HARNESSING BIOLOGICAL HETEROGENEITY FOR CLINICAL UTILITY IN LARGE B-CELL LYMPHOMAS

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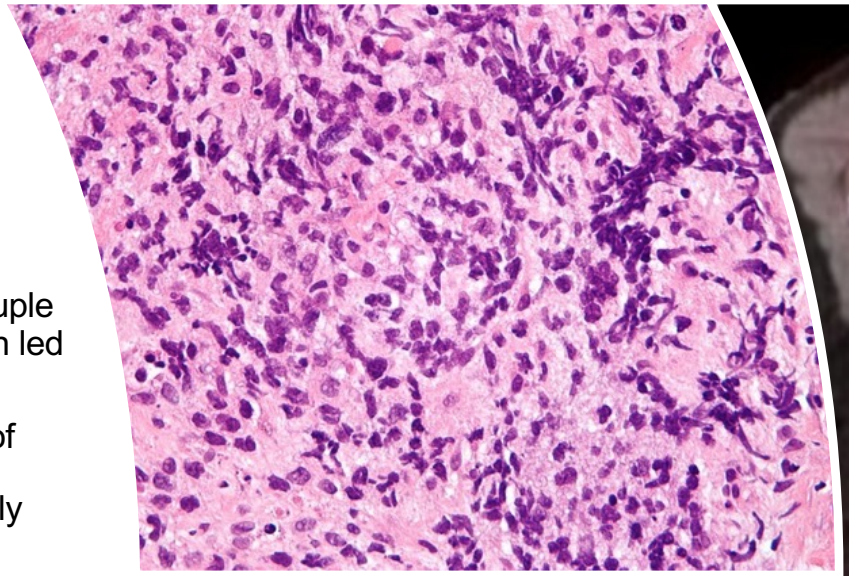
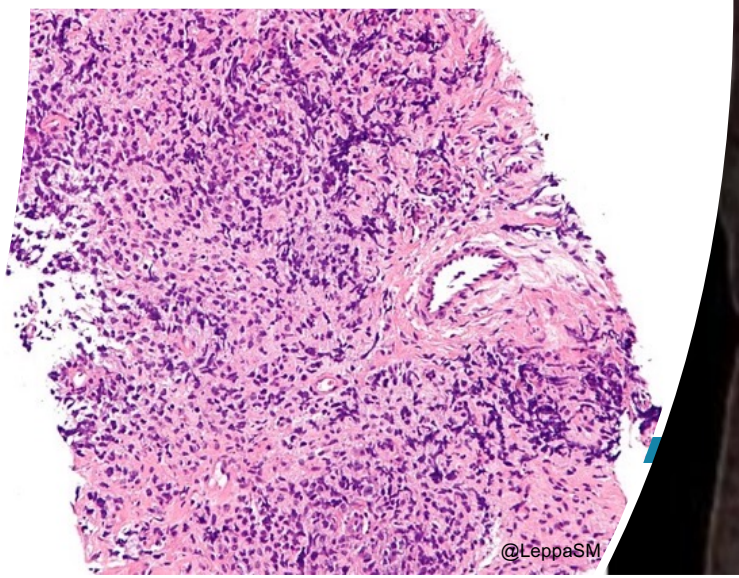
DISCLOSURES

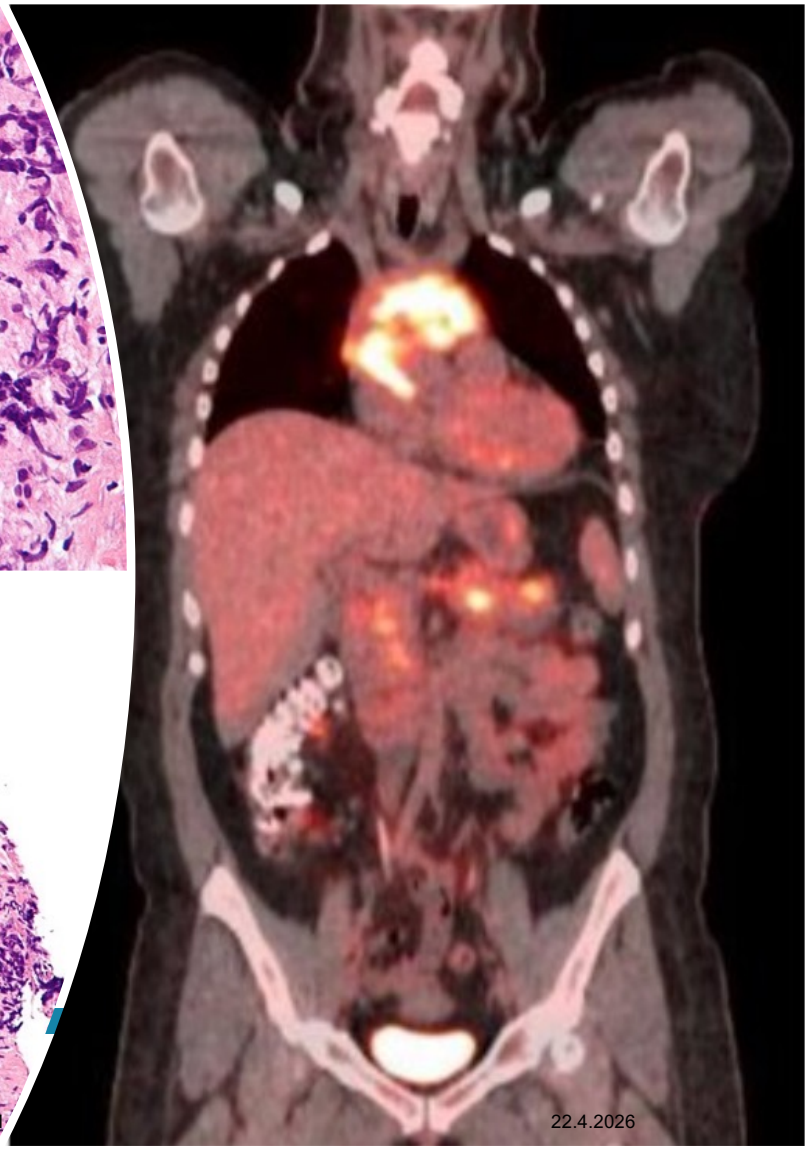
- Abbvie (consultation, honorary, advisory board)
- BMS (consultation, research support to the institute)
- Hutchmed (research support to the institute)
- Genmab (advisory board, research support to the institute)
- Gilead (honorary)
- Incyte (advisory board, honorary, research support to the institute)
- Novartis (research support to the institute)
- Roche (advisory board, honorary, research support to the institute)
- Sobi (advisory board)

CASE #I

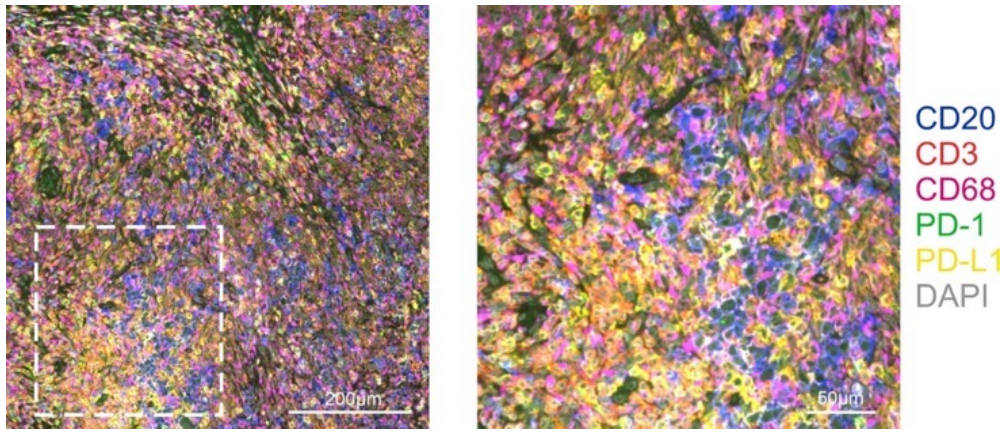
- 28-year-old male, had felt fatigued during the last couple months. A persistent cough led to a doctor's appointment.
- He did not have a history of smoking. His performance status was good, LD slightly elevated
- Imaging with PET-CT identified mediastinal mass and mesenteric lymphadenopathy
- Based on CT–guided small biopsy, dg was DLBCL
- Patient started treatment with dose-intensified ICT in NLG LBC-05 trial (aaIPI 2)

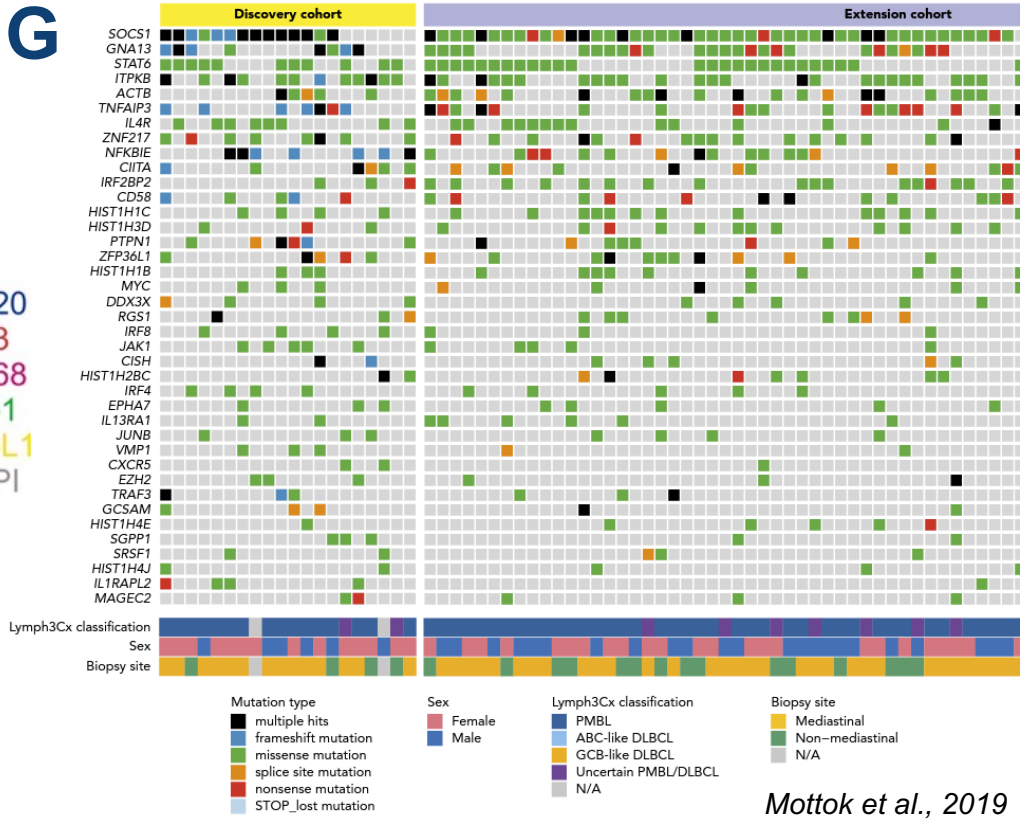






TME & ctDNA PROFILING



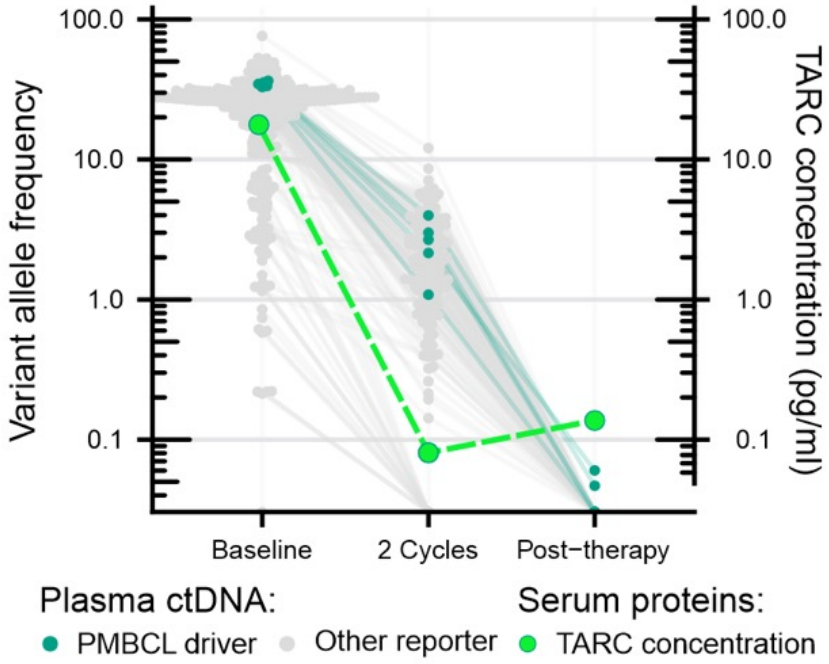


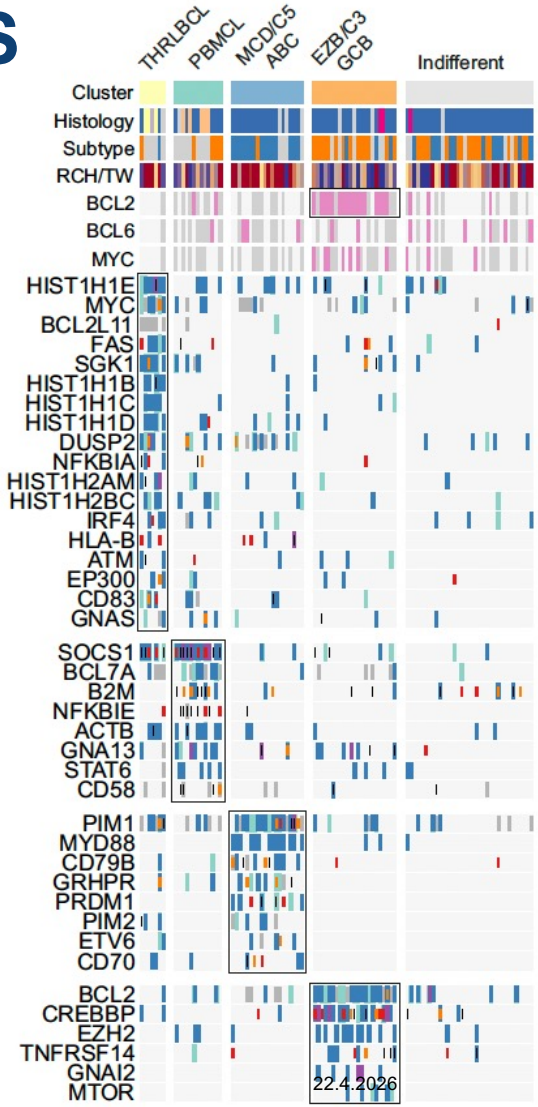
STAT6, SOCS1, B2M, NFKBIE mutations
and high serum CCL17/TARC concentration

Mottok et al., 2019

-> Primary mediastinal B-cell lymphoma (PMBCL)

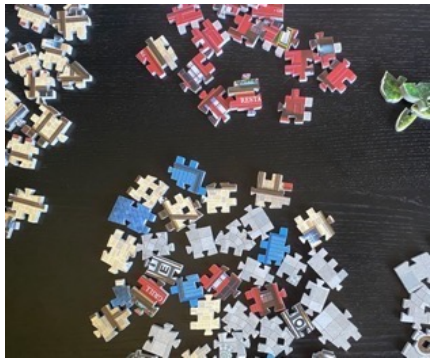
PLASMA ctDNA AND s-TARC LEVELS DURING THERAPY





CONTENT

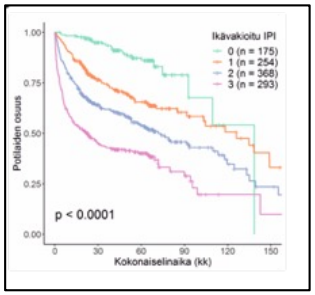
- How can we uncover molecular heterogeneity and host response in patients with LBCL
  - Genetic
  - Phenotypic
  - Spatial
  - Temporal
- How can we deploy liquid biopsy
  - Lessons from NLG trials
    - ctDNA & serum proteins

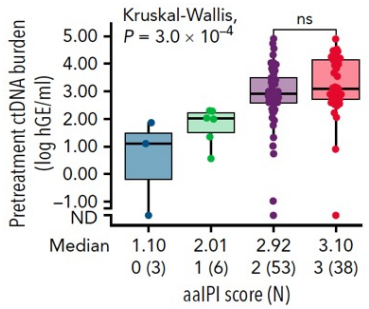


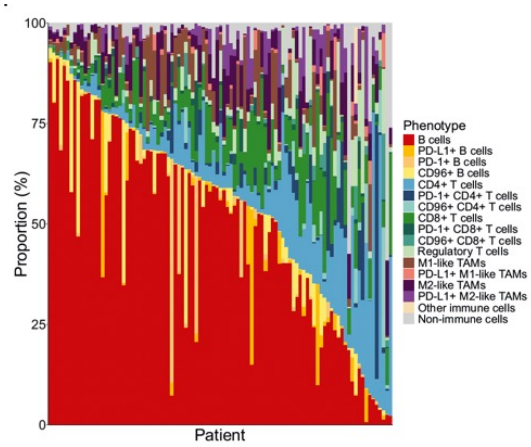
Biomarker-driven approaches- Leveraging specific biomarkers to tailor therapies based on the individual characteristics of the lymphoma subtype, genetic factors, and the patient's overall health
Molecular vulnerability- Specific genetic, biochemical, or signaling weaknesses within the cancer cells that can be targeted for therapeutic intervention

LARGE B-CELL LYMPHOMAS ARE CLINICALLY AND BIOLOGICALLY HETEROGENEOUS

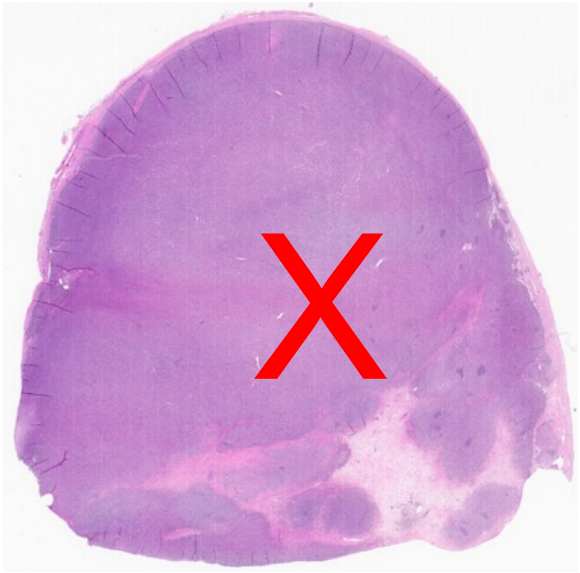
- **Most common type of adult lymphoid cancers**
  - Diagnosis is based on morphology, immunohistochemistry and FISH
  - Majority of LBCL belong to the category of DLBCL not otherwise specified (NOS)
  - Other entities: PMBCL, THRBCL, HGBCL
  - Staging is based on PET-CT
- **IPI clinical risk score remains the primary clinical tool for predicting outcomes and stratifying patients to different risk groups and therapies**
  - Cannot be used to identify patients at very high risk of relapse
  - Cannot discern biological heterogeneity
- **30% of patients suffer from relapse, and 10% show refractory disease with poor outcome**
- **The extreme genetic and phenotypic heterogeneity presents a challenge to the development of precision therapies**

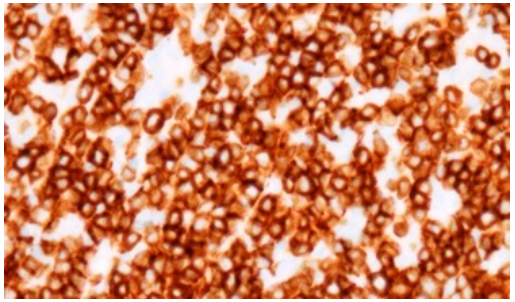


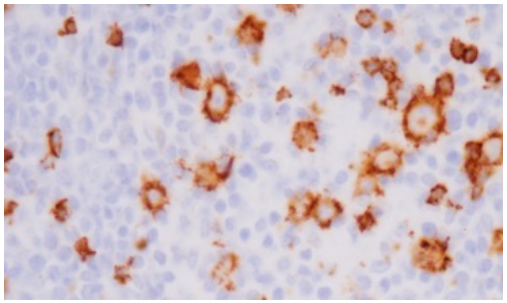


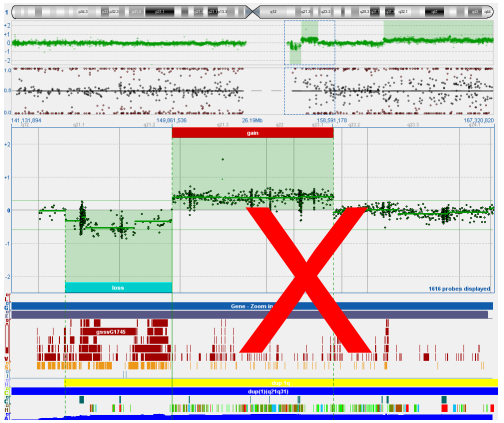


Autio et al., 2025; Meriranta et al., 2022









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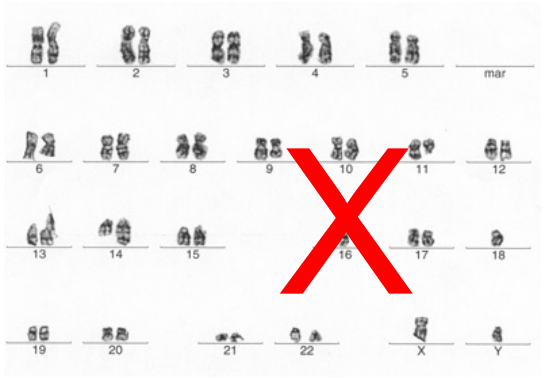

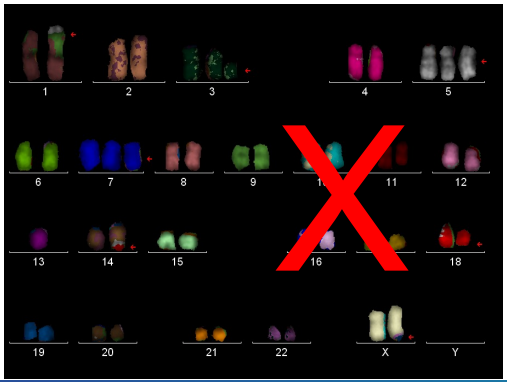

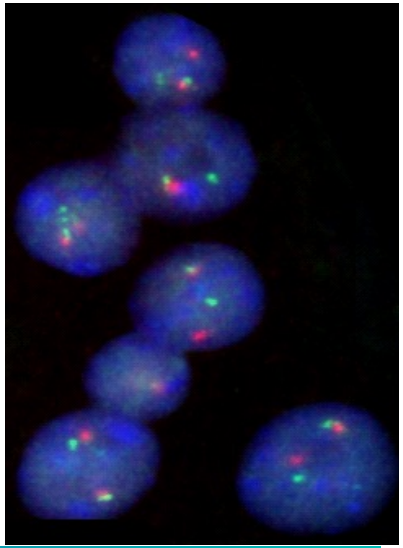

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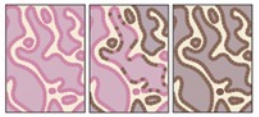
A COLLABORATION BETWEEN HUS & UNIVERSITY OF HELSINKI

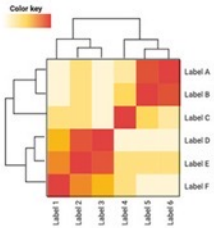
MOLECULAR CLASSIFICATION

Disease entities acc. to WHO/ICC classification



- Morphology
- IHC
- FISH

Gene expression based classification



- ABC *vs* GCB
- Dark zone/Molecular high grade signatures
- TME subtypes/ecotypes

Liquid biopsies

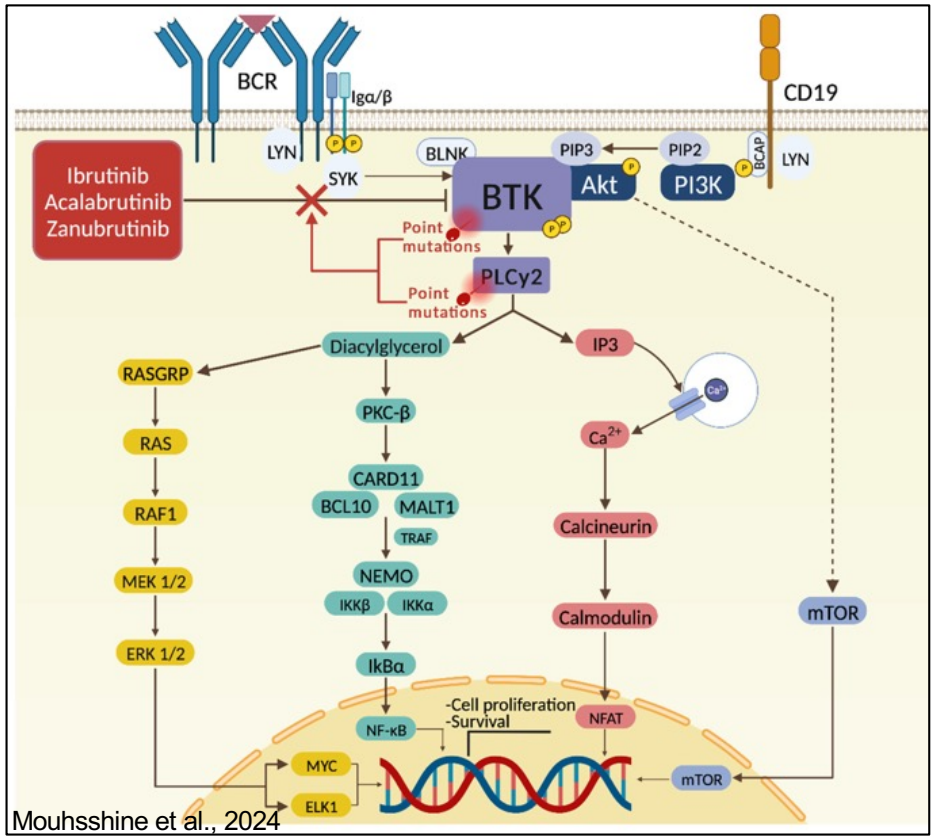
- Noninvasive profiling
- Dynamic profiling (MRD)

Genetic subtypes

DLBclass	LymphGen	HMRN	Landmark Alterations	COO Enrichment
C1	BN2	NOTCH2	*BCL6 SV, NOTCH2, SPEN, CD70, BCL10, TNFAIP3*	ABC, unclass
C2	A53	-	*Biallelic TP53 inactivation, aneuploidy*	ABC, GCB
C3	EZB EZB-MYC+	BCL2 BCL2-MYC	*BCL2 SV, EZH2, CREBBP, KMT2D, TNFSF14*	GCB
C4	ST2	TET2/SGK1 SOCS1/SGK1	*SGK1, TET2, KLHL6, BRAF* *SGK1, TET2, SOCS1, CD83 NFKBIA, NFKBIE, STAT3*	GCB
C5	MCD	MYD88	*MYD88^{L265P}, CD79B, PIM1, TBL1XR1, ETV6*	ABC
-	N1	NOTCH1	*NOTCH1, ID3*	ABC

Alig et al., 2025

ABC-SPECIFIC VULNERABILITY OF BTK TARGETING (I)

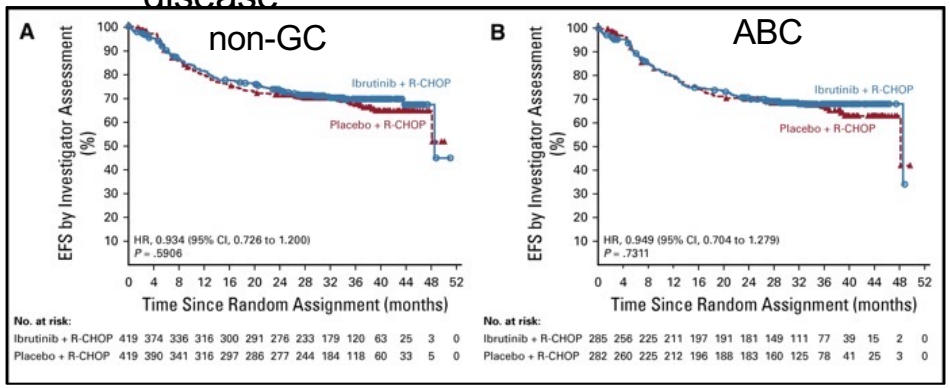


Mouhsshine et al., 2024

Effect of **ibrutinib** with R-CHOP in non-GCB DLBCL

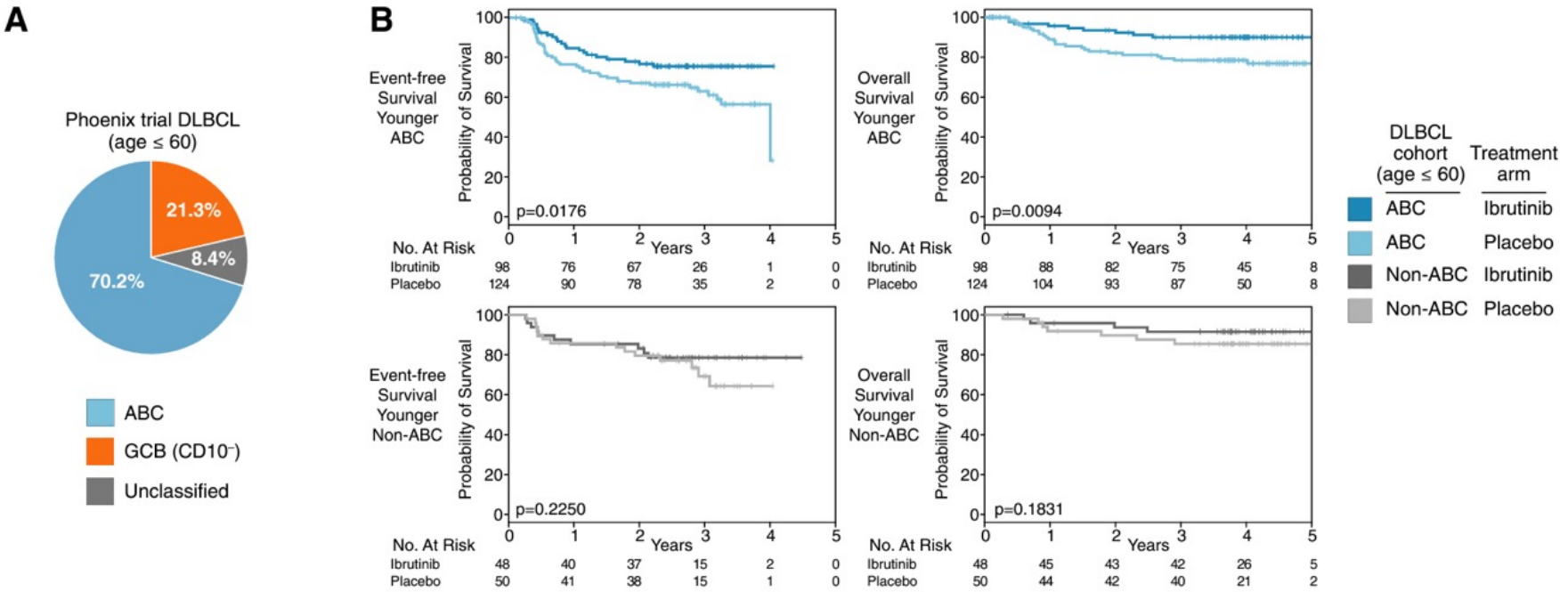
PHOENIX phase III study

- N=838
- median age 62 years (63%>60 years)
- 75.9% of evaluable patients had ABC subtype disease

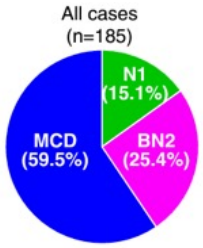


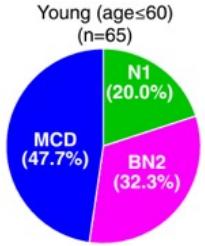
Younes et al., 2019

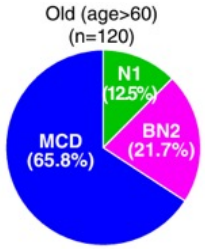
ABC-SPECIFIC VULNERABILITY OF BTK TARGETING (I)

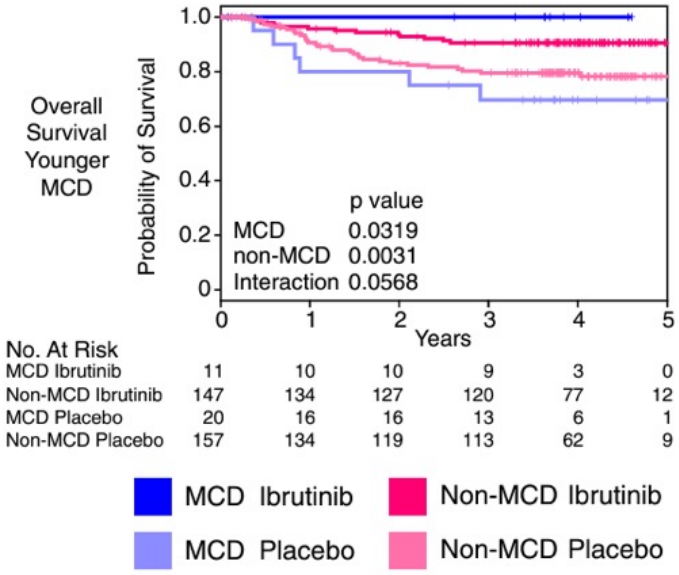


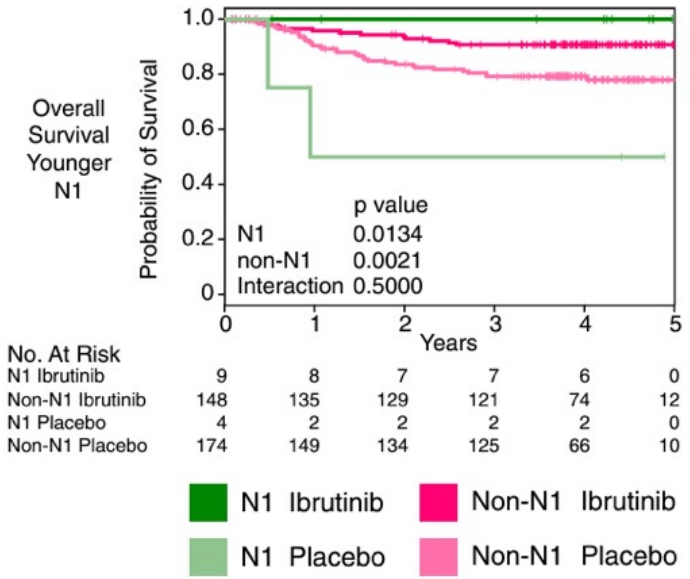
MCD/N1 GENETIC SUBTYPE-SPECIFIC VULNERABILITY OF BTK TARGETING











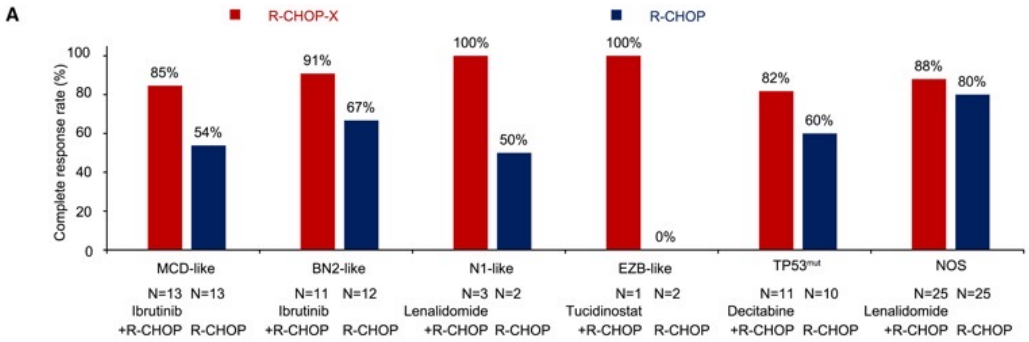
GENETIC SUBTYPE-GUIDED THERAPY

Effect of X with R-CHOP

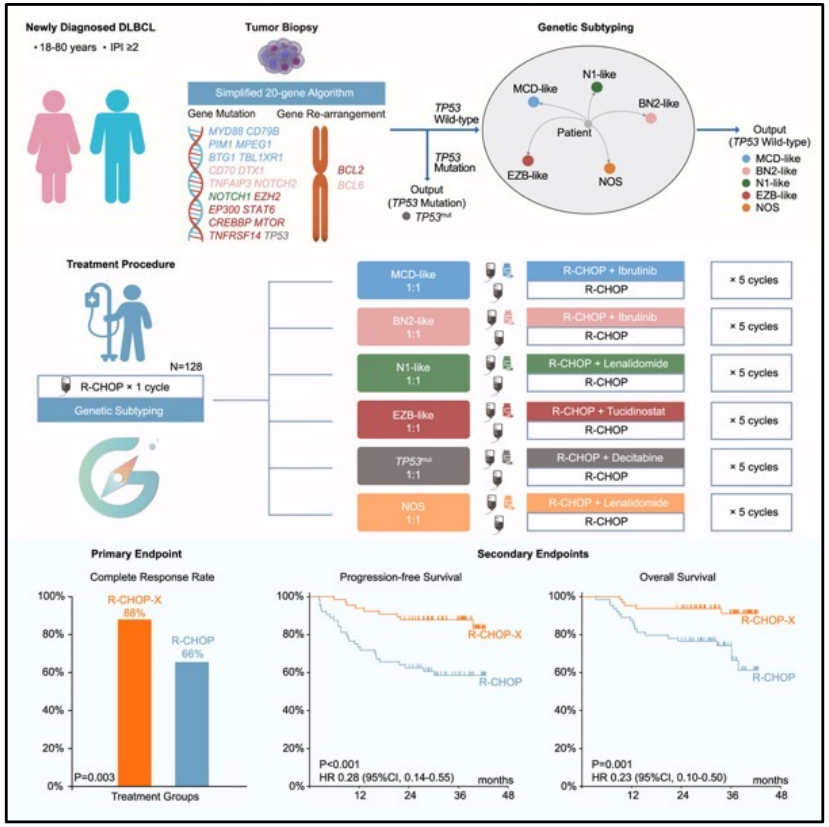
GUIDANCE-1 randomized phase II study; n=128

X=Genetic subtype-guided targeted drug

Targeted agents based on genetic subtyping improve R-CHOP efficacy



GUIDANCE-2 phase III study; n=1100



Zhang et al., 2023

BIOMARKER DISCOVERIES IN THE CONTEXT OF NLG-LBCL TRIALS

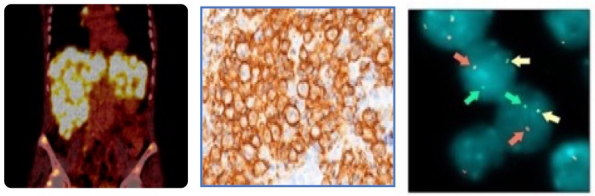
Disease | Intervention 1 | Translation | Outcome | Intervention 2

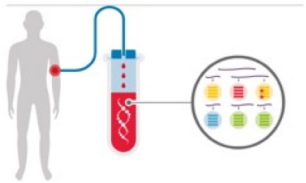
A. CLINICAL TRIALS

Biological risk factors
sMYC, DHL, p53del, p53+, MYC+/BCL2+, CD5 +

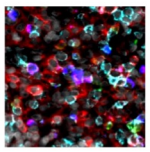
Registration
R-CHOP+HD-MTXx2
Staging and statification → PD → off study
Biol factors+ | Biol factors-
daEPOCH-Rx4 R-HD-Ara-C | R-CHOEPx4+ R-HD-Ara-C
Final staging (PET) → PD → off study
Follow up

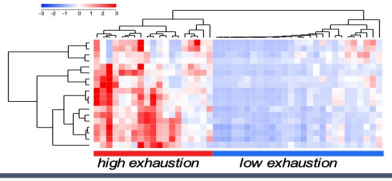
B. CLINICAL AND MOLECULAR PROFILING



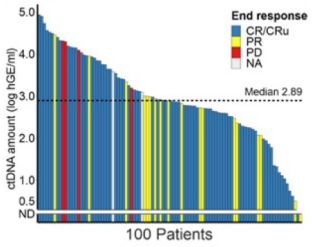


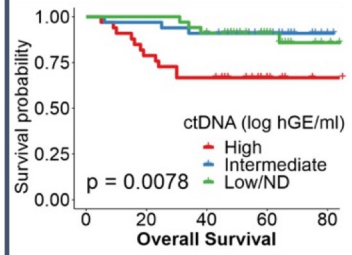
Plasma samples:
Baseline
Mid-treatment
Post-treatment
Follow-up
Relapse

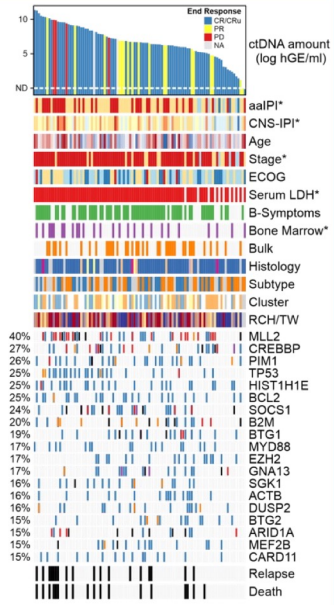




C. LIQUID BIOPSY







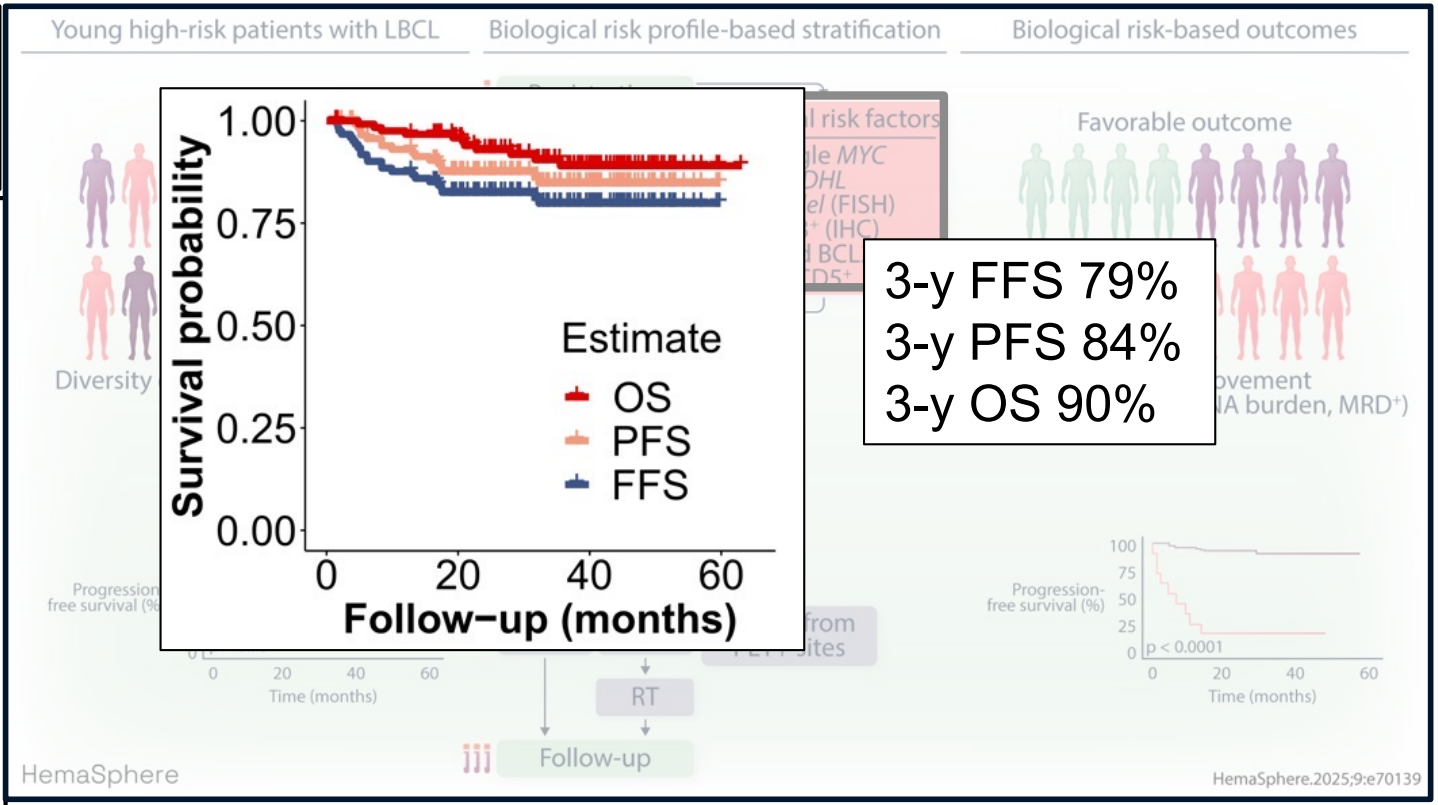
Blood samples: ctDNA & protein profiling

Leppä et al., 2025; Autio et al., 2022, 2025; Meriranta et al., 2022; Arffman et al 2024

NLG-LBC-06 (BIO-CHIC)

NLG-LBC-6 Trial: BIO-marker driven and dose intensified CHemoImmunotherapy with early CNS prophylaxis in patients less than 65 years with high risk LBCL (BIO-CHIC)

- Age 18-65 years
- Histologically confirmed CD20+ diffuse large B-cell lymphoma
  - High grade B-cell lymphomas allowed
  - Follicular lymphomas grade 3B allowed
- Patients with at least stage II with aaIPI score of 2 or 3 and/or patients with
  - More than one extranodal site
  - Testicular lymphoma, stage IIE and higher
  - Paranasal sinus and orbital lymphoma with destruction of bone
- Previously untreated, except steroids allowed
- WHO performance status 0-3

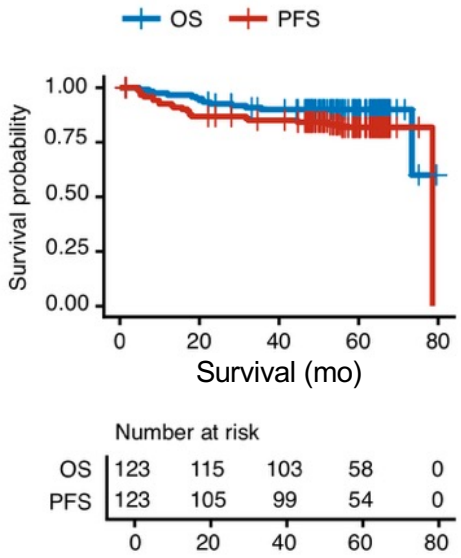


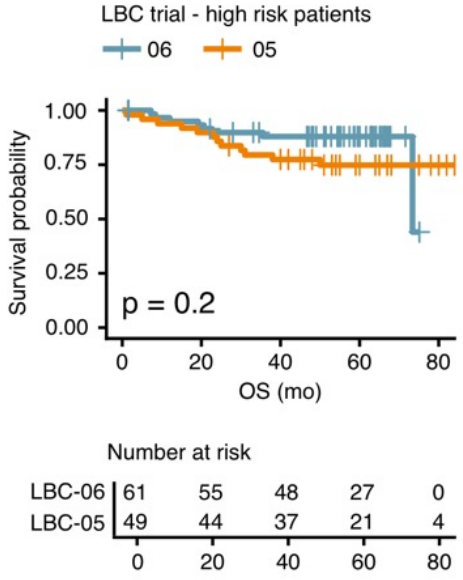
Leppä et al. 2025

OUTCOMES (MEDIAN F-UP 60 MO)

Event	All N=123 (%)
Treatment failure	**33 (27)**
Adverse event	8 (6.5)
Lymphoma progression	19 (15)
Death	1 (0.8)
Consent withdrawn	5 (4.1)
Progression	**22 (18)**
Primary refractory lymphoma	5 (4.1)
Later progression	17 (14)
CNS progression	1 (0.8)
Systemic & CNS progression	1 (0.8)
Indolent lymphoma	3 (2.4)
Death	**13 (11)**
Lymphoma	12 (9.8)
Other disease	1 (4.3)

5-year	FFS	PFS	OS
All	78%	82%	90%
Biol high risk	73%	76%	88%





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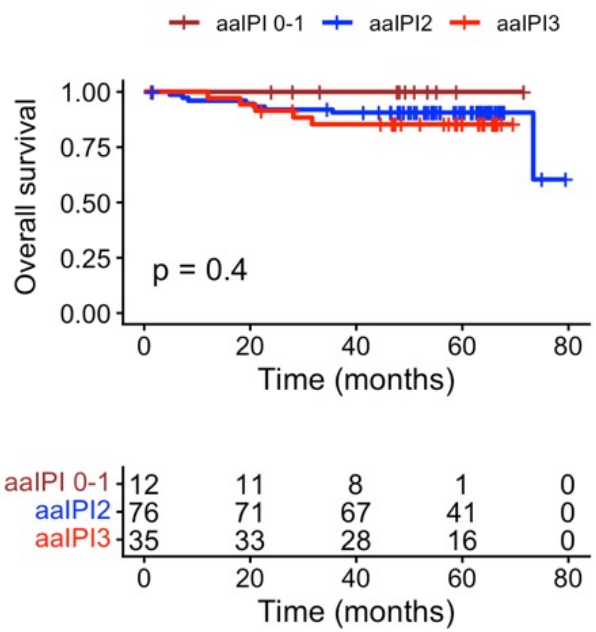
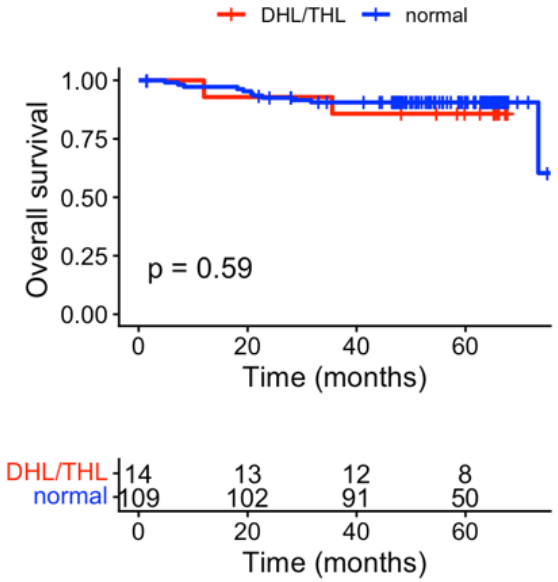
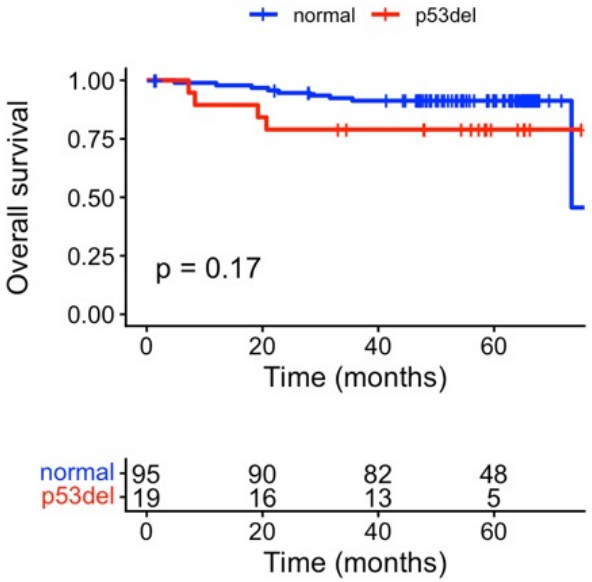
PREDEFINED RISK FACTORS







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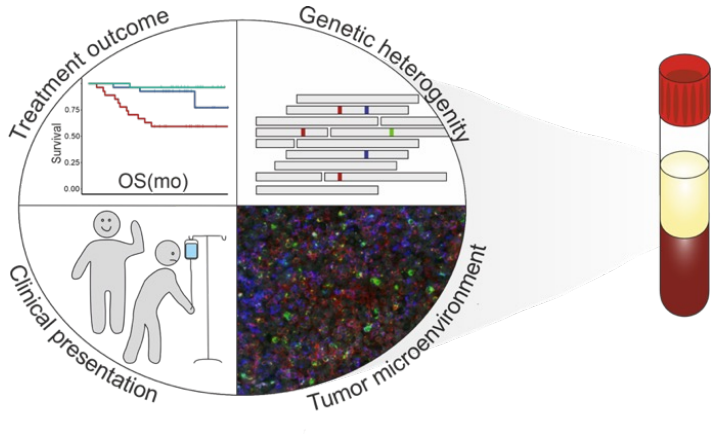
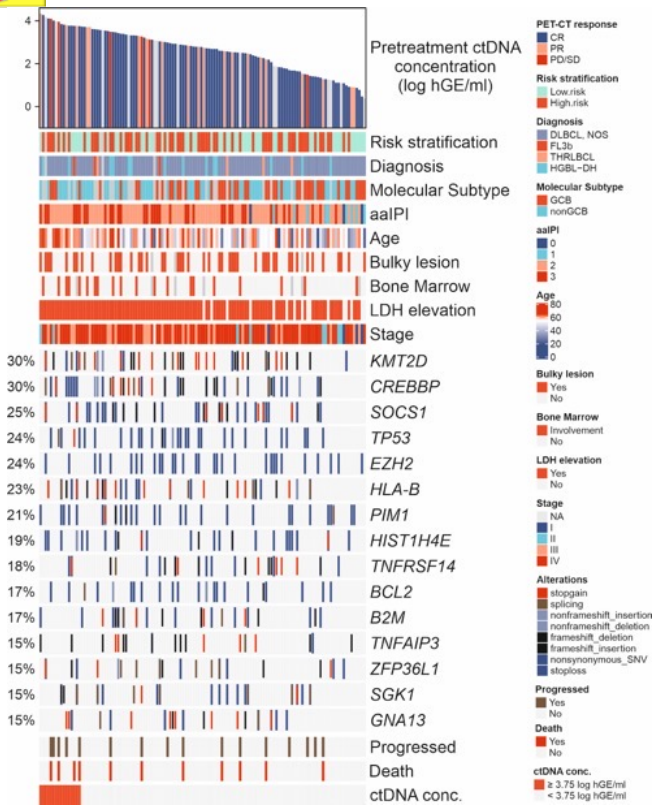
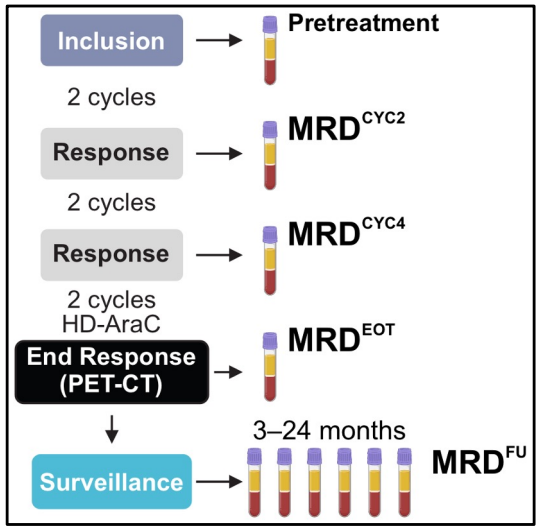
Correlative Studies – Liquid Biopsy

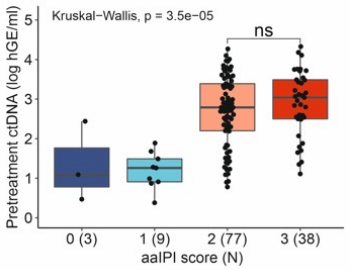
HOW MIGHT WE BEST DEPLOY LIQUID BIOPSY *-LESSONS FROM NLG-LBCL TRIALS*

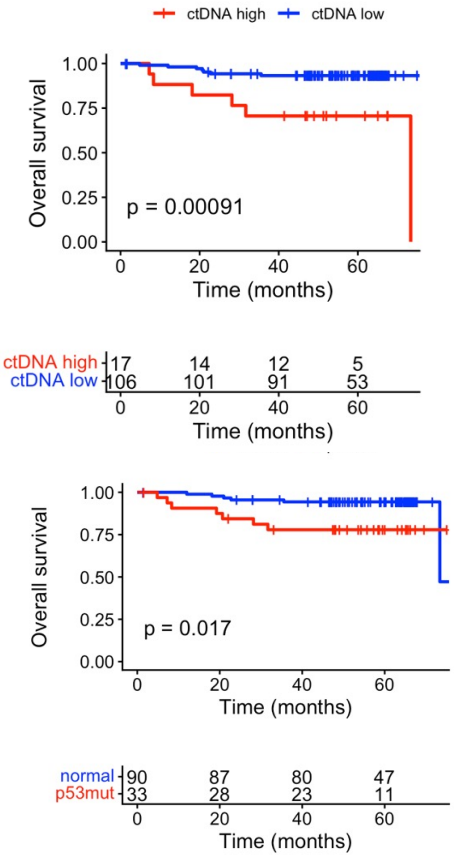


Circulating tumor DNA (ctDNA)



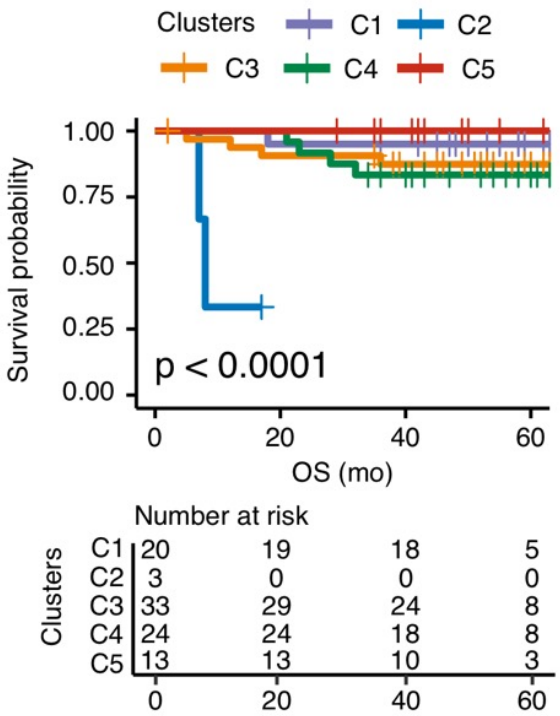




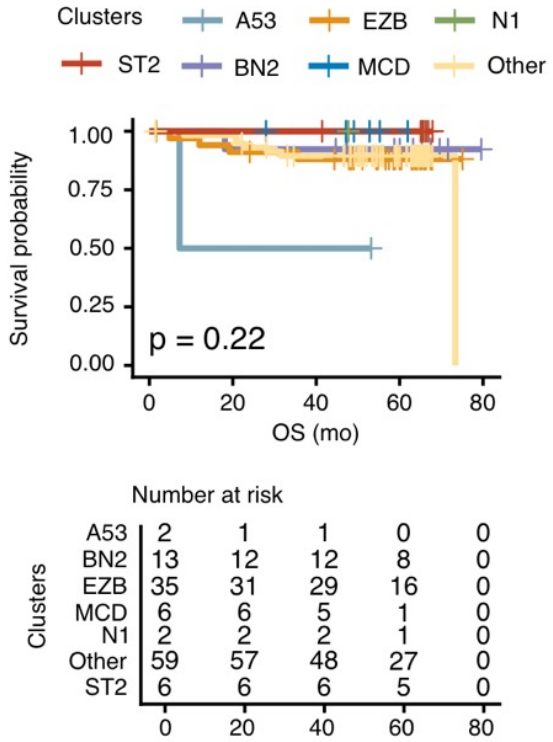


OS ACCORDING TO DLBCLASS & LYMPHGEN

DLBClass



Lymphgen



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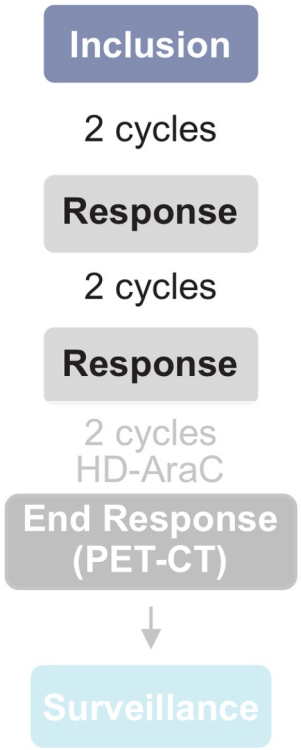
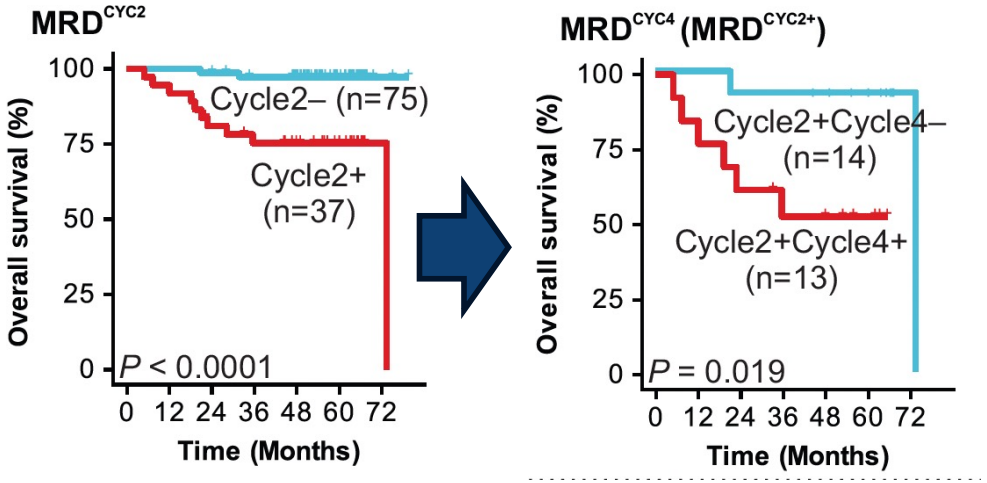
MOLECULAR RESPONSE AND SURVIVAL





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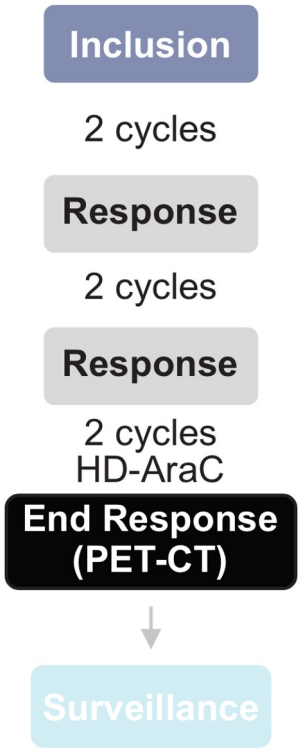
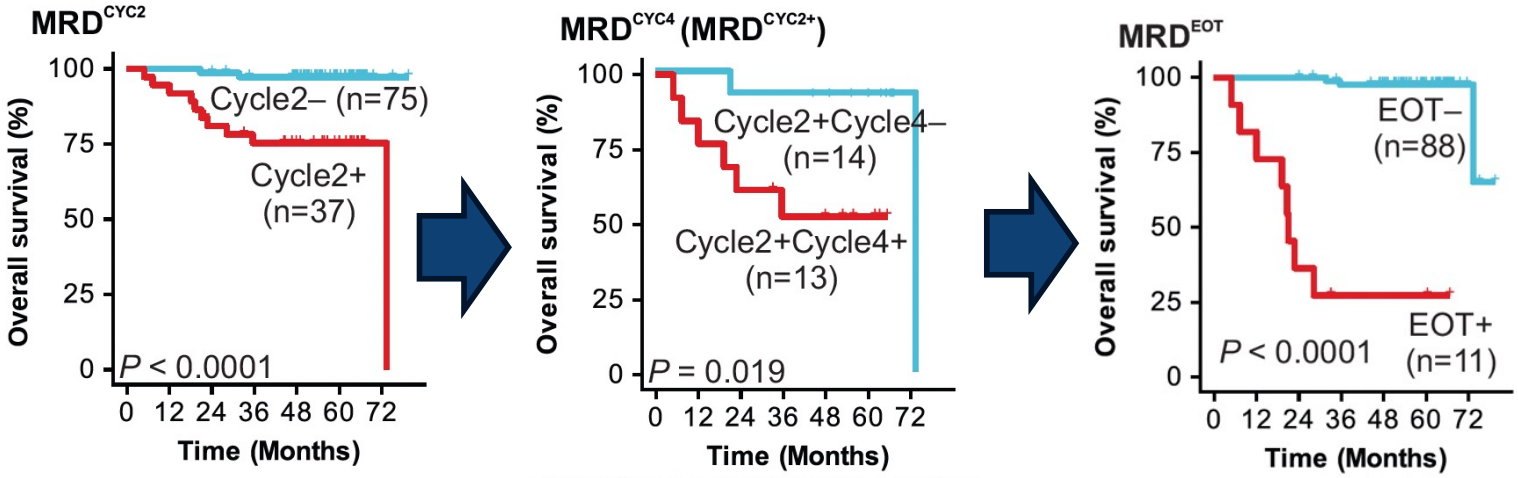
MOLECULAR RESPONSE AND SURVIVAL





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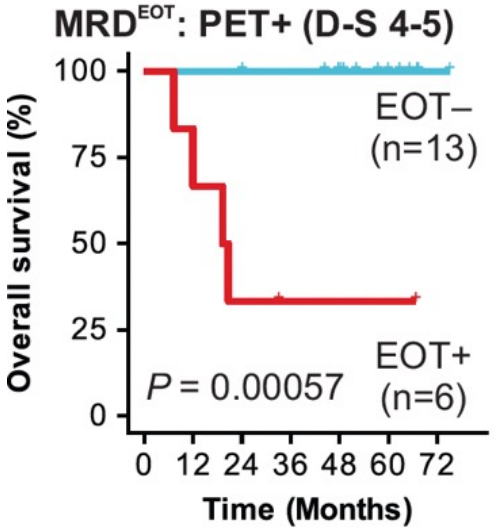
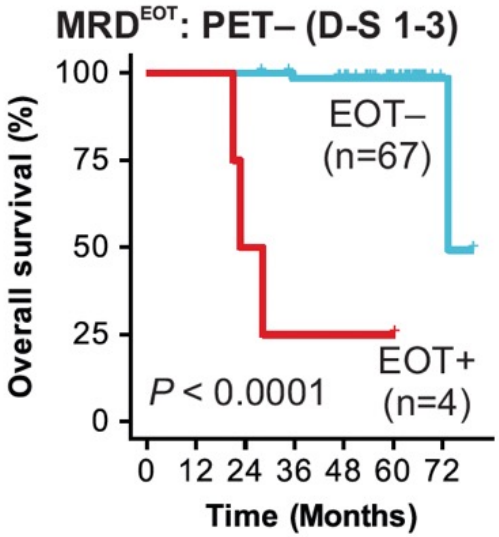
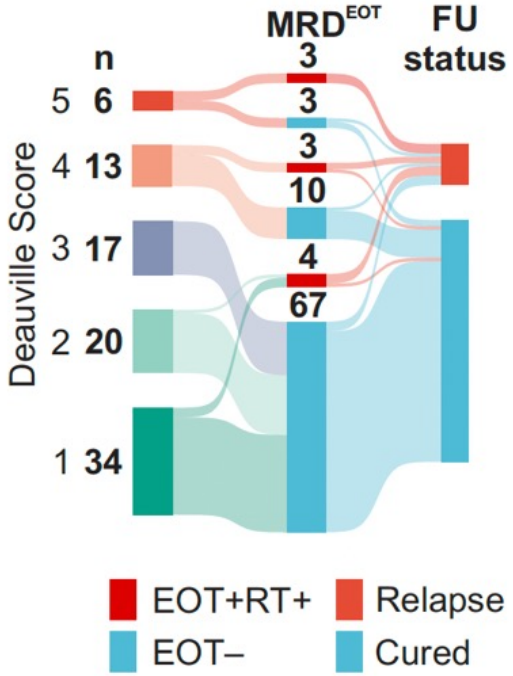
PET WITH MRD







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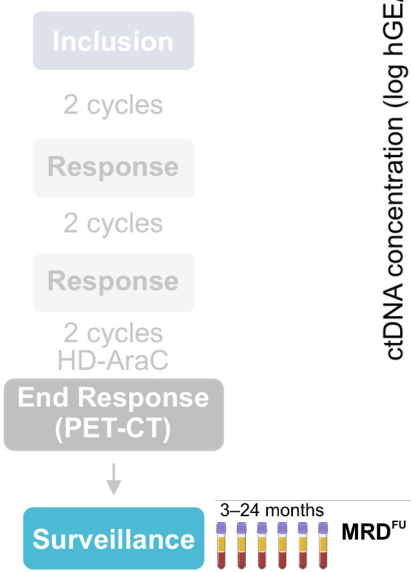
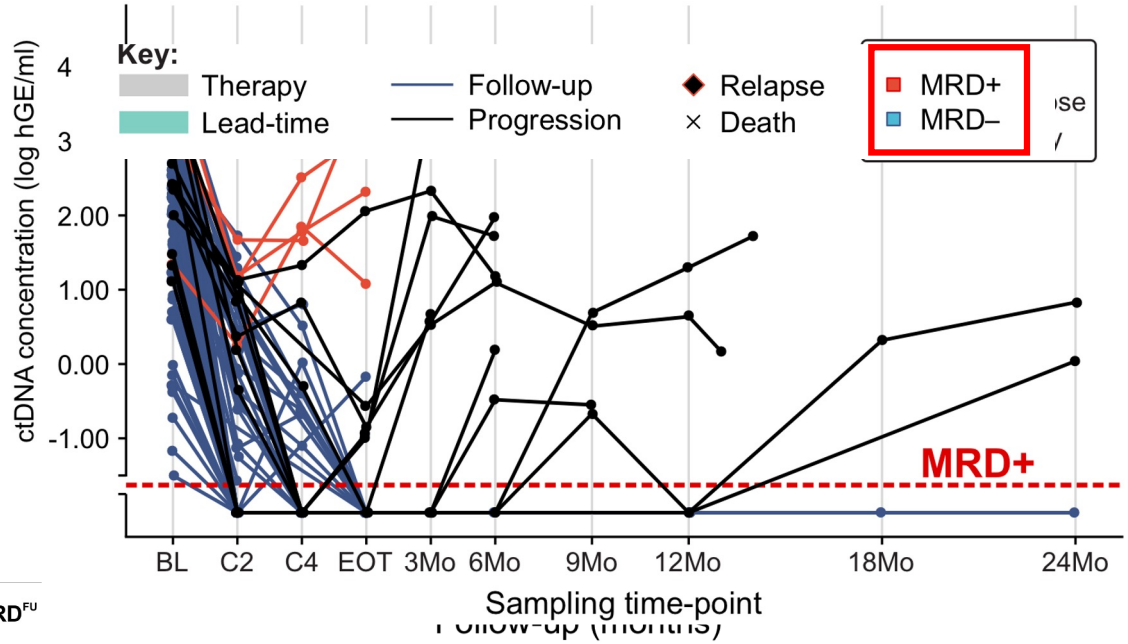
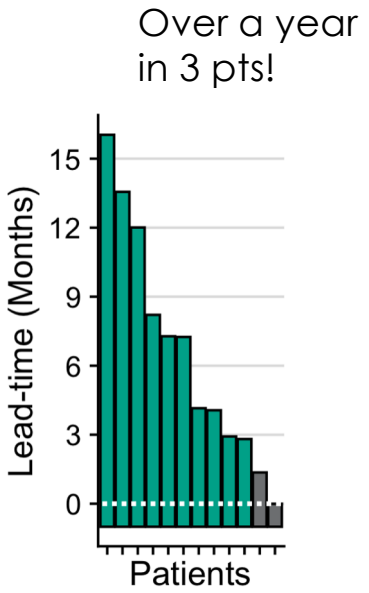
SURVEILLANCE AFTER THERAPY PROVIDES LEAD-TIME TO PROGRESSION







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CIRCULATING TUMOR DNA-GUIDED THERAPY IN LARGE B-CELL LYMPHOMA

CINDERELLA (NLG-LBC-09)

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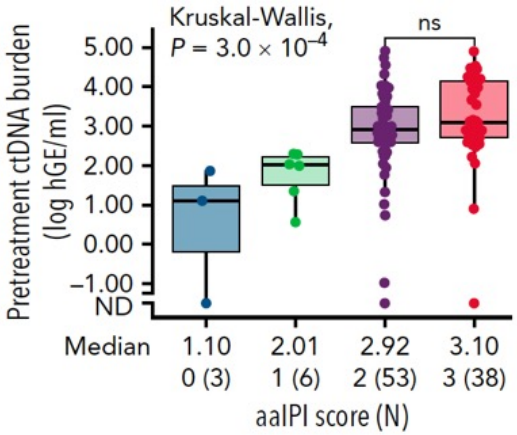
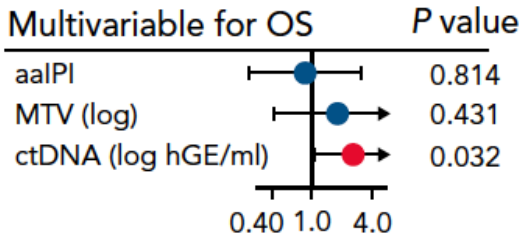
HUS & HELSINGFORS UNIVERSITET I SAMARBETE
A COLLABORATION BETWEEN HUS & UNIVERSITY OF HELSINKI

@LeppaSM

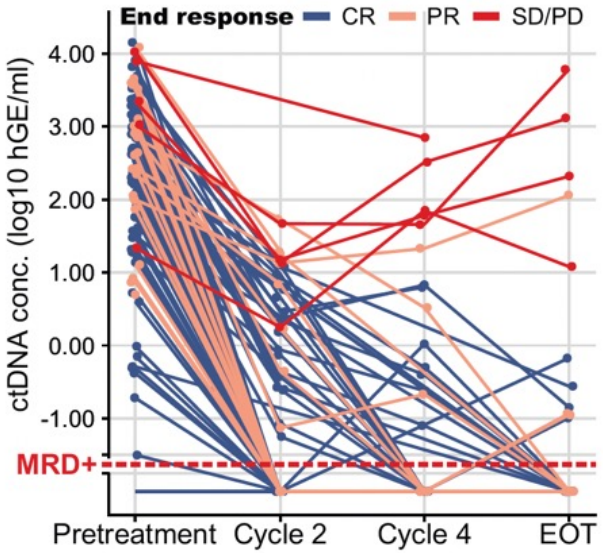
ctDNA BURDEN-BASED STRATIFICATION – WHY?

Hidden heterogeneity

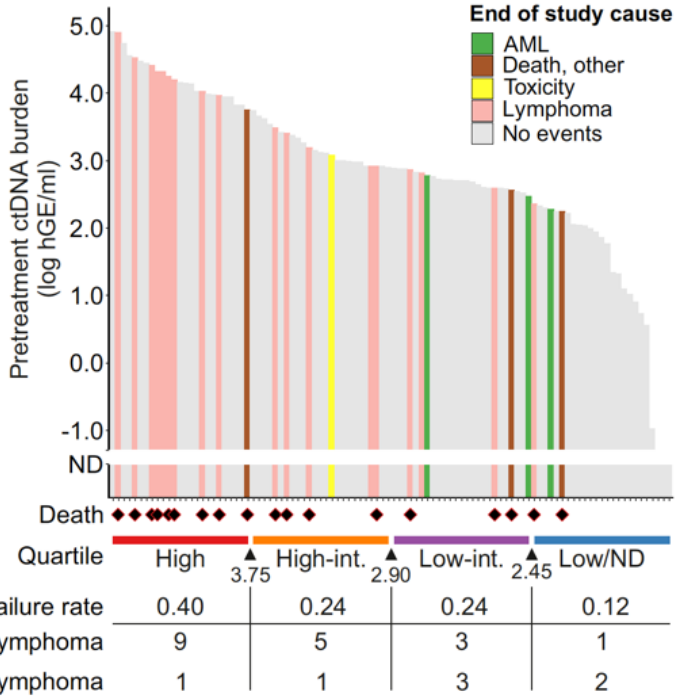




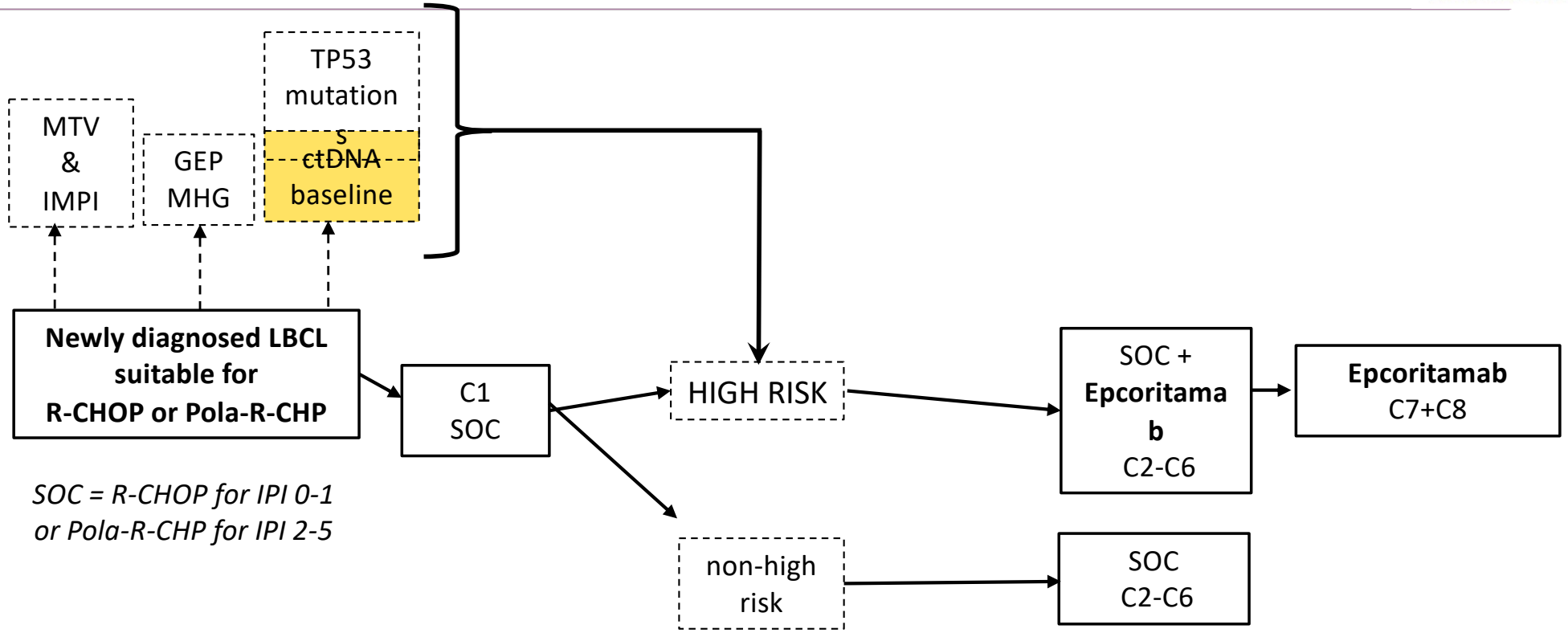
Chemoresistance



Overtreatment



RADICAL: A RISK-ADAPTED PHASE 2 STUDY FOR NEWLY DIAGNOSED DLBCL



Courtesy of Cris Fox

Chief Investigator Prof Chris Fox Nottingham, UK

CINDERELLA PRIMARY OBJECTIVES

- To evaluate if a ctDNA-guided molecular treatment approach is feasible in terms of failure-free survival as a first-line treatment in patients with aggressive large B-cell lymphoma
  - Run-in part: To determine the turnover time and success of the ctDNA assay
  - Phase II: To establish if ctDNA levels measured during therapy can be used to guide therapy in patients with aggressive LBCL.

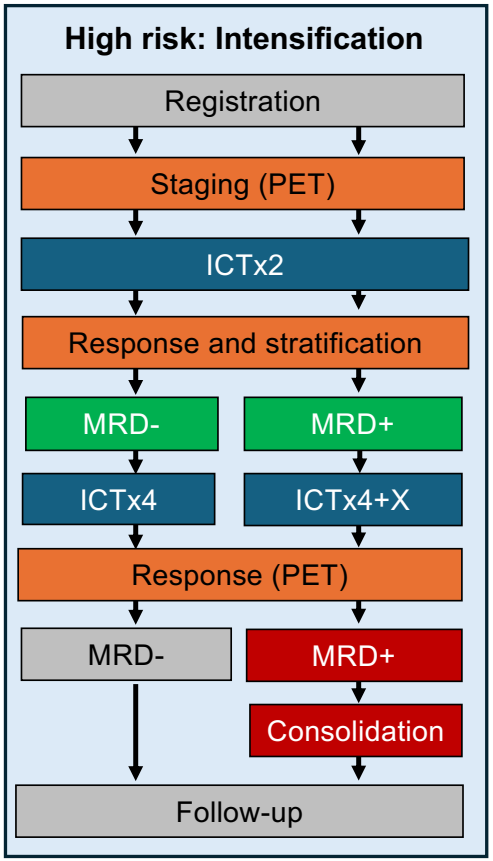
STUDY DESIGN

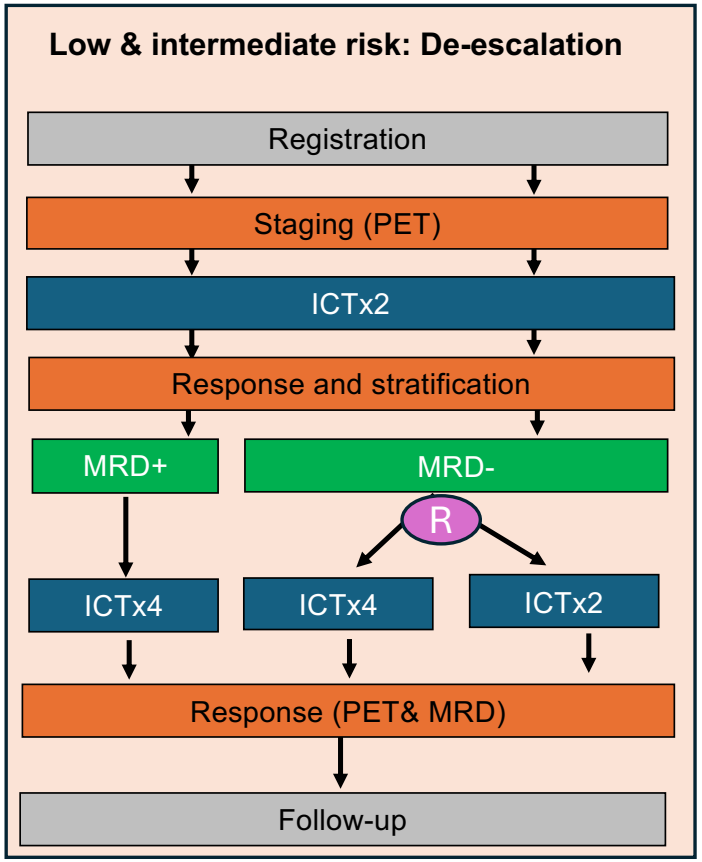
Pretreatment ctDNA & tumor

ctDNA after 2 cycles

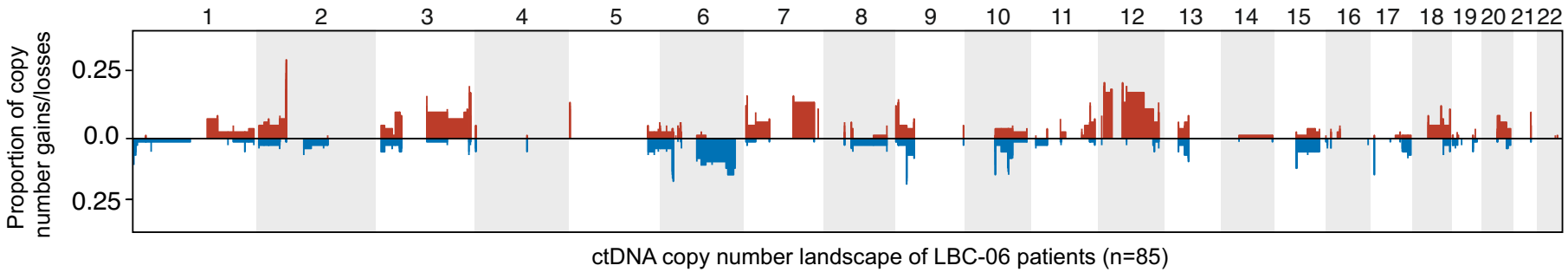
ctDNA end-of-therapy

Surveillance

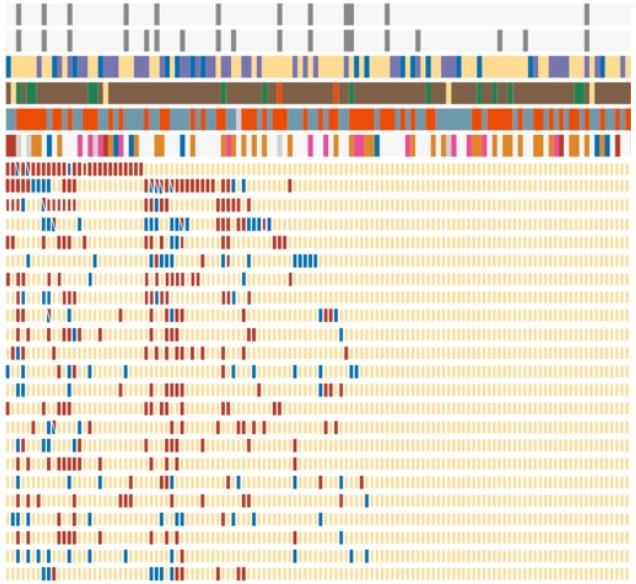


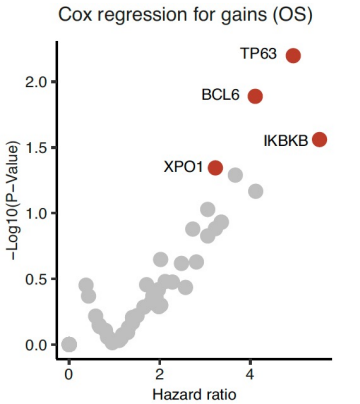


Extension of liquid biopsy repertoire to ctDNA copy number profiling

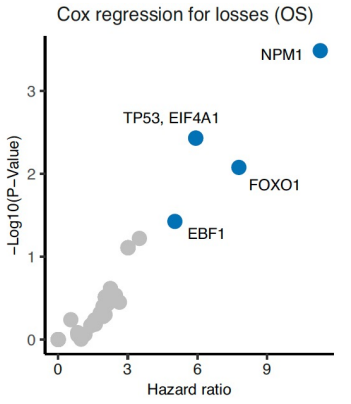


COPY NUMBER VARIANT LANDSCAPE

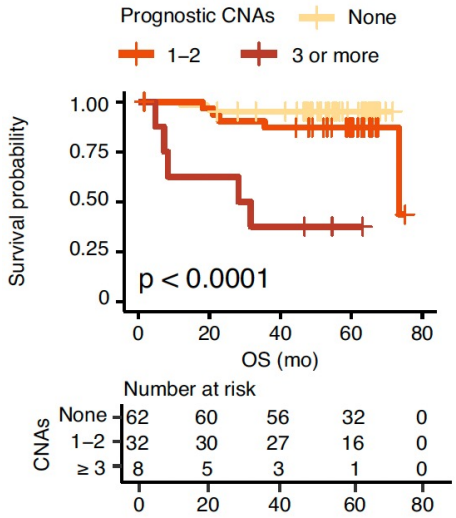


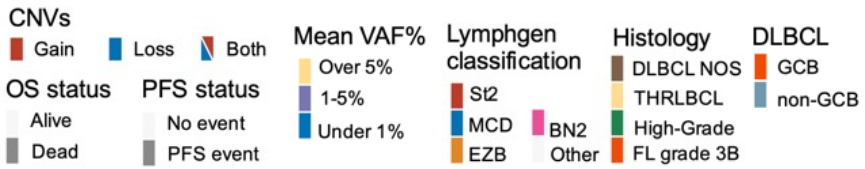


Gene	HR (95% CI)	p-value
XPO1	3.23 (1-10)	0.0452
BCL6	4.11 (1.3-12)	0.0129
IKBKB	5.53 (1.2-25)	0.0275
TP63	4.95 (1.6-16)	0.00633



Gene	HR (95% CI)	p-value
EBF1	5.03 (1.1-23)	0.0375
FOXO1	7.79 (1.7-36)	0.00836
TP53, EIF4A1	5.93 (1.8-20)	0.0037
NPM1	11.3 (3-42)	0.000326





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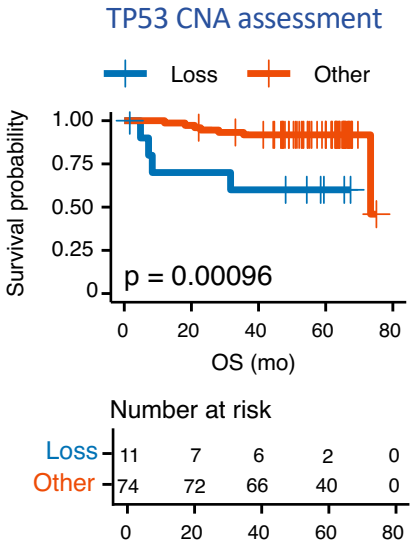
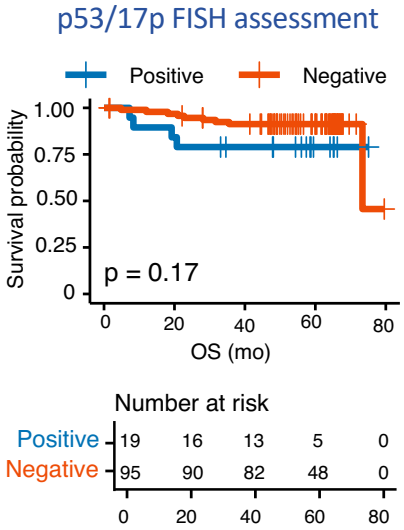
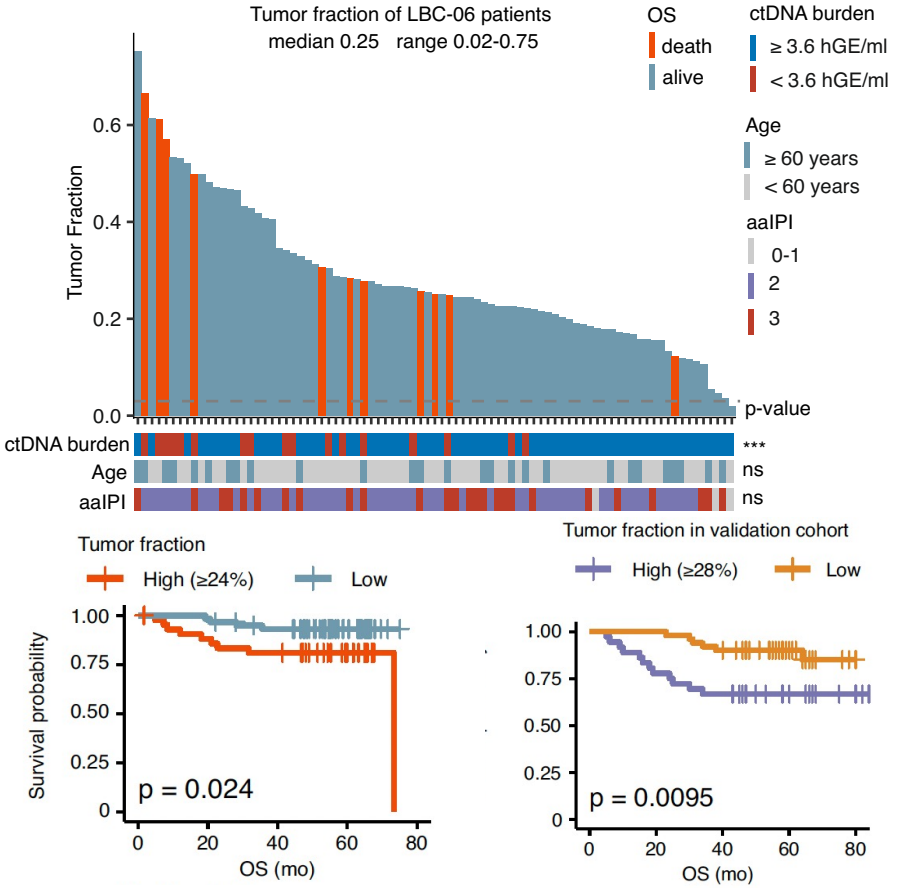
COPY NUMBER ABERRATIONS IN THE ctDNA







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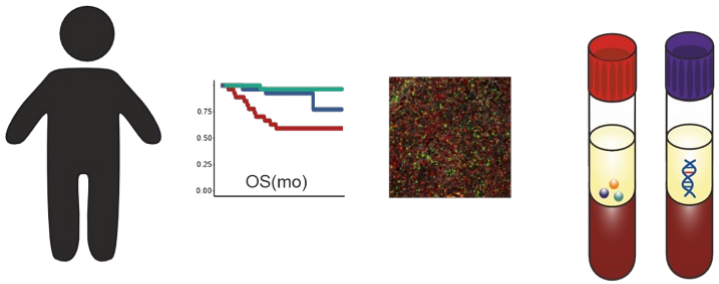
HOST RESPONSE/SERUM PROTEOMICS

LBC-05 trial

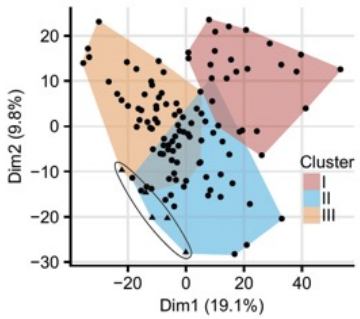
109 patients <65 y, high risk (aaIPI 2-3) LBCL

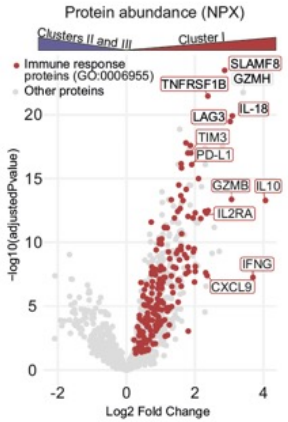
Olink proximity extension assay (1463 proteins)

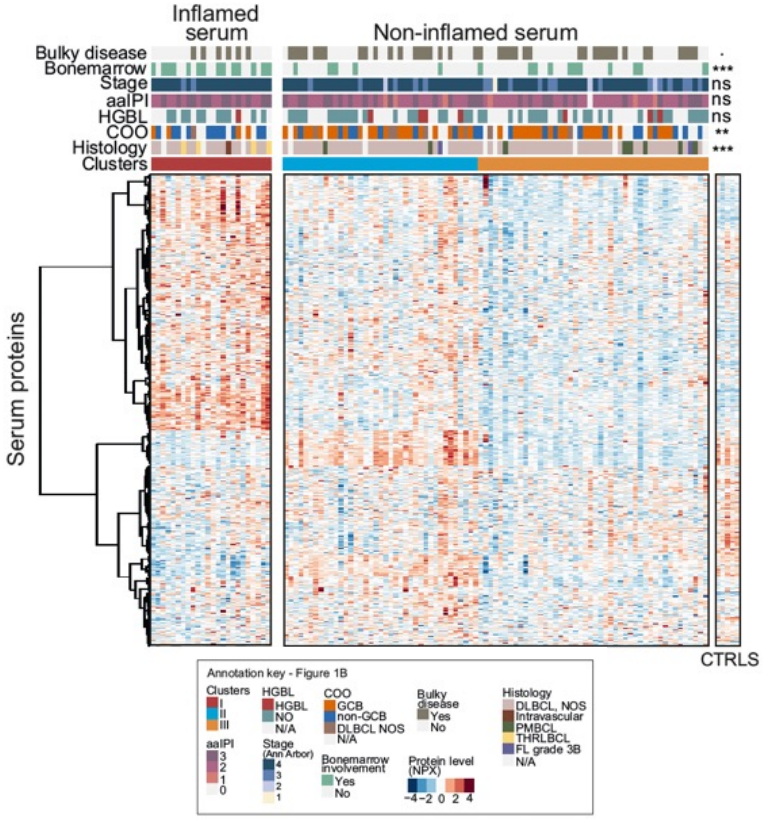
Correlation with TME and ctDNA



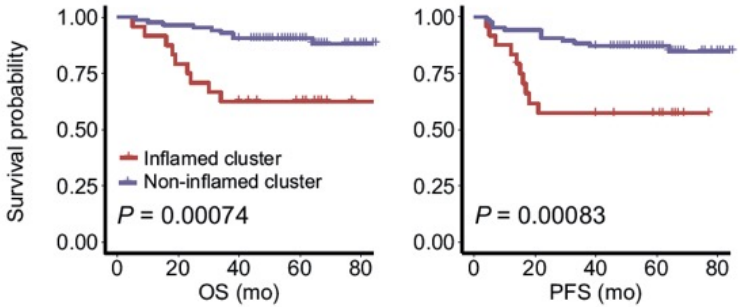
SERUM PROTEOME LANDSCAPE

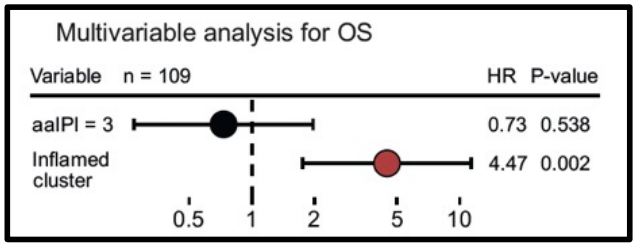




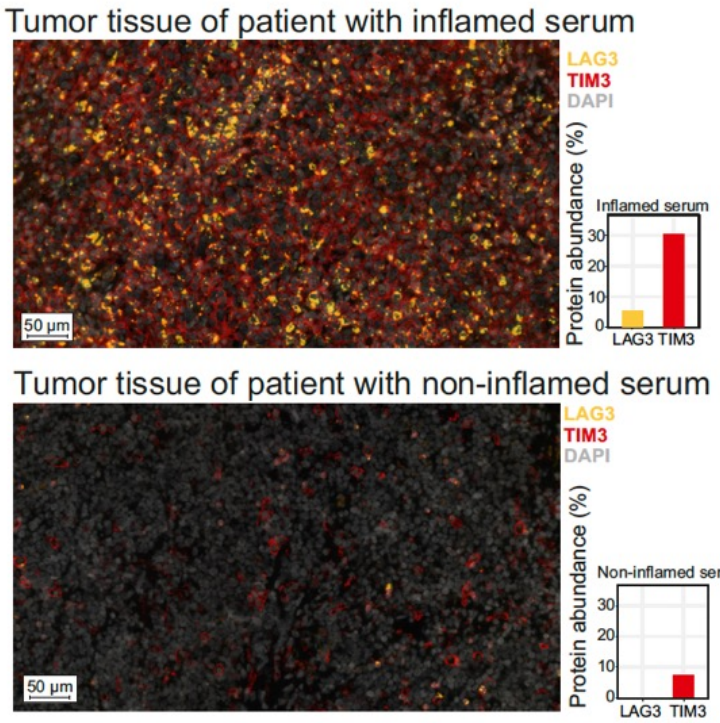


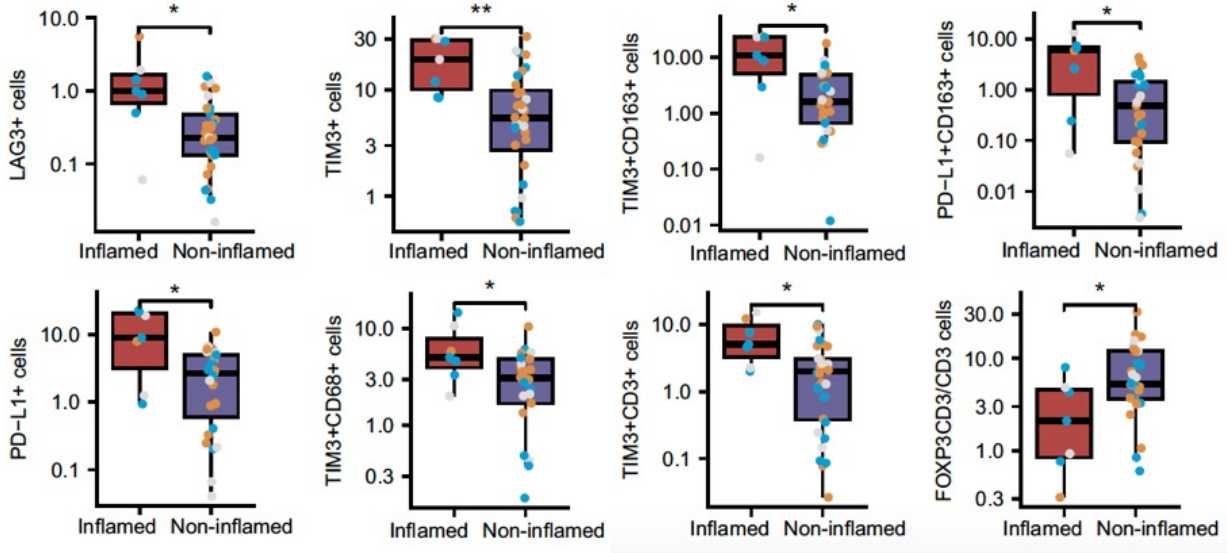
Inflamed cluster enriched in patients with
- *TCRBCL*
- *Non GCB DLBCL*
- *B-symptoms*
- *Bone marrow+*





INFLAMMATION SIGNATURE IN THE SERUM CORRELATES WITH TME

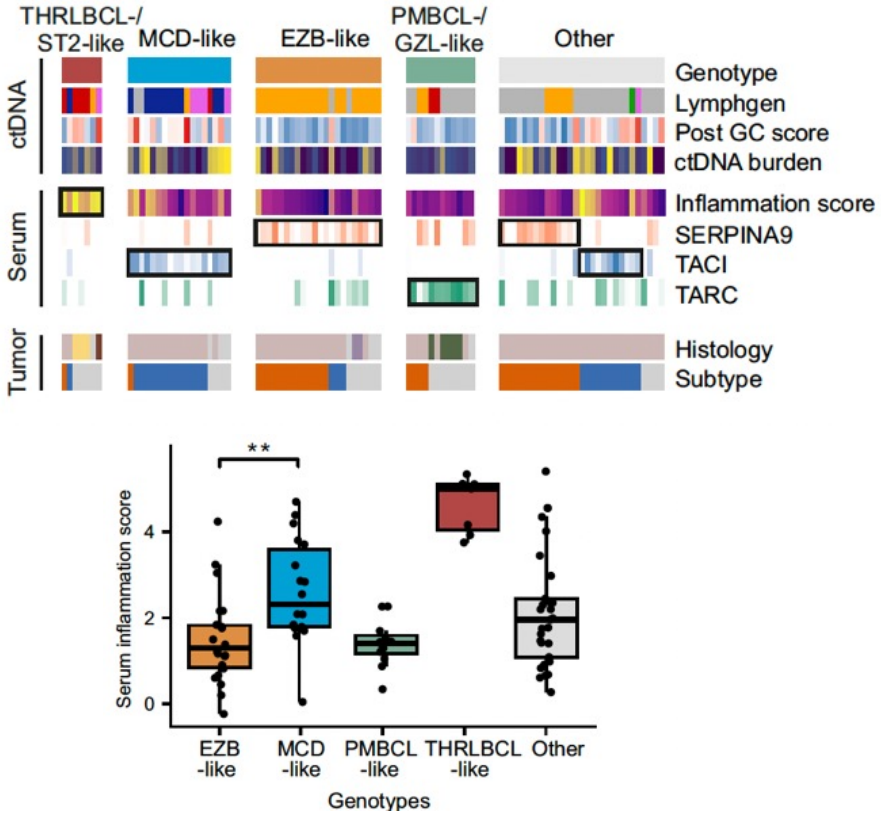


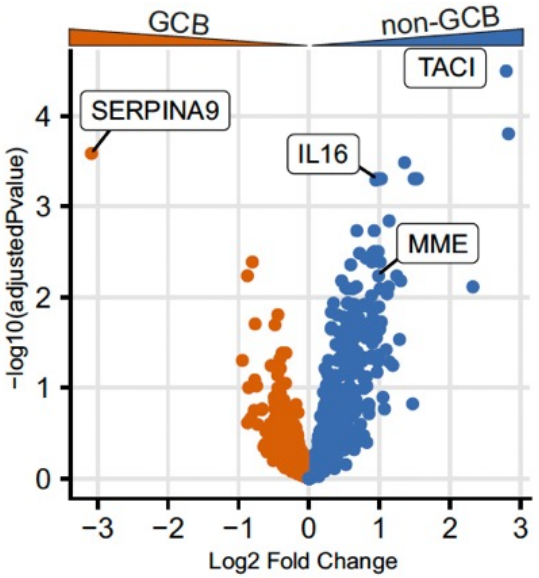


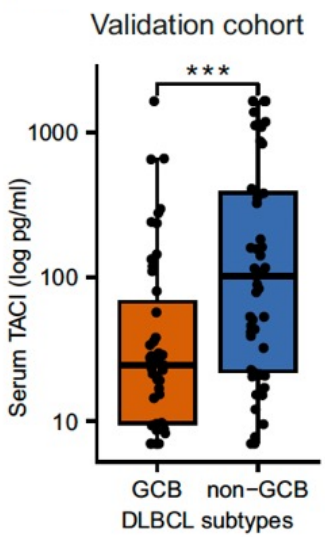
HUS & HELSINGIN YLIOPISTO YHTEISTYÖSSÄ
HUS & HELSINGFORS UNIVERSITET I SAMARBETE
A COLLABORATION BETWEEN HUS & UNIVERSITY OF HELSINKI

Arffman et al., 2024

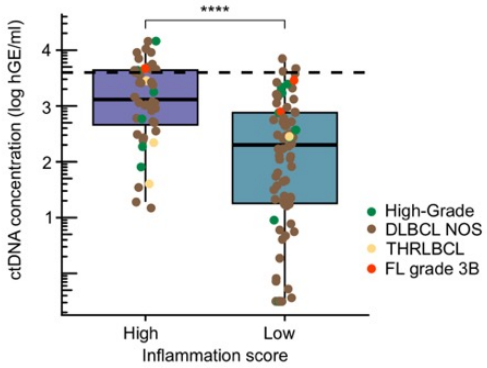
MULTIPLE SERUM PROTEINS CORRELATE WITH LBCL SUBTYPES







JOINT ANALYSIS OF INFLAMMATION AND ctDNA



Combined groups

Inflammation score & ctDNA burden

High & High	High & Low
Low & High	Low & Low

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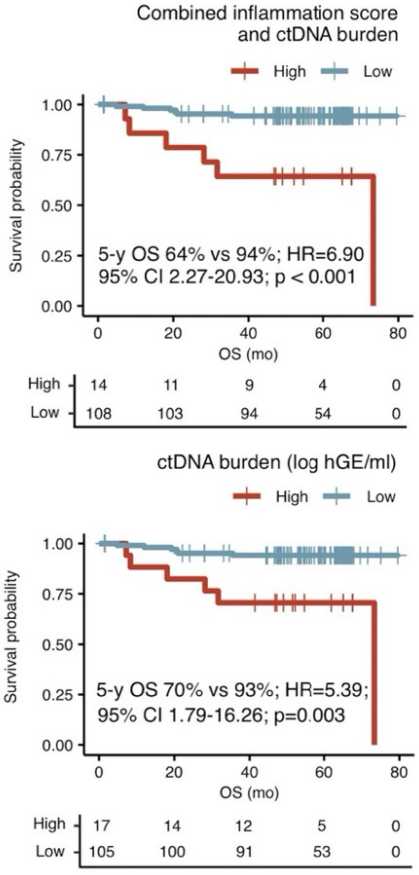
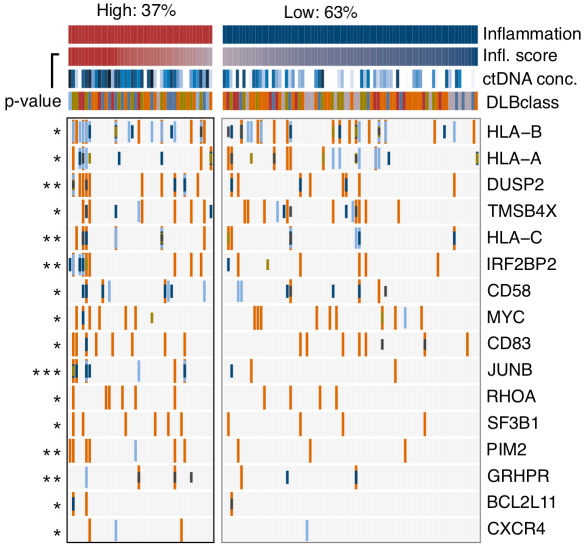
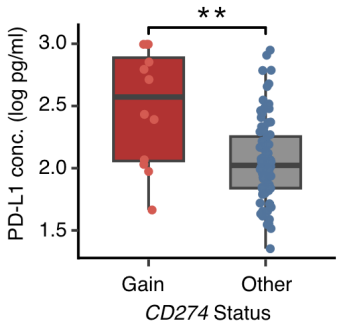






CONCLUSIONS

DLBCL includes a heterogeneous collection of biologically distinct tumors

Genomic profiling from tumor tissue and blood resolve biological heterogeneity into clinically meaningful subgroups

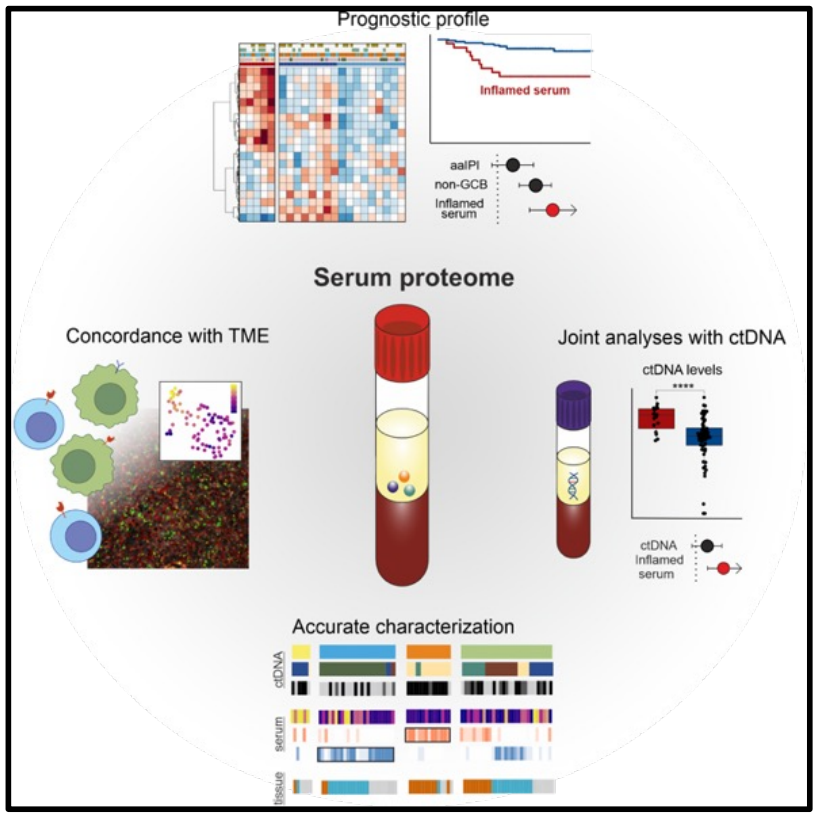
Serum protein profiling can capture a crucial extra element of lymphoma biology beyond genomic subtypes

Inflamed serum protein profile

- reflects host response to lymphoma
- correlates with ctDNA characteristics
- translates to poor outcome

Joint analyses of serum proteome and ctDNA

- reveal facets of lymphoma, which have potential in diagnostics and baseline patient characterization
- improve survival estimates



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Patients

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FIMM
Teijo Pellinen
Pekka Ellonen
Esa Pitkänen

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