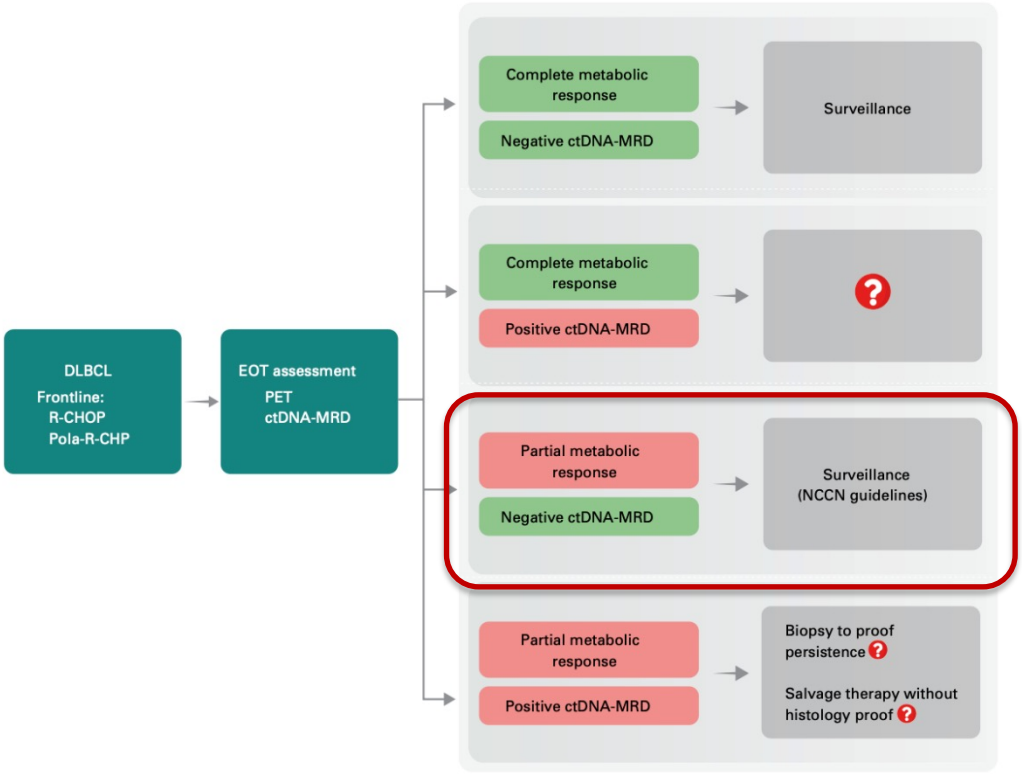
Dondolin *et al., ICML*. 2025

ctDNA and PET guided therapy in untreated DLBCL
The SAKK 38/19 phase II trial



Stathis *et al., ICML*. 2025

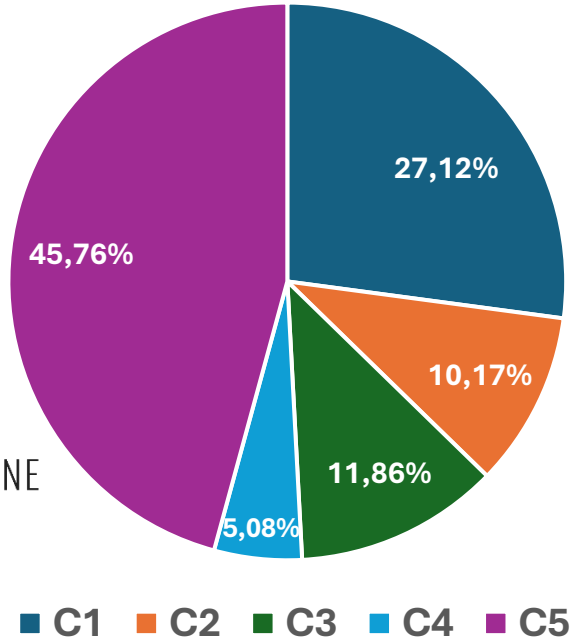
Response assessment pathway for DLBCL



NCCN Guidelines for B-Cell Lymphomas; Rossi *et al., JCO. 2025.*

ctDNA analysis in the FIL RI-CHOP trial

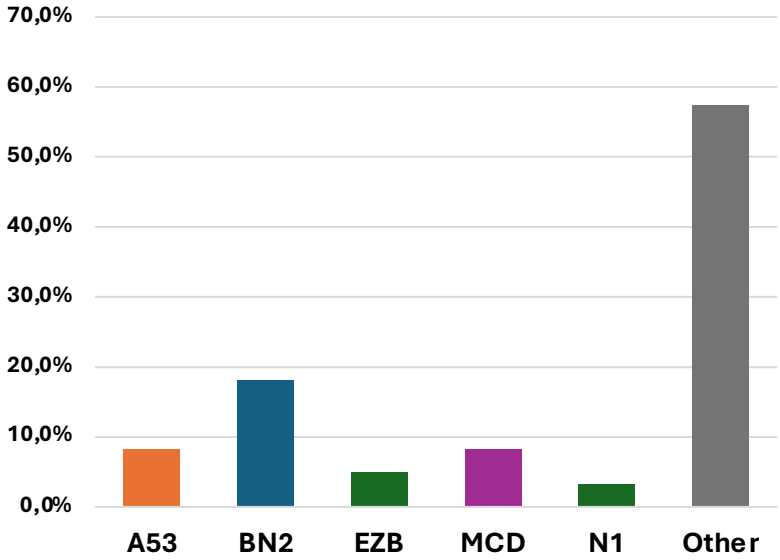
96.7% of patients classifed



FONDAZIONE ITALIANA LINFOMI

DLB*class*

42.6% of patients classifed



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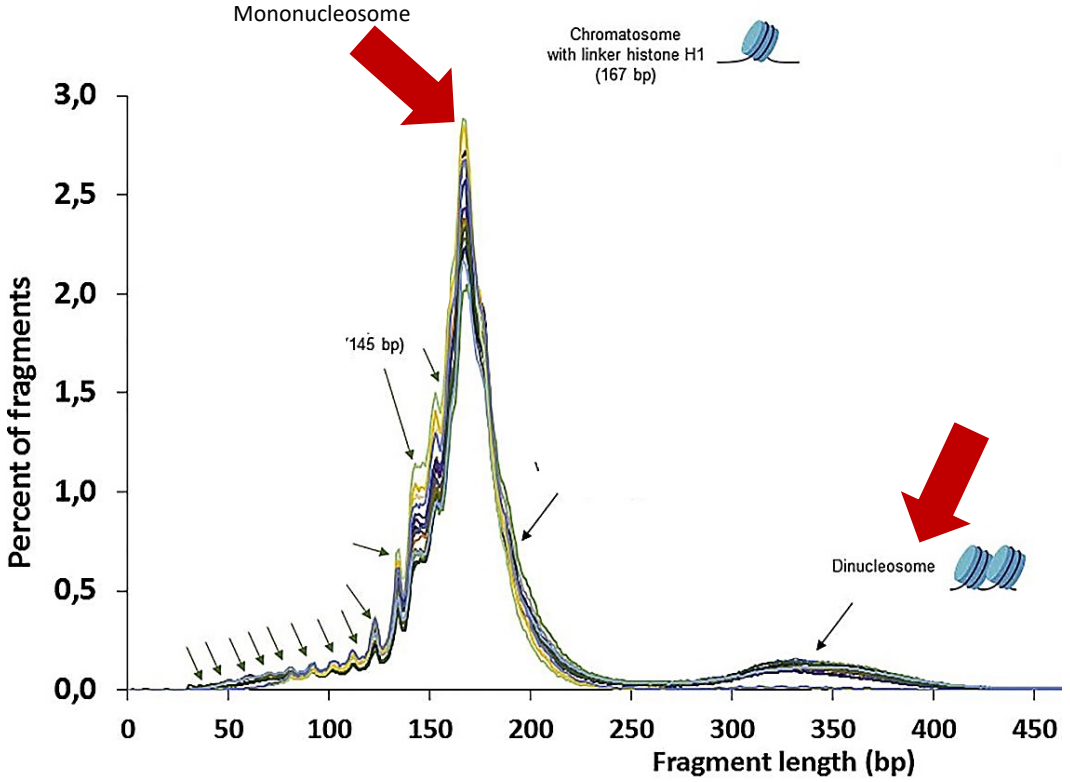
- Exploiting cfDNA for lymphoma (and CH) genotyping
- cfDNA analysis integrates other lymphoma biomarkers
- **The potential role of cfDNA fragmentomics**

cfDNA fragment size profile and characteristics in healthy individuals

cfDNA fragmentation is influenced by chromatin organization

Mostly **mono-nucleosome** sized, with some **di-**nucleosomes

Shows **10 bp periodicity = Helical repeat**, and can be detectable down to 31 bp



Thierry *et al., Cell Genom*. 2023

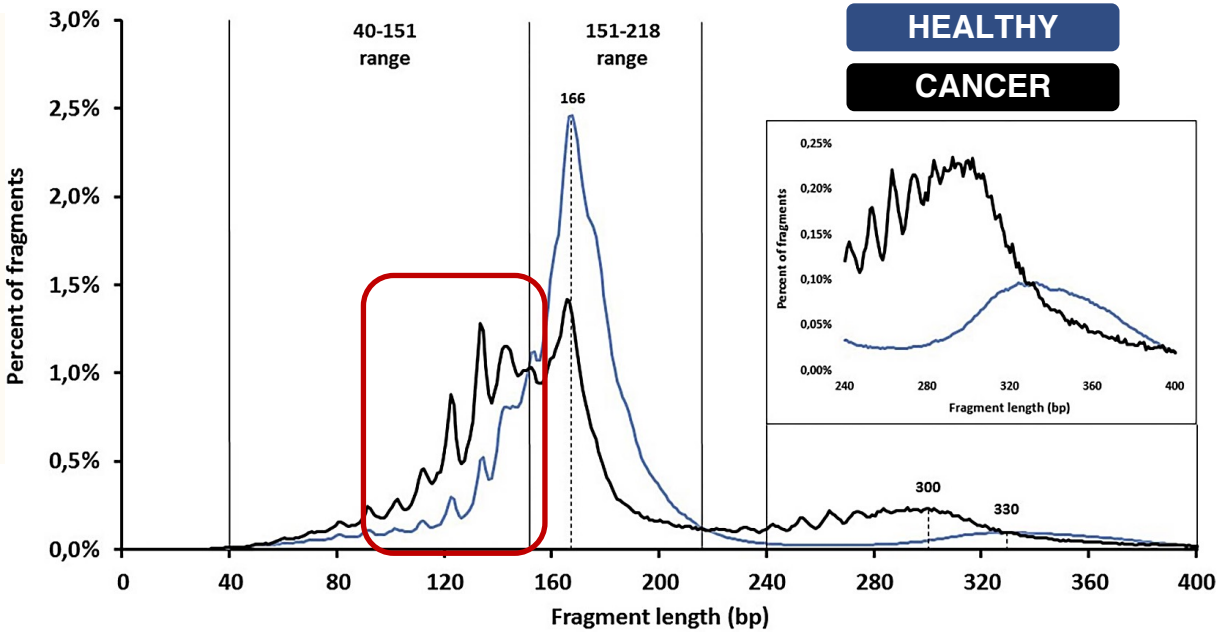
cfDNA fragmentomics differs between cancer and healthy individuals

sWGS & N-qPCR findings:

More <150 bp fragments in cancer
Fewer 151–218 bp fragments in cancer

Di-nucleosome peak:
~**300** bp (**cancer**) vs. ~**330** bp (**healthy**)

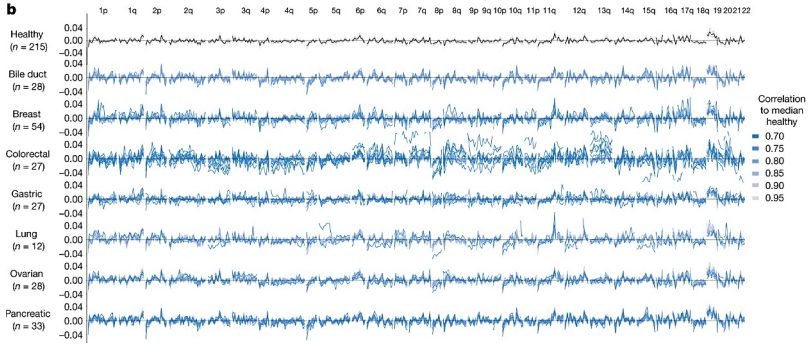
Fewer >1,000 bp fr. in cancer



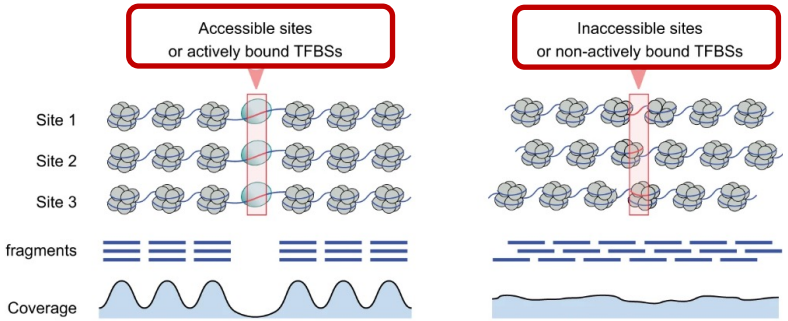
sWGS: Shallow Whole Genome Sequencing
N-qPCR: Nucleosome Quantitative PCR

Thierry *et al., Cell Genom*. 2023

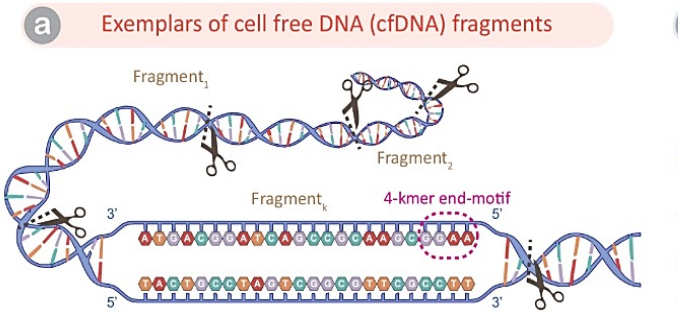
Fragment length is not the only parameter

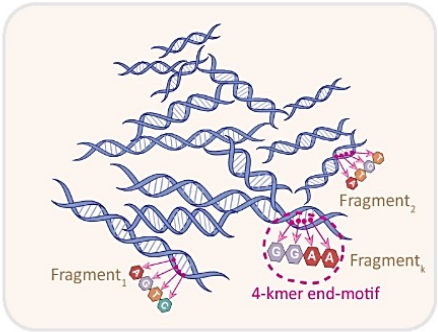


Non-Random Fragmentation Pattern



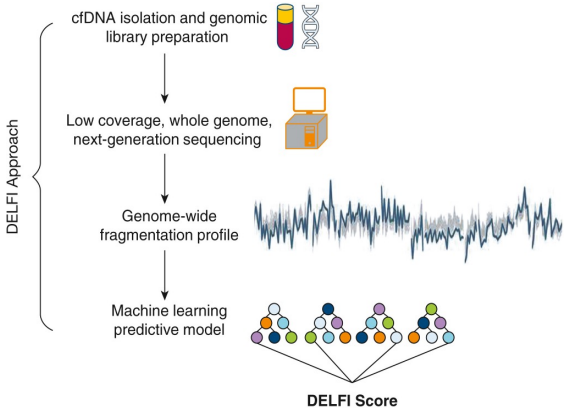
Transcription Factor Association Pattern

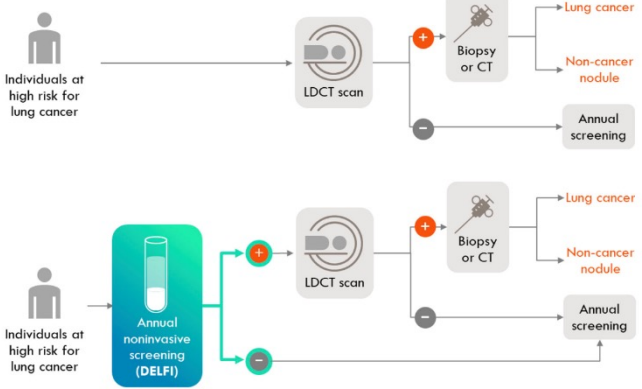


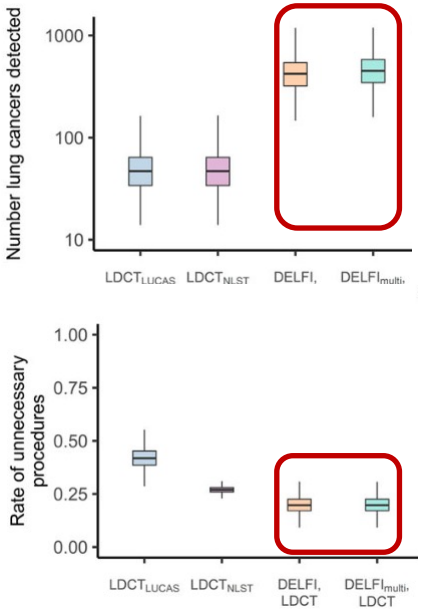


End Motif Pattern

DELFI (DNA Evaluation of Fragments for Early Interception)



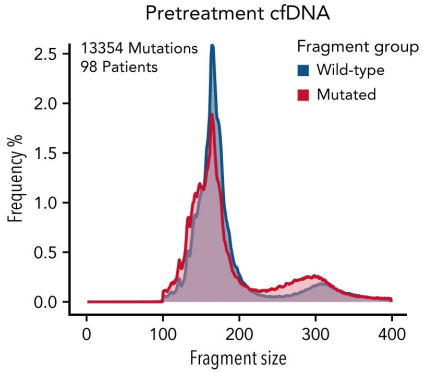


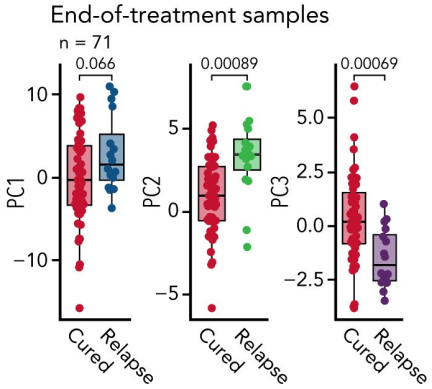


DELFI score improved the identification of patients with lung cancer when combined LDCT and reduced the rated of unnecessary LDCT

Leal *et al. Chest*. 2023; Cristiano *et al., Nature*. 2019; Mathios *et al. Nat Commun*. 2021

Potential applications of fragmentomics in DLBCL *(i)*

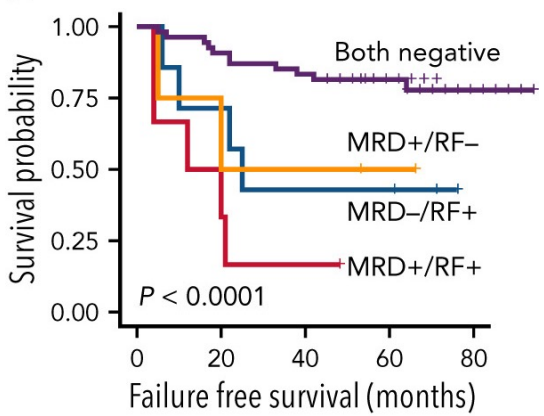




Mutated fragments tend to be **shorter** and favor submononucleosomal and subdinucleosomal lengths in **B cell lymphoma**

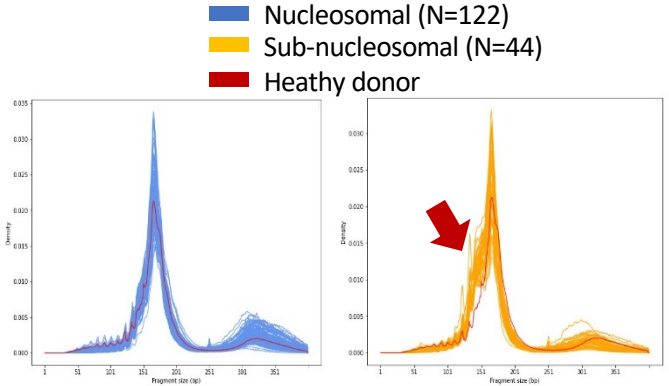
EOT of cfDNA profiles differ between **cured** and **relapsing** patients based on major principal components

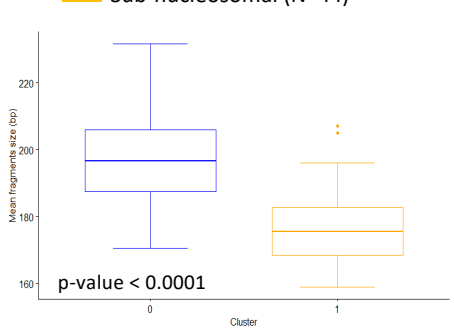
Fragmentome disparities can complement mutation-based MRD detection in predicting **survival**

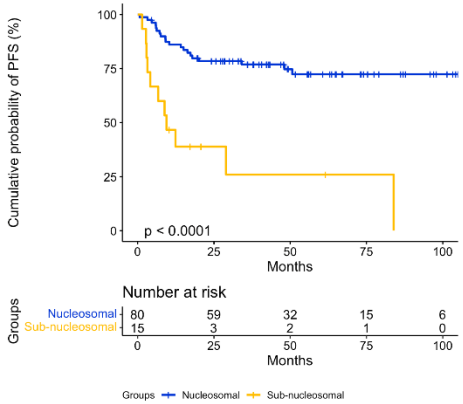


RF: Random Forest Classifier Relapse Predictor
MRD: Minimal Residual Disease

Potential applications of fragmentomics in DLBCL *(ii)*







Variable	N	Hazard ratio	p
ctDNA>=Log10hGE	95	3.18 (1.44, 7.02)	0.004
Sub-nucleosomal cfDNA	95	3.12 (1.36, 7.15)	0.007

The fragmentation profile of cfDNA predicts the outcome of DLBCL not classified to a molecular cluster

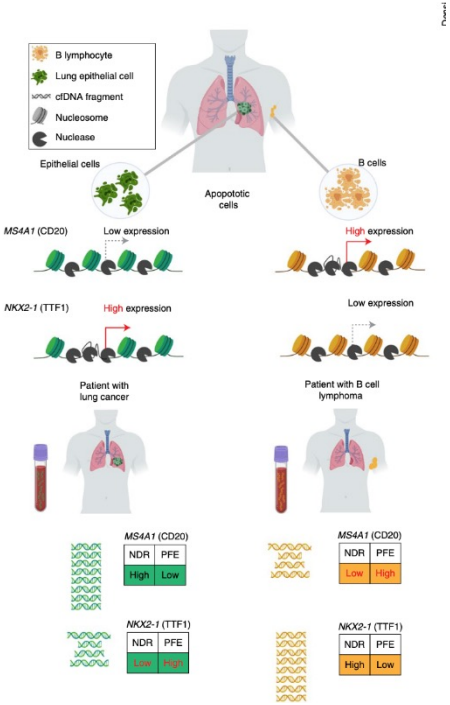
Moia *et al. ICML*. 2023

EPIC-Seq (Epigenetic Expression Inference from cfDNA-sequencing)

cfDNA fragments from active promoters (less nucleosome-protected) exhibit substantially more random fragmentation patterns than those from inactive promoters

EPIC-Seq
Uses deep **WGS** to analyse cfDNA fragmentation

Correlates cfDNA fragmentomic patterns with gene expression data from **RNA-seq** of tumor



NDR: nucleosome-depleted regions (Covarage)
PFE: promoter fragmentation entropy

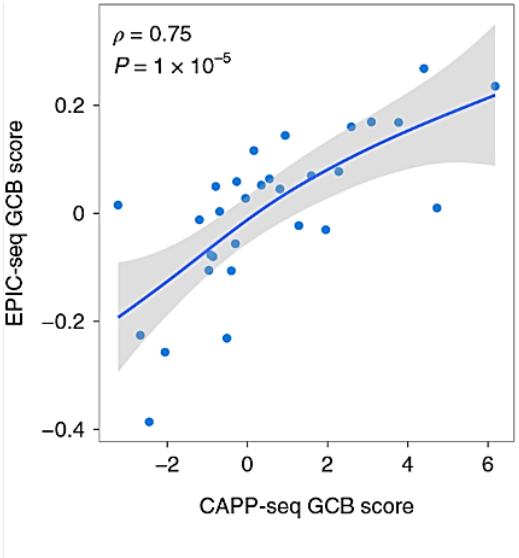
WGS: Whole Genome Sequencing
DLBCL: Diffuse Large B-cell Lymphoma

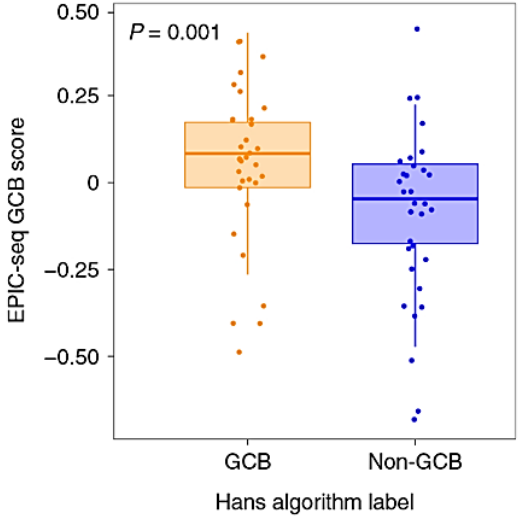
Esfahani *et al. Nat Biotechnol*. 2022

Application of EPIC-seq for DLBCL COO classification

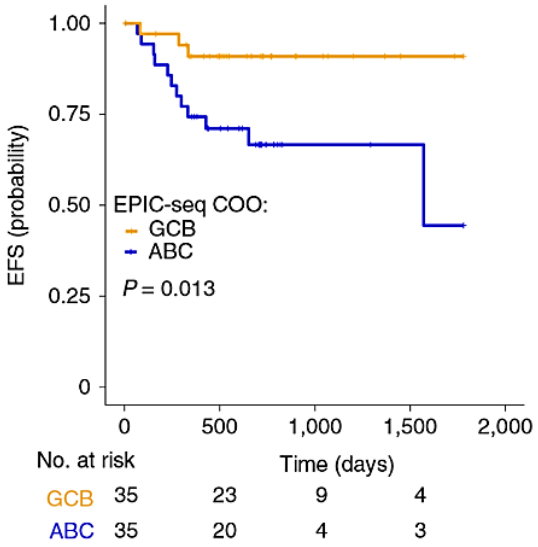
EPIC-Seq GCB score:

- Showed strong correlation with mutation-based GCB scores
- Significantly discriminated between GCB and non-GCB DLBCL





Patients with higher EPIC-seq GCB scores had significantly better survival



COO: Cell of Origin **ABC:** Activated B-cell Like **GCB:** Germinal Center B-cell Like

Esfahani *et al. Nat Biotechnol*. 2022

Conclusions

- Liquid biopsy represents a reliable and powerful tool to dissect lymphoma molecular landscape and host-related clonal hematopoiesis.

- Ongoing and future clinical trials will clarify the role of liquid biopsy in guiding treatment decisions, particularly when integrated with established prognostic tools for DLBCL. Standardization of methodologies (especially for MRD detection), will be pivotal.

- The fragmentomic profile represents a promising future application of liquid biopsy, with potential utility in the early detection of lymphoma and in noninvasively inferring the lymphoma transcriptomic profile.

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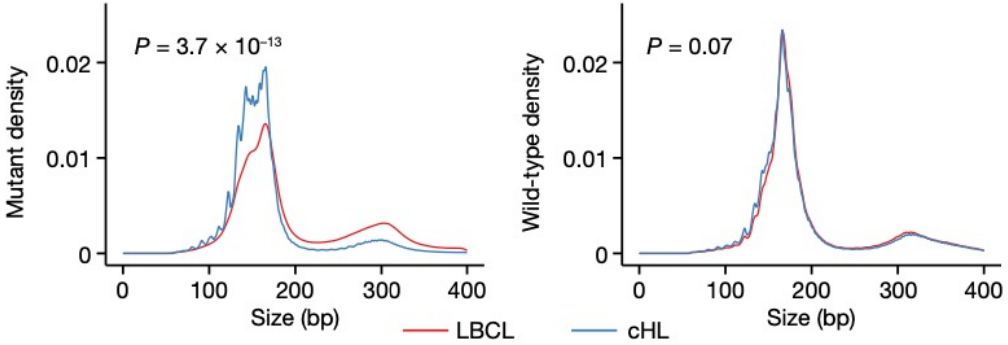
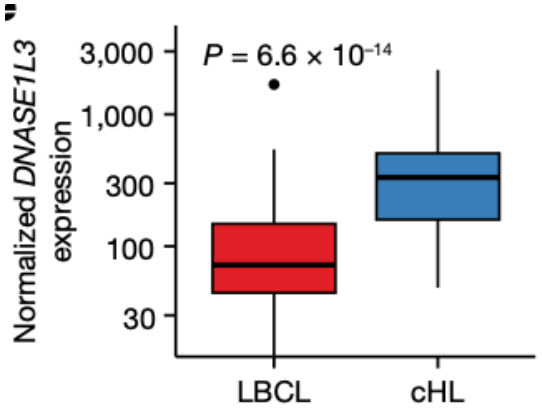
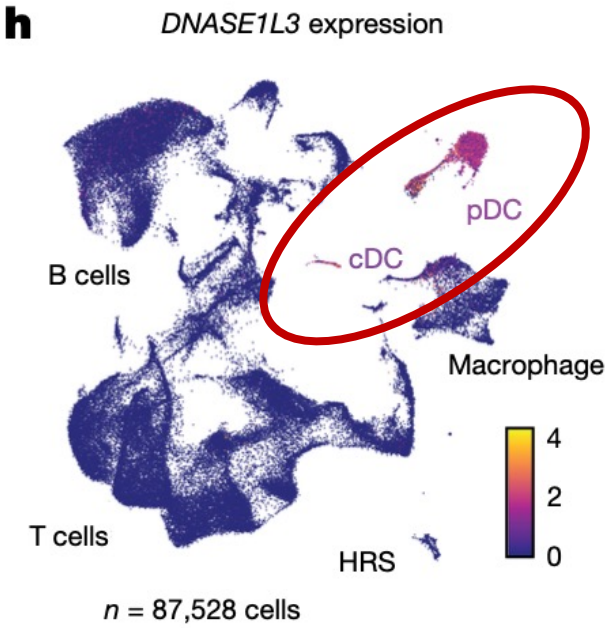
Responsabile Simone Ferrero

Grant support:

ctDNA fragmentation features of cHL vs non-Hodgkin lymphoma





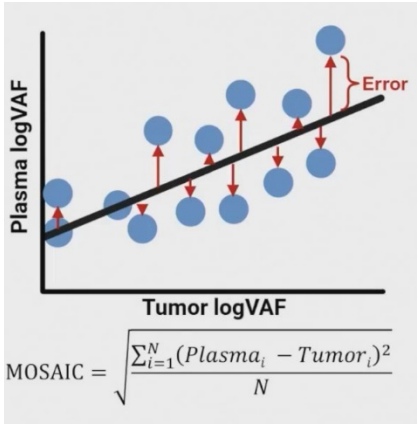


Alig *et al., Nature*. 2024

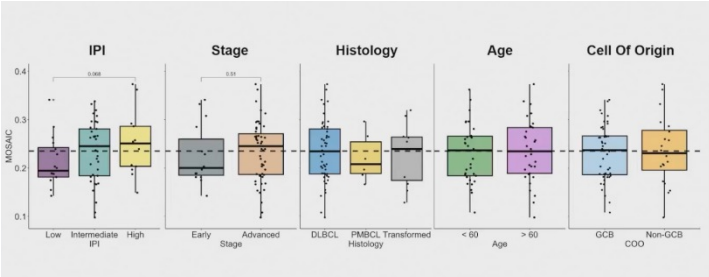
MOSAIC (metric of spatial anatomical intra-tumoral genetic complexity)

- While cfDNA can provide comprehensive spatial genomic profiling, does not provide site specificity

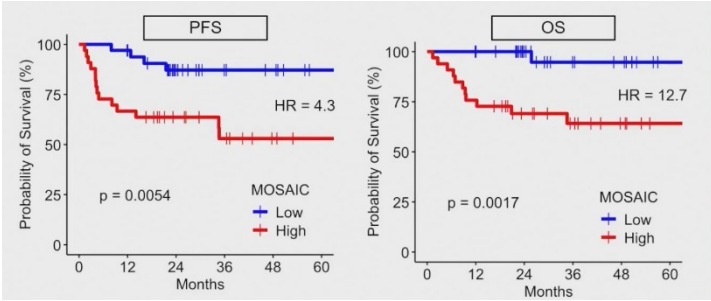
- Root mean square error of linear model of plasma logVAF relative to tumor logVAF for all mutations identified in plasma



MOSAIC is independent of clinical features



High MOSAIC associates with poor outcomes



Limited splatial evolutaion → MOSAIC low

High spatial evolution → MOSAIC high

Goldstein et al., ASH. 2025 #354