UNIVERSITÀ DEL PIEMONTE ORIENTALE

La fragmentomica

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Biopsia liquida:

CHE TRAFFICO IN PERIFERIA!

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Disclosures Riccardo Moia

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Abbvie					x	x	
BeiGene					x	x	
Astrazeneca					x		
Johnson & Johnson					x	X	

Agenda

- Definition and characteristics of fragmentomics
- Potential prognostic impact of fragmentomics
- EPIC-Seq

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- **Definition and characteristics of fragmentomics**
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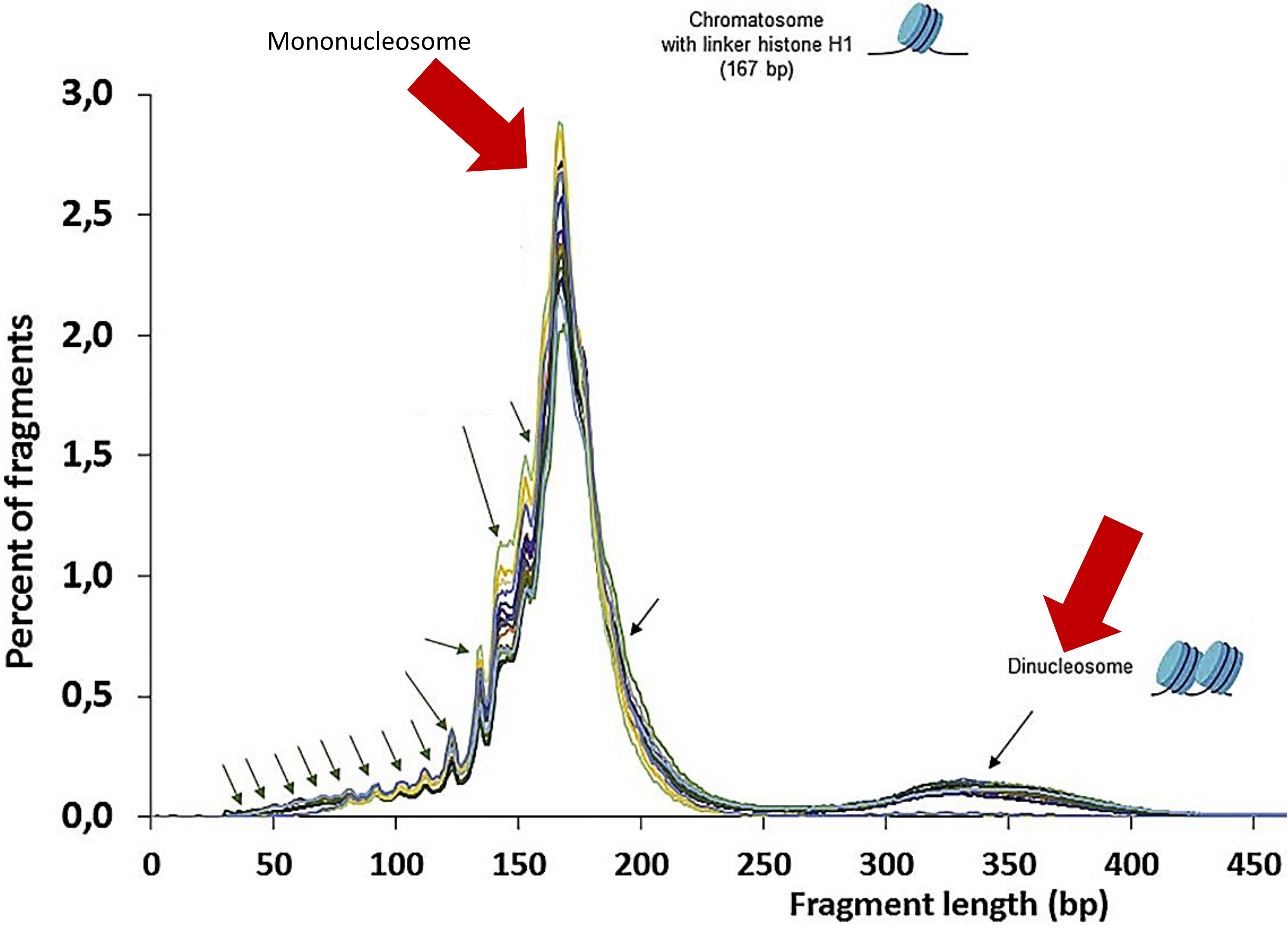
cfDNA fragment size profile and characteristics in healthy individuals

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

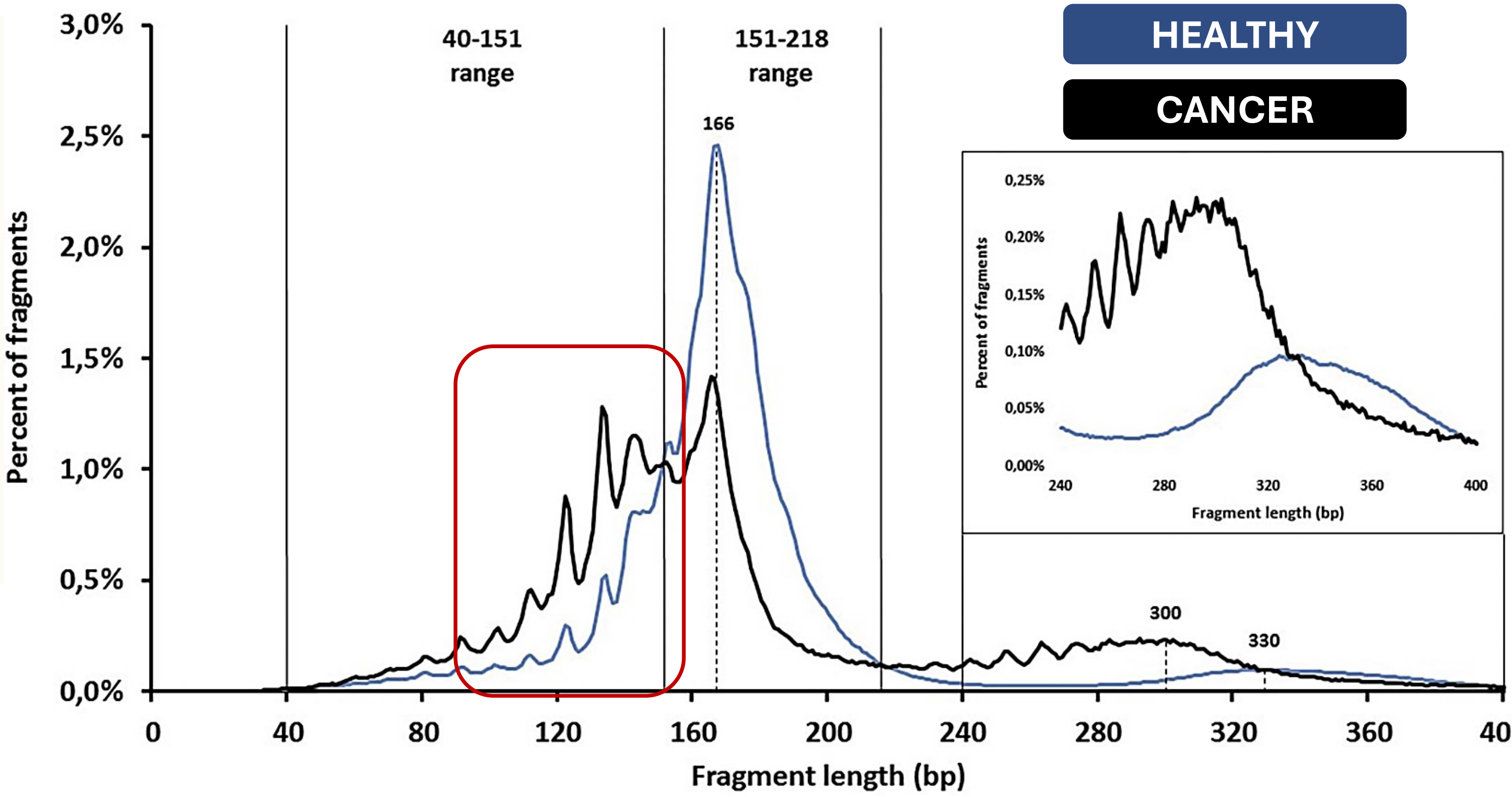
cfDNA fragmentomics differs between cancer and healthy individuals

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**)

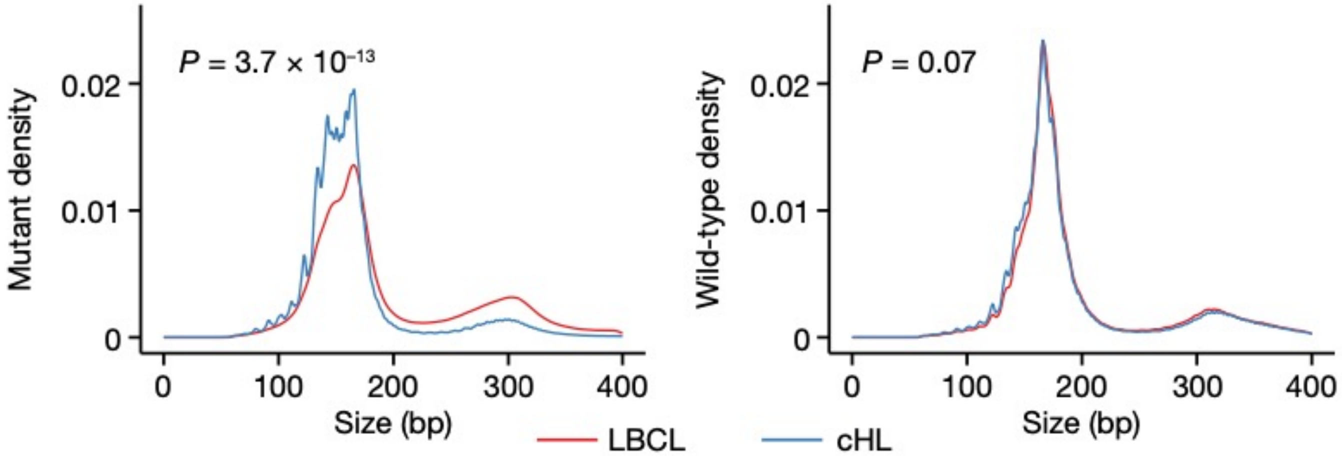
Fewer >1,000 bp fr. in cancer

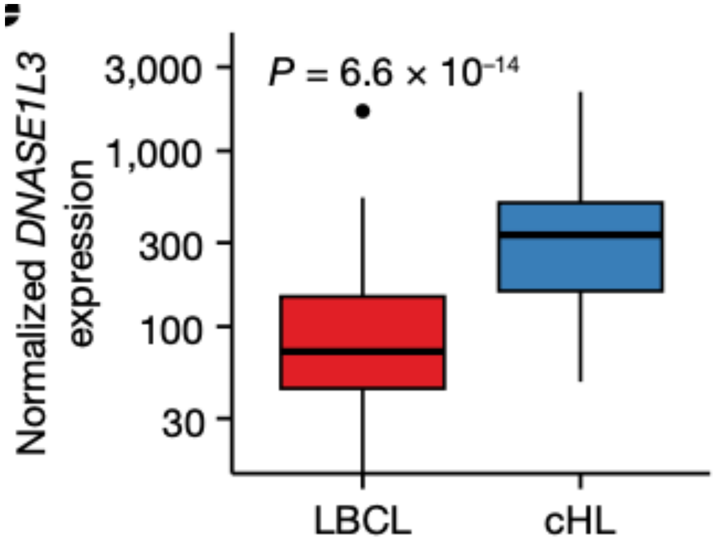


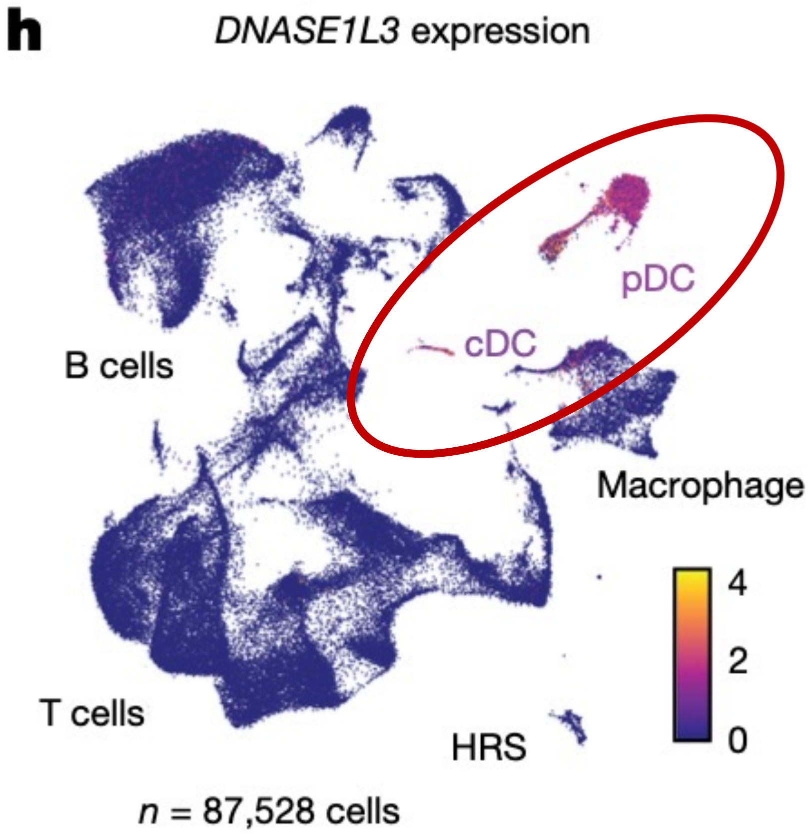
sWGS: Shallow Whole Genome Sequencing
N-qPCR: Nucleosome Quantitative PCR

Thierry *et al.*, *Cell Genom*. 2023

ctDNA fragmentation features of cHL *vs* non-Hodgkin lymphoma

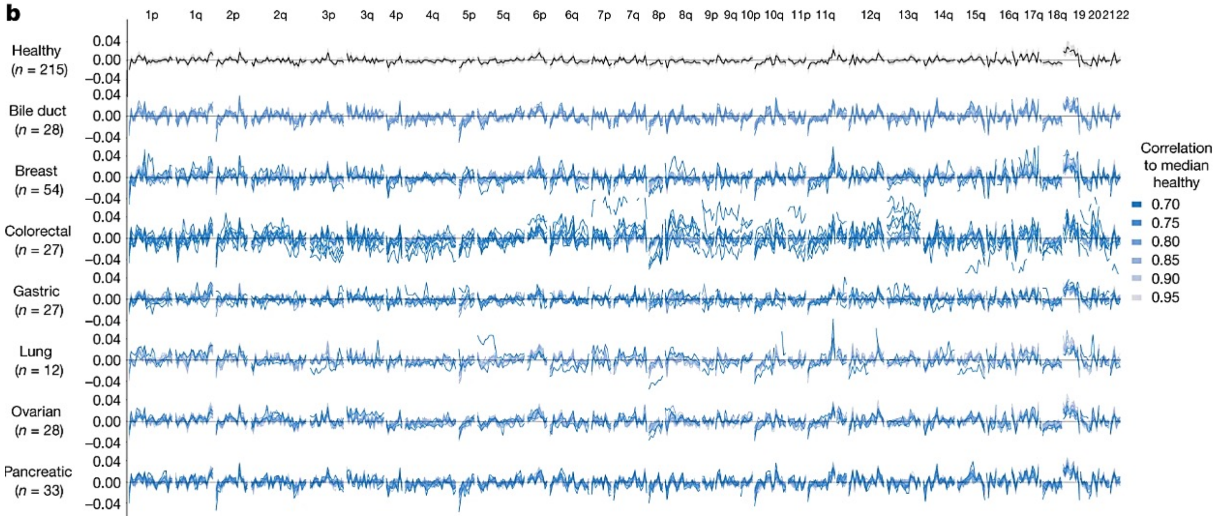




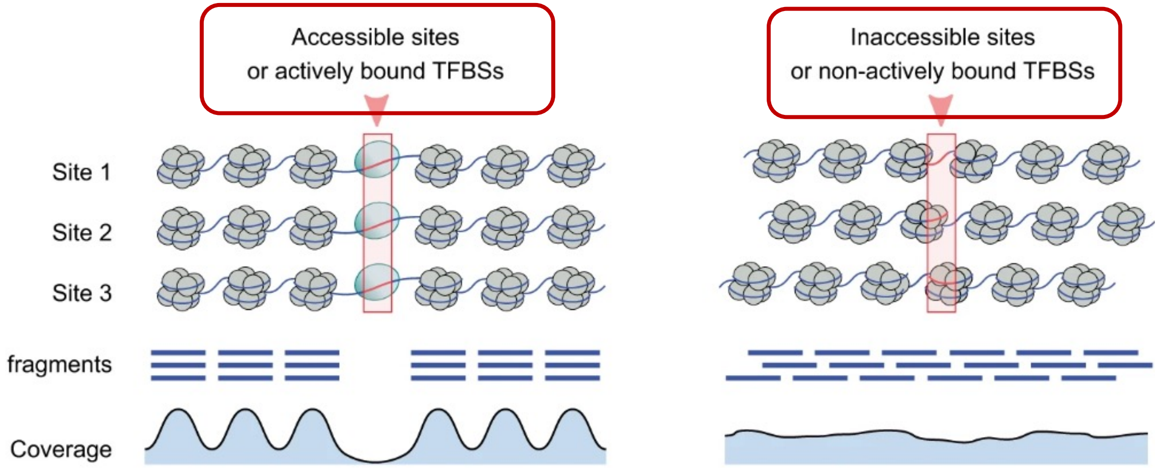


Alig *et al., Nature*. 2024

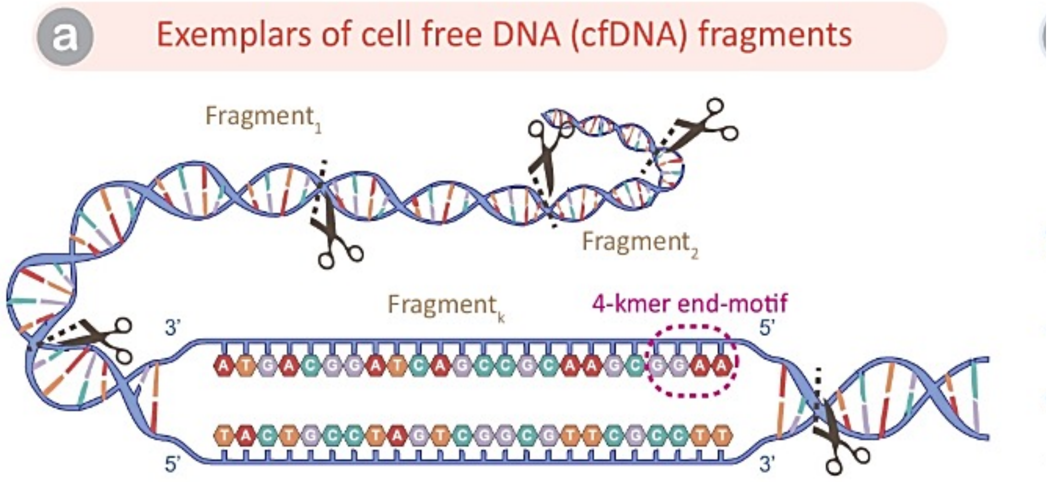
Fragment length is not the only parameter

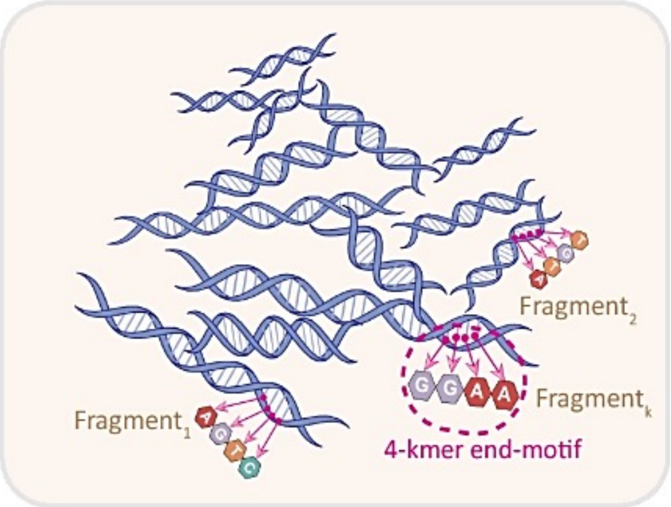


Non-Random Fragmentation Pattern



Transcription Factor Association Pattern





End Motif Pattern

Shen et al. *npj Precis. Onc.* 2024; Cristiano et al. *Nature*. 2019; Doebley et al. *Nat Commun*. 2022

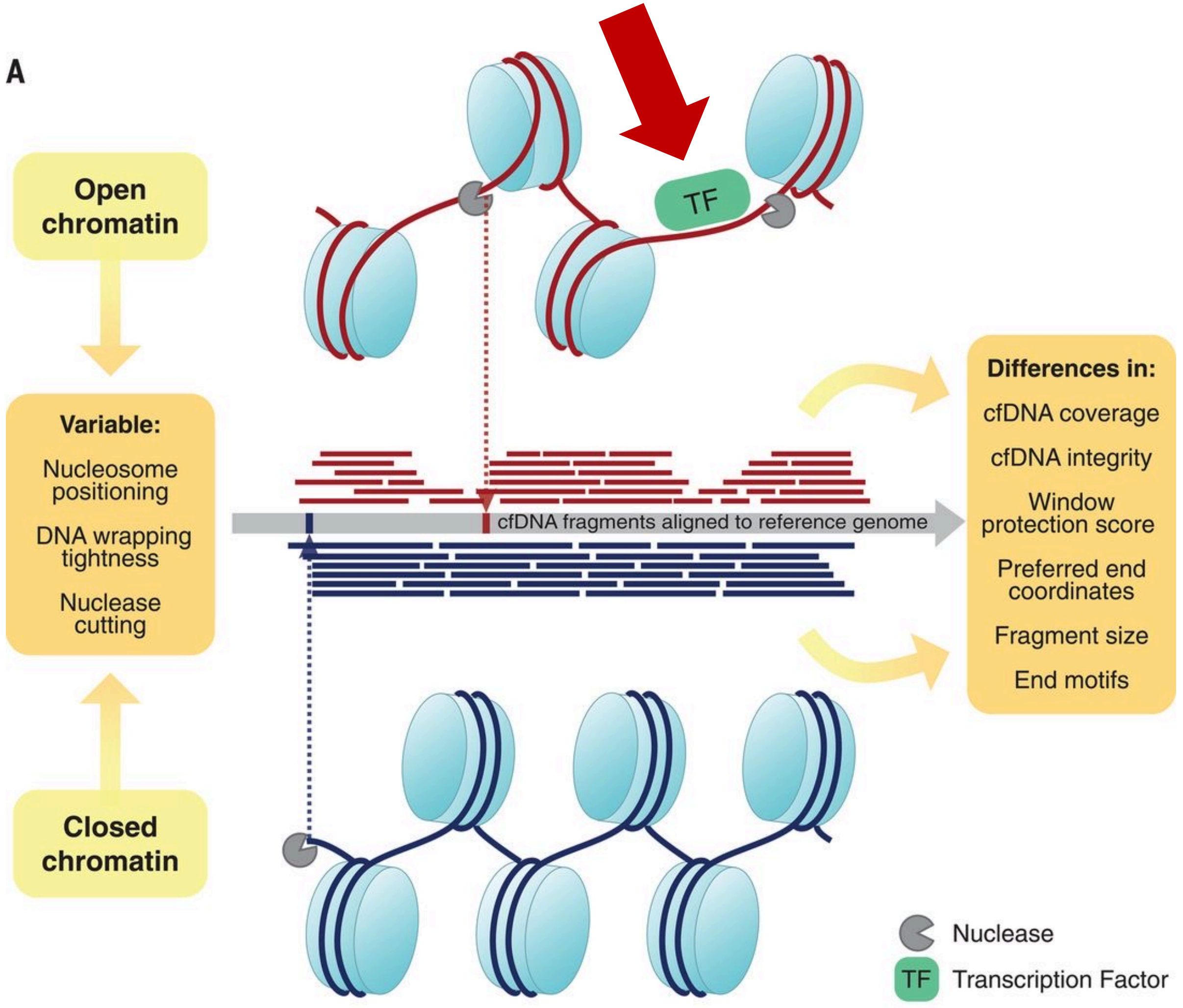
Transcription factor association pattern

This can infer which cell types contribute to cfDNA

Deep sequencing maps **nucleosome occupancy** in cfDNA

Short cfDNA fragments reveal **TF footprints** and **mutant alleles**

“Griffin”: predict estrogen receptor subtype in 139 patients with at least 5% detectable circulating tumor DNA with an area under the receive operator characteristic curve (AUC) of 0.89 and validate performance in independent cohorts (AUC = 0.96)



TF: Transcription Factor

Lo *et al*. *Science*. 2021; Doebley *et al*., *Nature*. 2022

Fragment ends/end motif pattern

Tumour-derived DNA showed a distinct end motif distribution from non-tumoral DNA

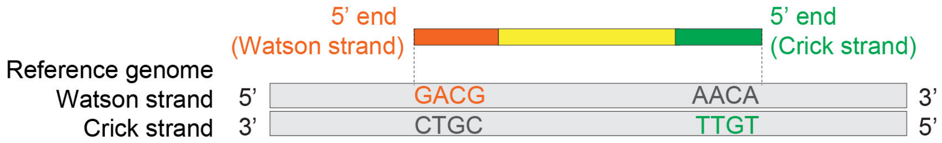
CCCA motif was less common in tumour DNA (HCC) than in other tumors

An enrichment of CCCG motifs in mutant cHL molecules compared with their LBCL counterparts

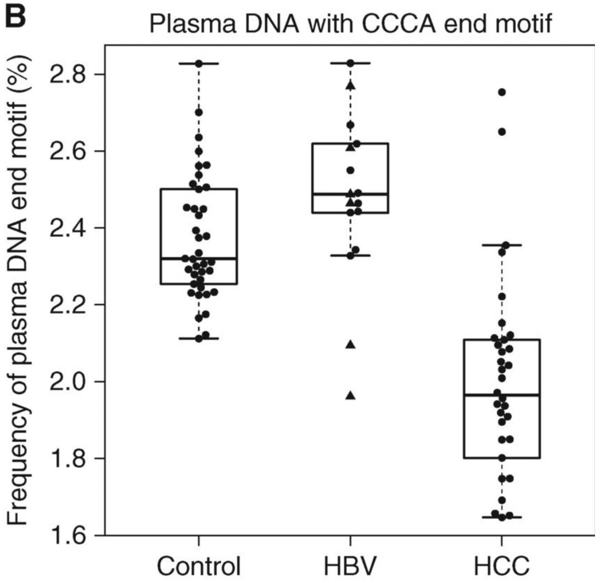
HCC: Hepatocellular Carcinoma

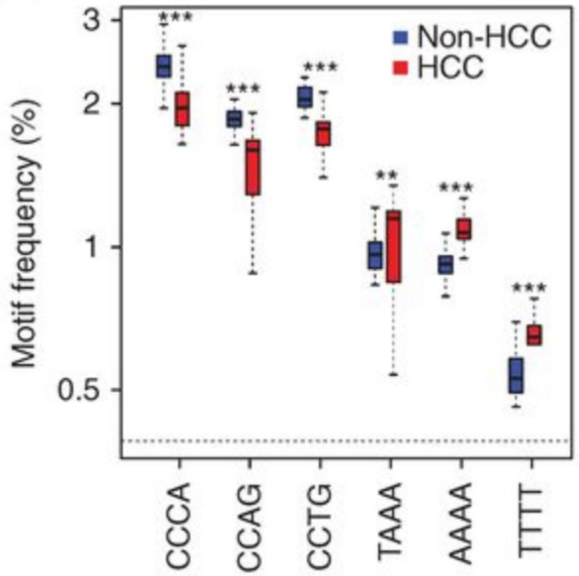
4bp end motif

Sequenced cfDNA fragment

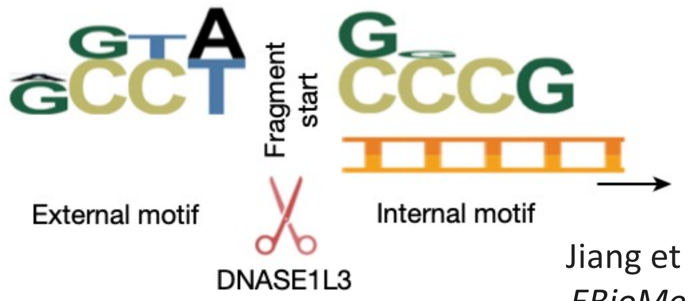


GACG and TGTT are examples of cfDNA 4bp end motif





f End-motif enrichment in cHL versus LBCL

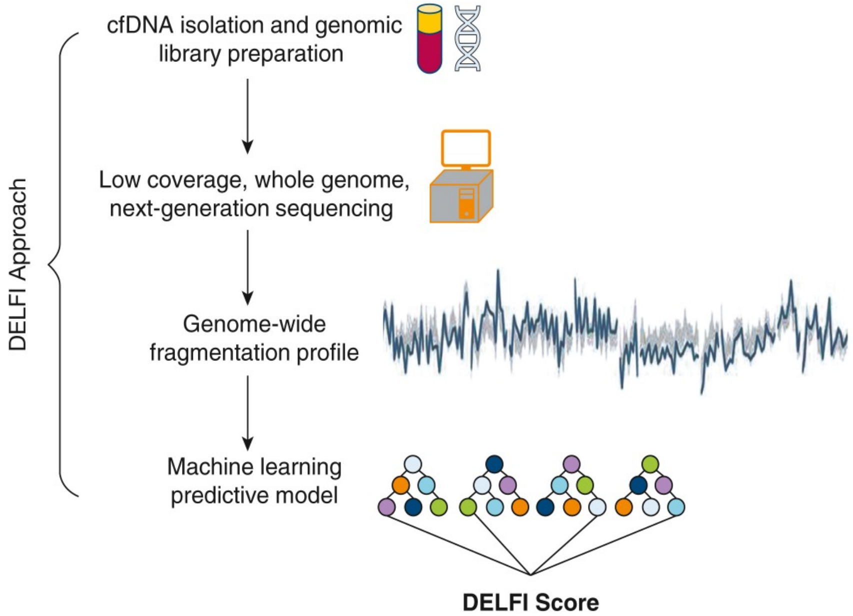


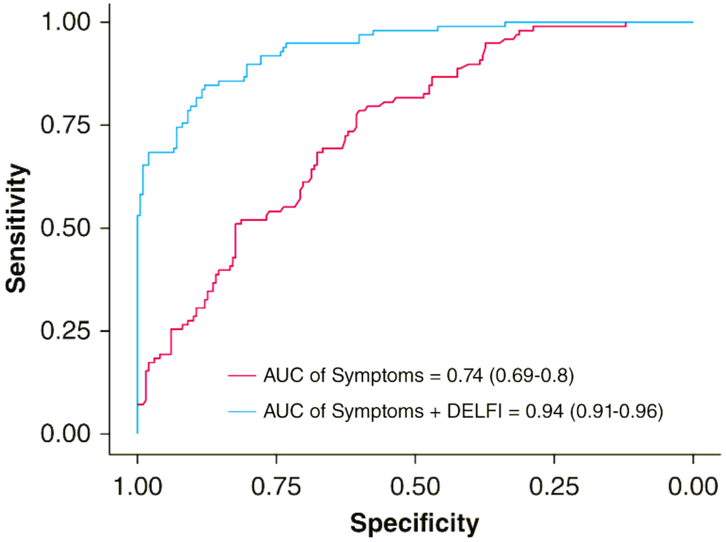
Jiang et al. *Cancer discovery.* 2020; Guo et al. *EBioMedicine.* 2022; Alig et al., Nature. 2024

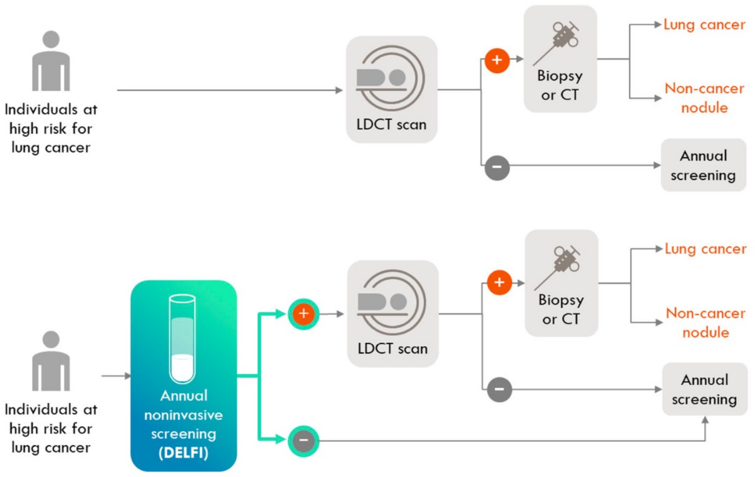
Agenda

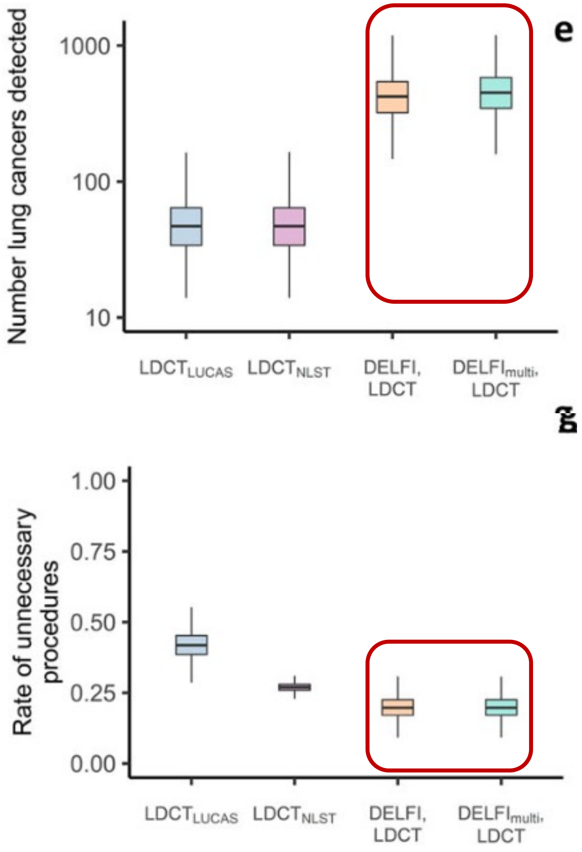
- Definition and characteristics of fragmentomics
- **Potential prognostic impact of fragmentomics**
- EPIC-Seq

DELFI (DNA Evaluation of Fragments for Early Interception)





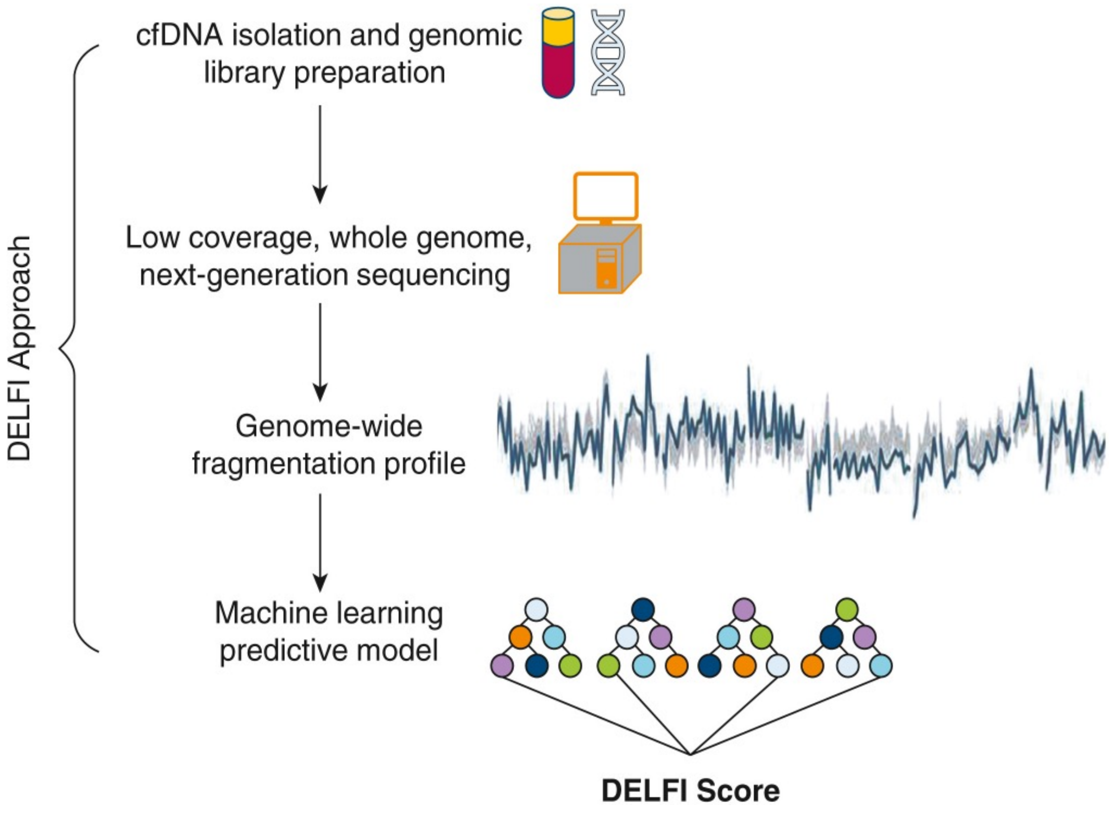


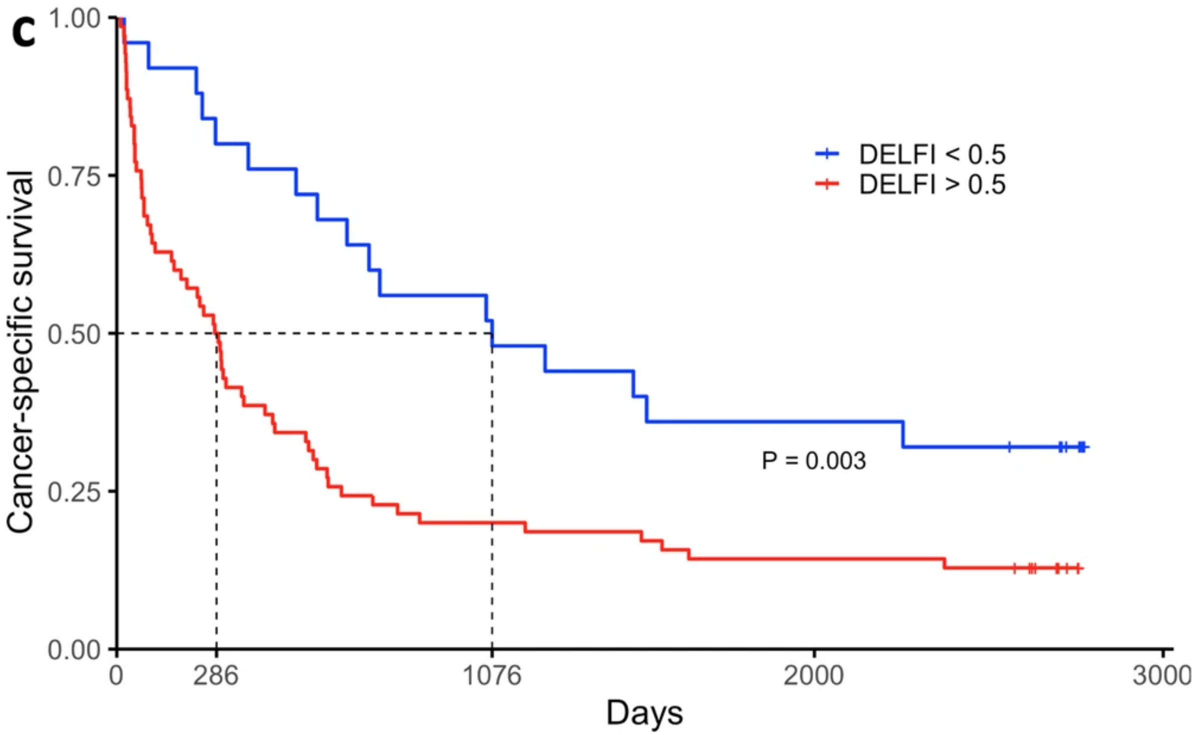


DELFI score improved the identification of patients with lung cancer when combined LDCT and reduced the rated of unnecessary LDCT

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

DELFI (DNA Evaluation of Fragments for Early Interception)

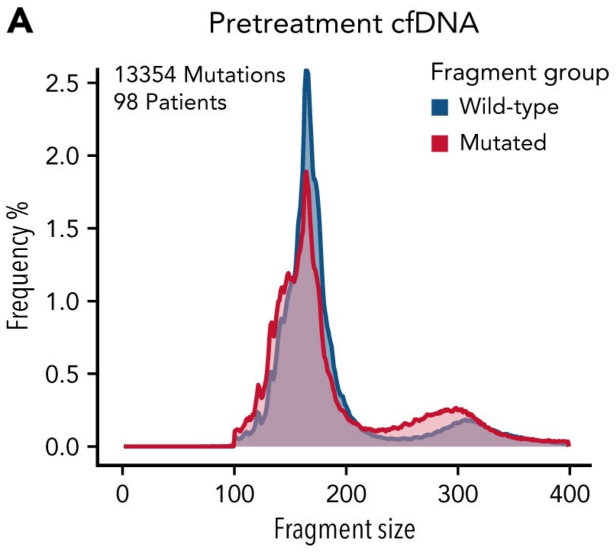


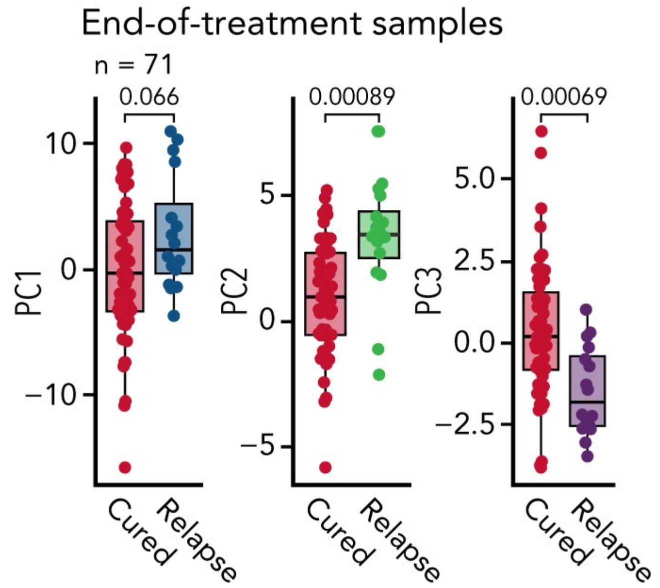


Patients with primary lung cancer were stratified in two groups based on a DELFI cutoff of 0.5 (n = 93)

Leal et al. *Chest.* 2023;
Mathios et al. *Nat Commun.* 2021

Potential application of fragmentomics in DLBCL

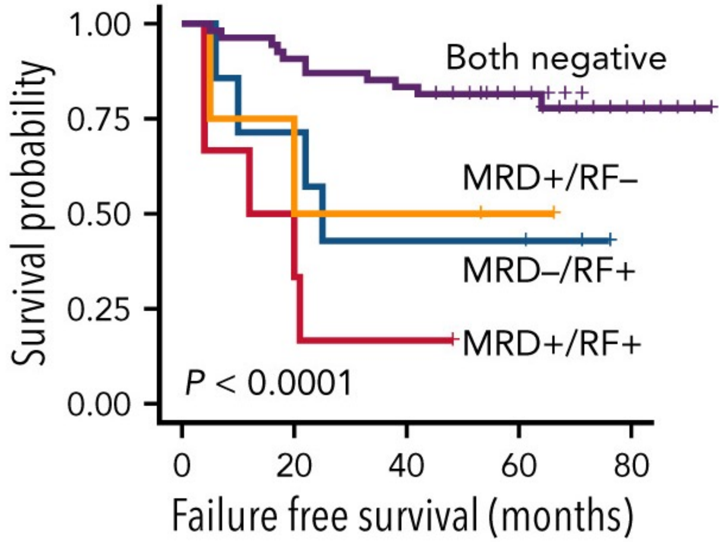




Mutated fragments tend to be **shorter** and favor submononucleosomal and subdinucleosomal lengths in **B cell lymphoma**

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

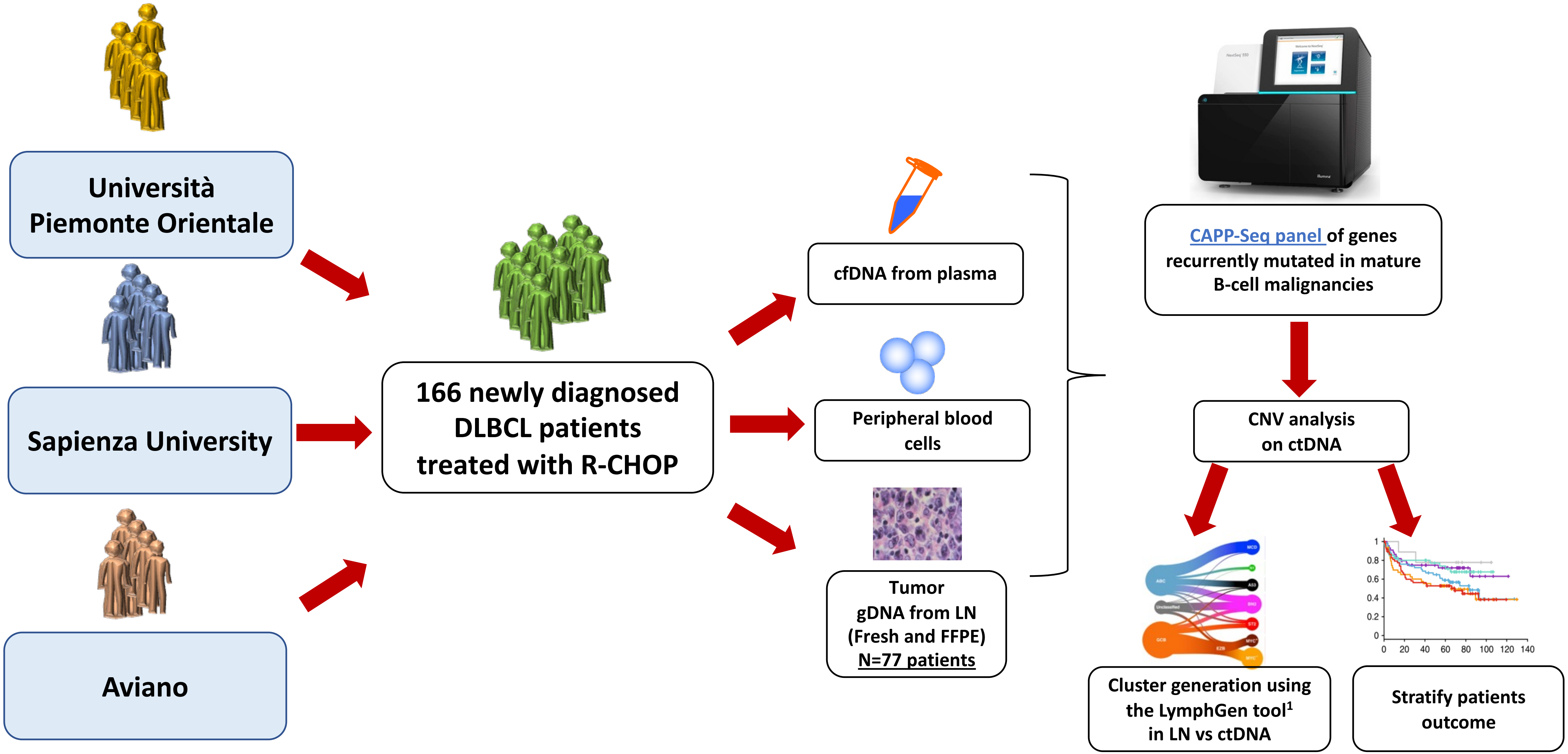
Fragmentome disparities can complement mutation-based MRD detection in predicting **survival**



RF: **Random Forest Classifier Relapse Predictor**
MRD: **Minimal Residual Disease**
EOT: **End of Therapy**

Meriranta et al. *Blood.* 2022

Experimental workflow



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166 newly diagnosed DLBCL patients treated with R-CHOP

cfDNA from plasma

Peripheral blood cells

Tumor
gDNA from LN
(Fresh and FFPE)
N=77 patients

CAPP-Seq panel of genes recurrently mutated in mature B-cell malignancies

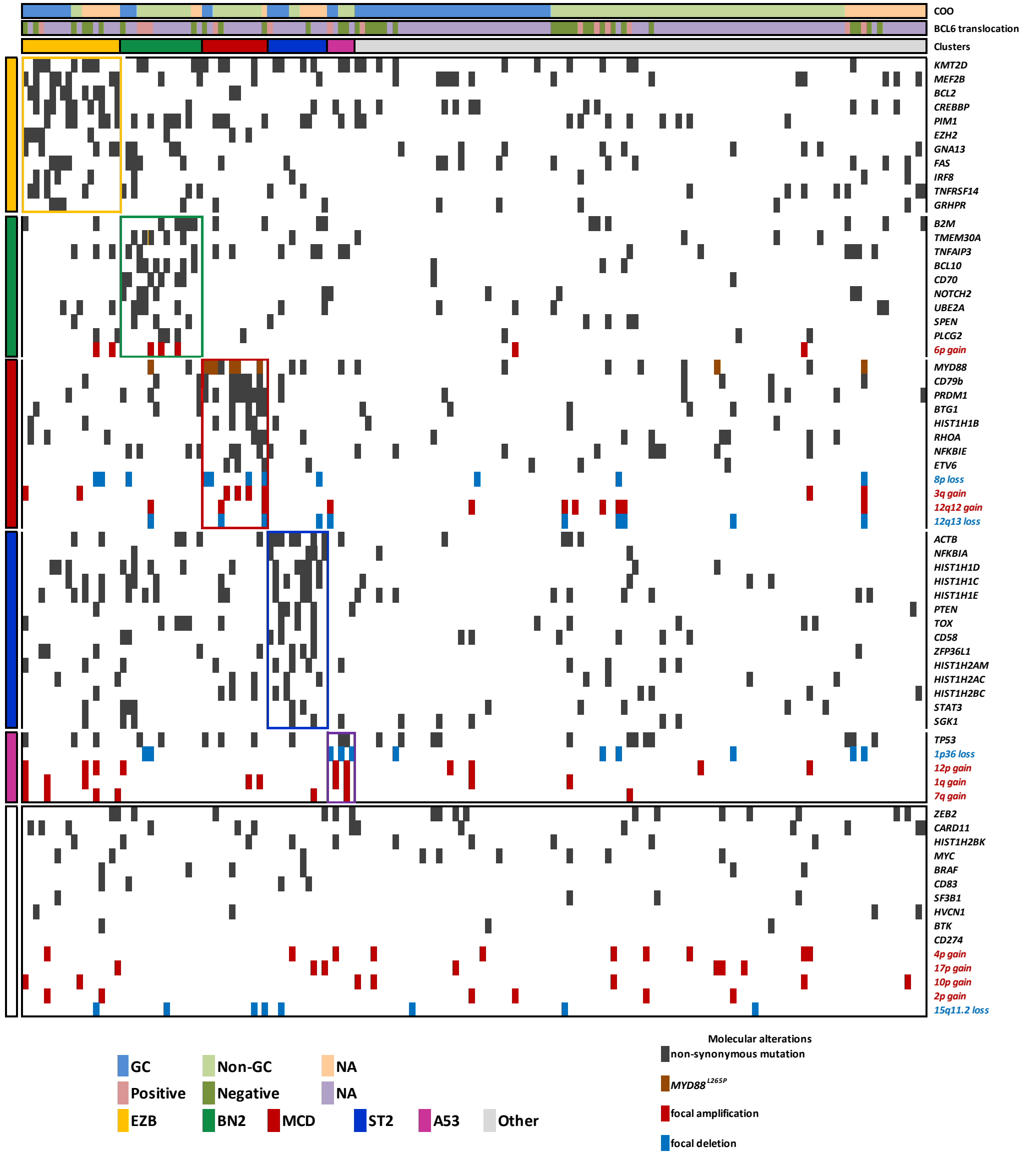
CNV analysis on ctDNA

Cluster generation using the LymphGen tool[1] in LN vs ctDNA

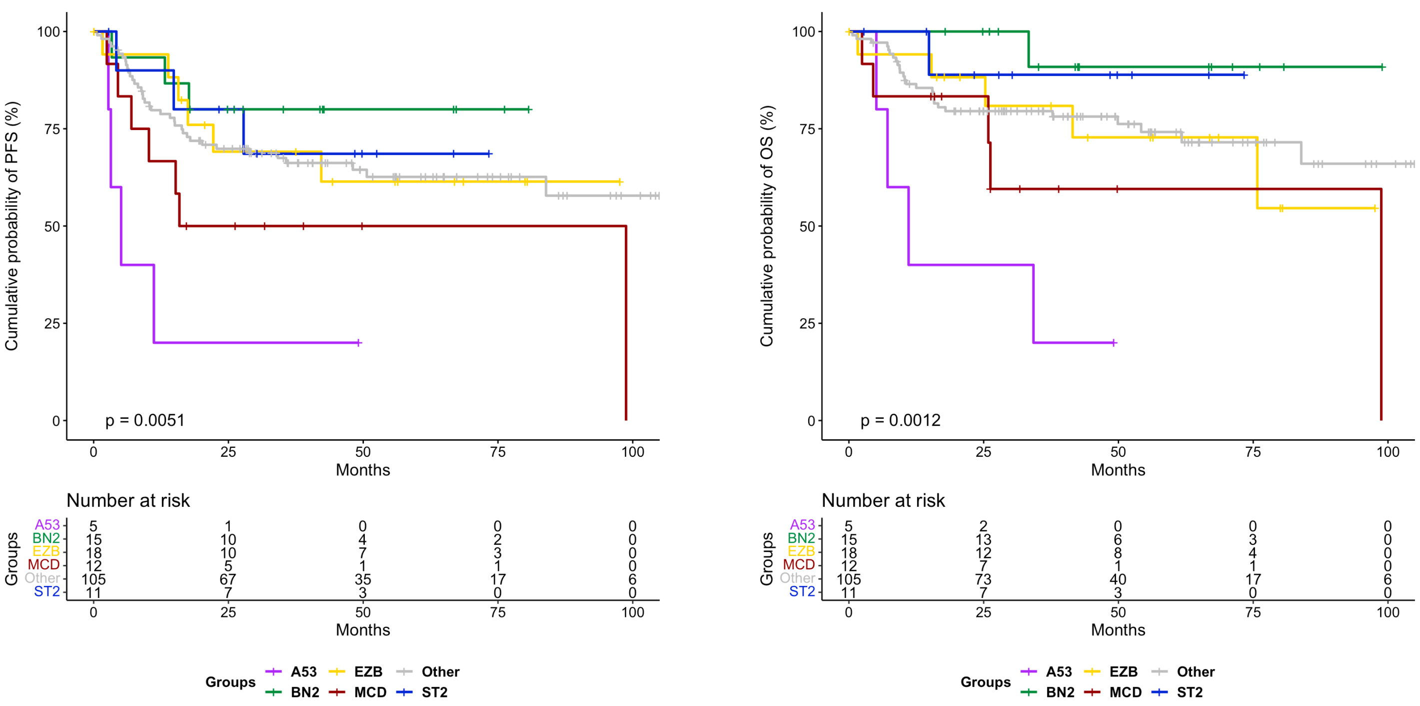
Stratify patients outcome

Moia *et al., Blood Advances.* 2025

Molecular clusters on ctDNA



LymphGen toll allows the classification of 40-50% of patients



Number at risk

Groups	0	25	50	75	100
A53	5	1	0	0	0
BN2	15	10	4	2	0
EZB	18	10	7	3	0
MCD	12	5	1	1	0
Other	105	67	35	17	6
ST2	11	7	3	0	0

Months

Number at risk

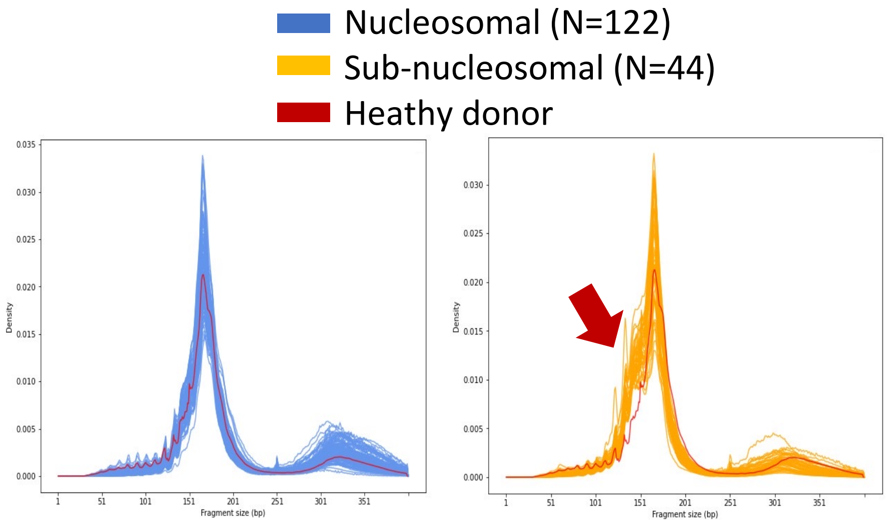
Groups	0	25	50	75	100
A53	5	2	0	0	0
BN2	15	13	6	3	0
EZB	18	12	8	4	0
MCD	12	7	1	1	0
Other	105	73	40	17	6
ST2	11	7	3	0	0

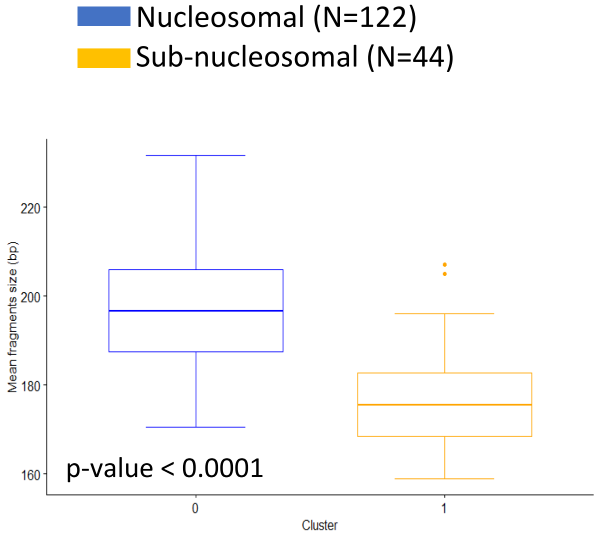
Months

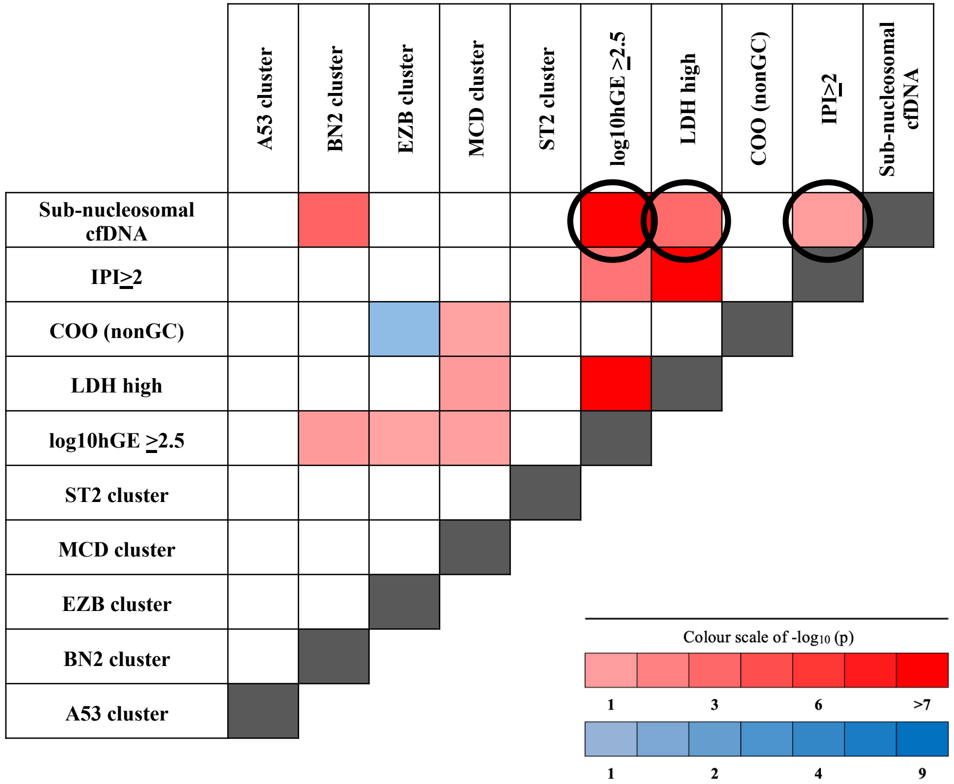
Can patients not assigned to any specific molecular cluster be further stratified?

Moia *et al., Blood Advances.* 2025

Two different groups
with different fragmentation profile have been identified







Patients with the subnucleosomal cfDNA fragmentation profile associate with BN2 cluster, high level of ctDNA, high LDH and with high IPI scores

Moia *et al.*, *ICML* 2023

The fragmentation profile of cfDNA predicts the outcome of DLBCL with no detectable mutation or not classified to specific molecular cluster

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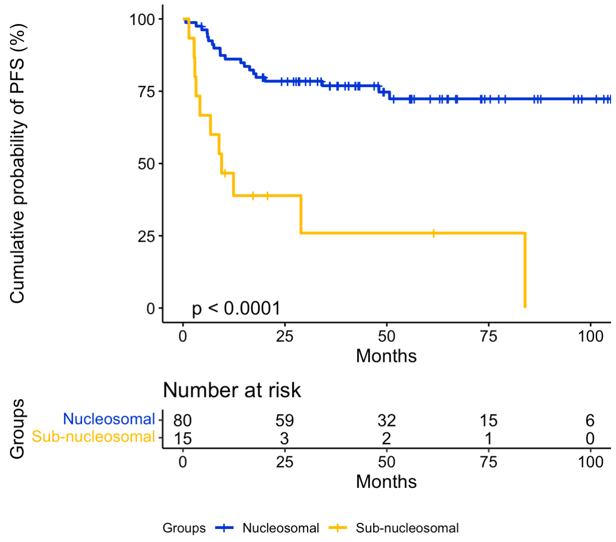
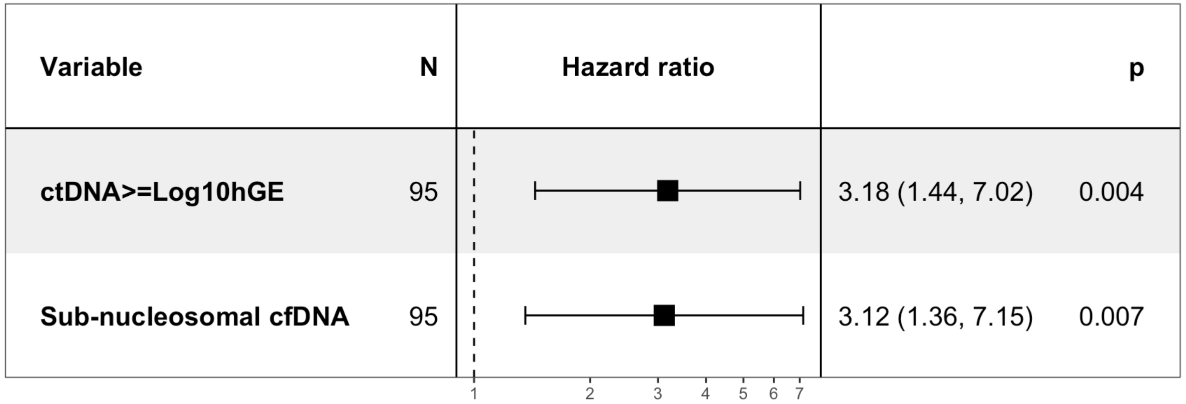
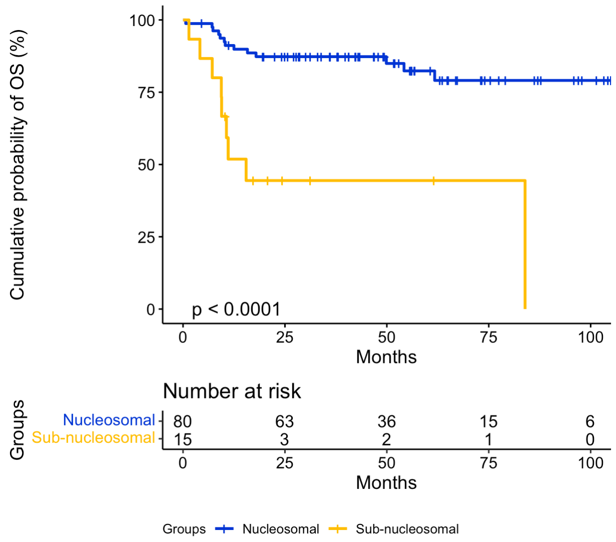
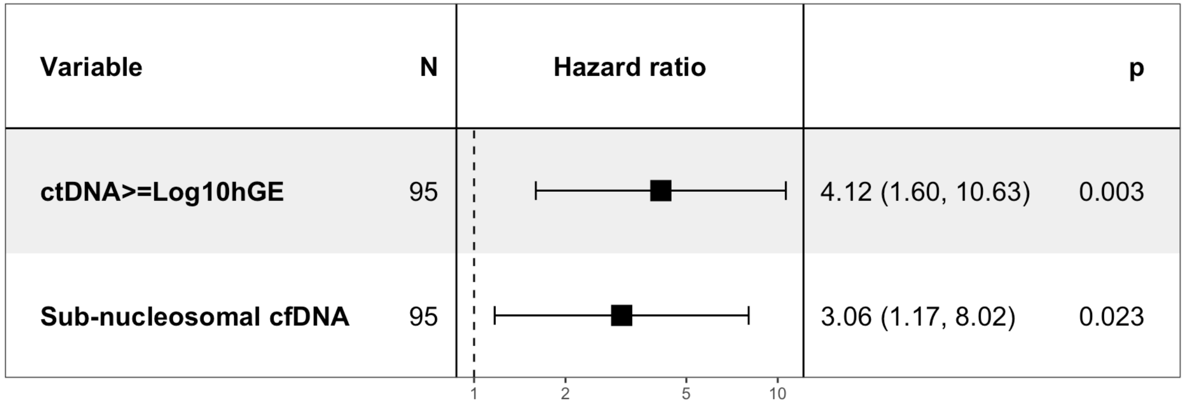




Variable	N	Hazard ratio		p
ctDNA>=Log10hGE	95		3.18 (1.44, 7.02)	0.004
Sub-nucleosomal cfDNA	95		3.12 (1.36, 7.15)	0.007





Variable	N	Hazard ratio		p
ctDNA>=Log10hGE	95		4.12 (1.60, 10.63)	0.003
Sub-nucleosomal cfDNA	95		3.06 (1.17, 8.02)	0.023

Moia *et al.*, *ICML* 2023

Agenda

- Definition and characteristics of fragmentomics
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- **EPIC-Seq**

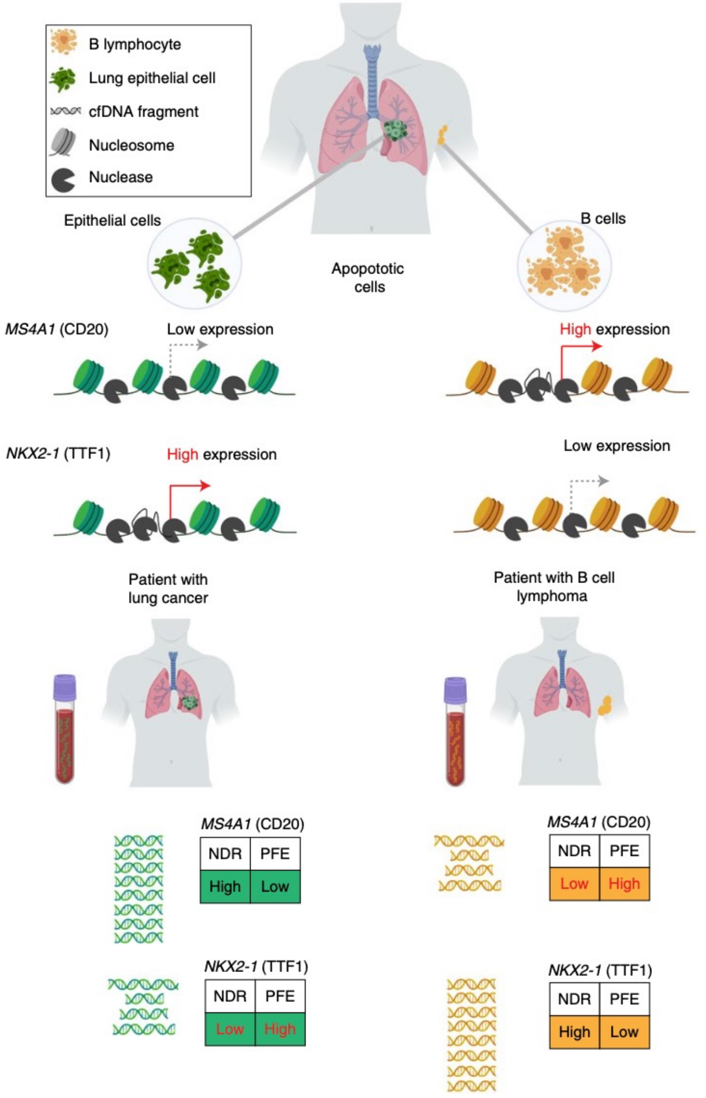
EPIC-Seq (Epigenetic Expression Inference from cfDNA-sequencing)

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 → Coverage
PFE: promoter fragmentation entropy → fragment length diversity

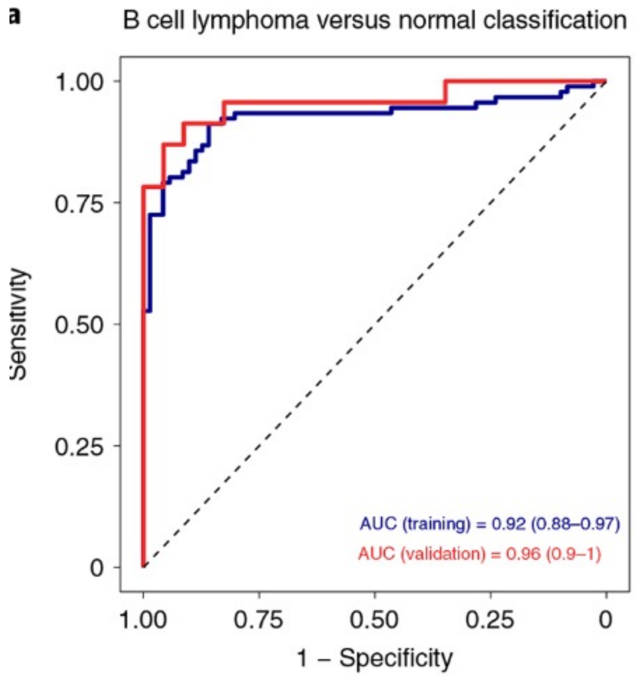
Esfahani et al. *Nat Biotechnol.* 2022

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

Application of EPIC-seq for DLBCL

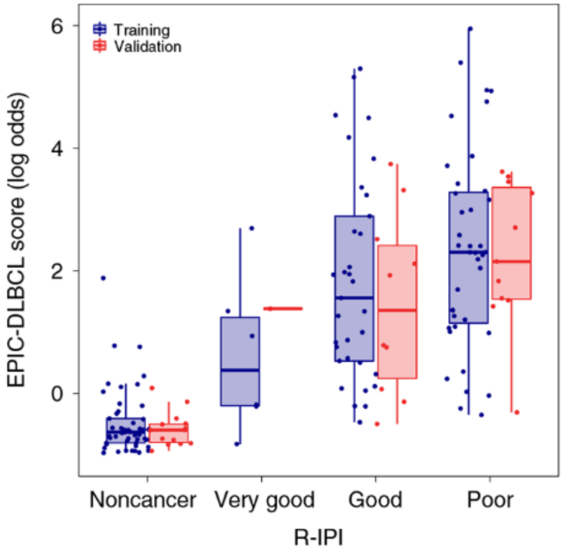
Training Cohort: A classifier trained on DLBCL and noncancer samples showed high accuracy in distinguishing cases

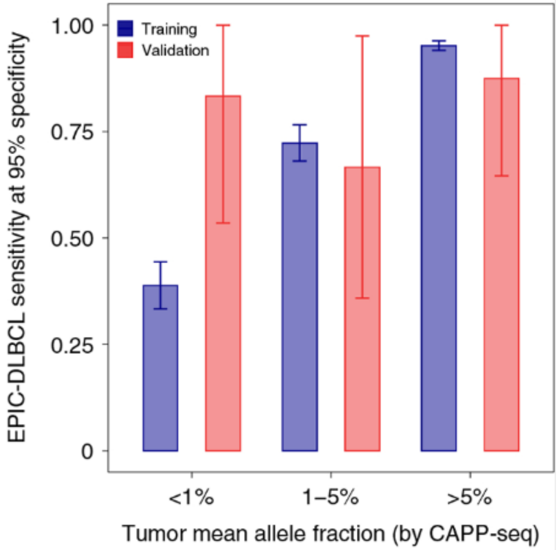
Validation Cohort: The classifier maintained strong performance in an independent patient set



Higher epigenetic classifier scores **correlated** with worse prognosis (**R-IPI**)

Most effective when ctDNA levels were high
Still detected a significant number of **DLBCL** cases even at low ctDNA levels

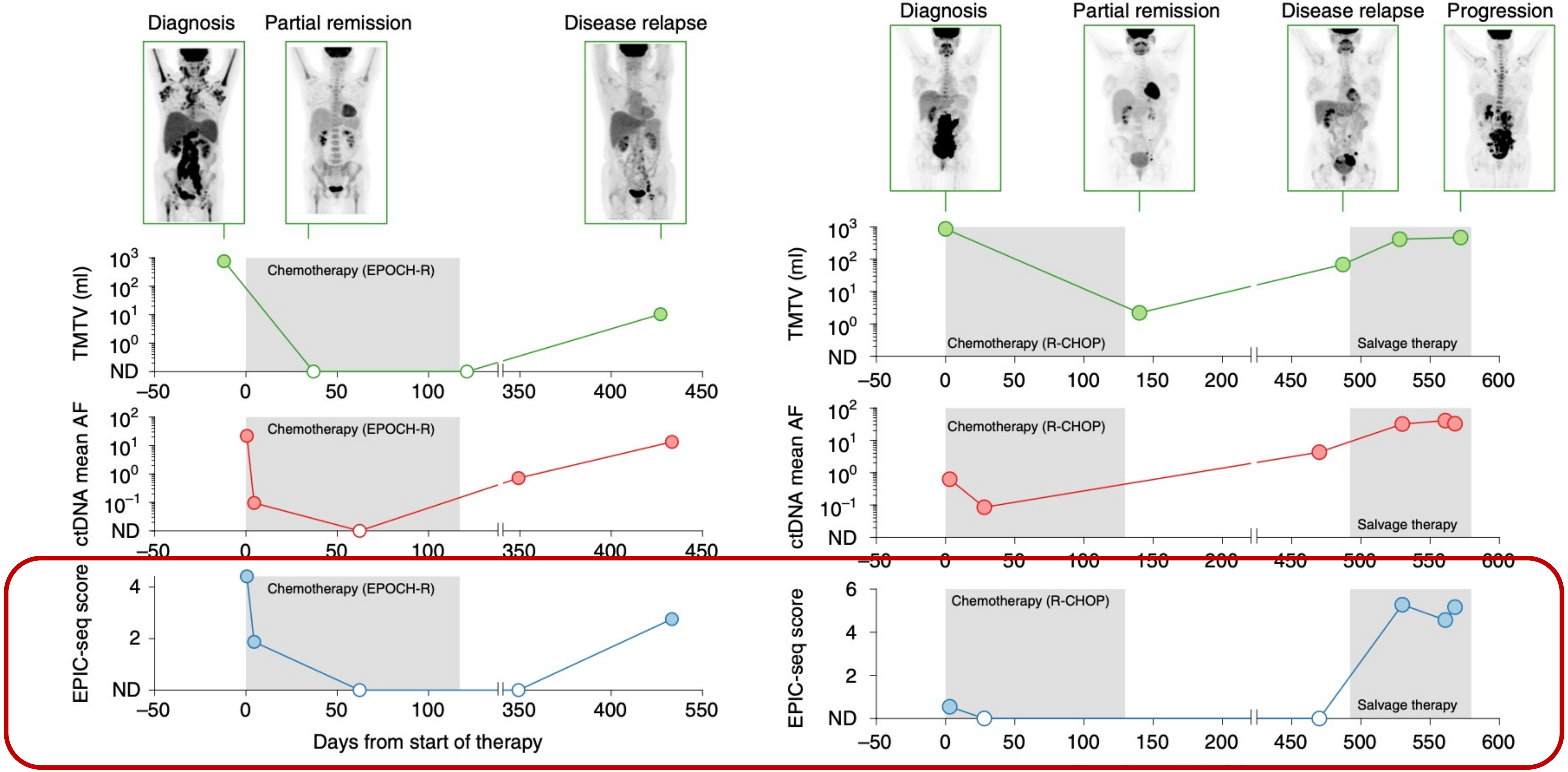




R-IPI: Revised International Prognostic Index

Esfahani et al. *Nat Biotechnol.* 2022

EPIC-seq for DLBCL



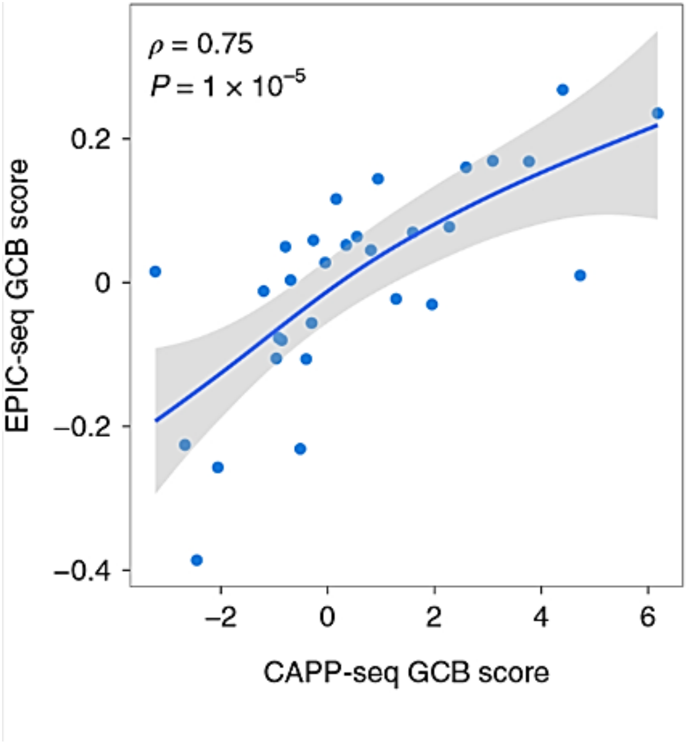
Expression inferences by EPIC-seq faithfully reflect disease burden before and after DLBCL therapy

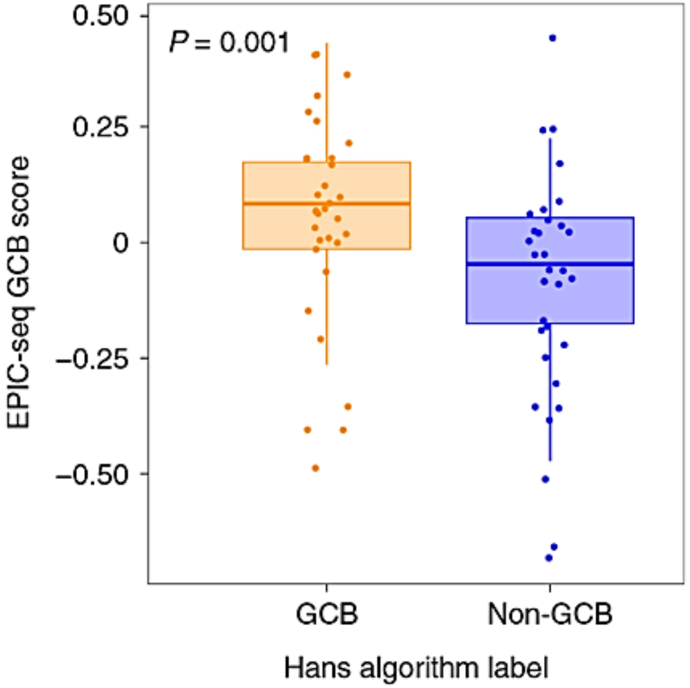
Esfahani et al. *Nat Biotechnol.* 2022

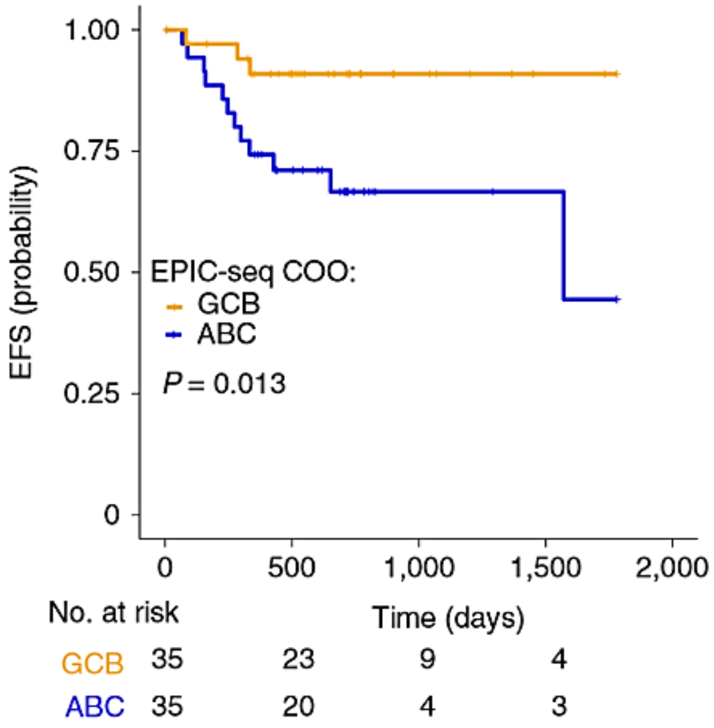
Application of EPIC-seq for DLBCL COO classification

EPIC-Seq GCB score:
Showed **Strong correlation** with mutation-based GCB scores
Discriminated significantly 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

- cfDNA shows different fragmentation patterns in healthy individual compared to cancer patients

- In solid cancers and in lymphomas cell-free DNA fragmentation patterns offer potential opportunity for early cancer detection, for assessing prognosis at diagnosis and for disease monitoring during therapy

- EPIC-Seq offers potential opportunity to extrapolate the transcriptomic profile of lymphoma patients according to cfDNA fragmentation patterns

UPO
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