Settima edizione di

AIEOP...

...in Lab

G.d.L. Biologia Cellulare Molecolare AIEOP
ASSOCIAZIONE ITALIANA EMATOLOGIA ONCOLOGIA PEDIATRICA

Beyond tissue biopsy: circulating biomarkers in pediatric lymphomas

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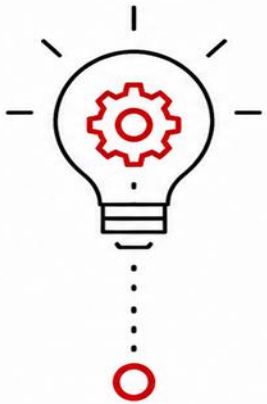
Fondazione ISTITUTO DI RICERCA PEDIATRICA

Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
None							

MIT Technology Review

Published by MIT



10 BREAKTHROUGH TECHNOLOGIES

Our annual list of emerging technologies
that have the potential to change the world.

MIT Technology Review
Published by MIT

Top 10 Breakthrough Technologies 2015

Our annual list of technologies
that have the potential to change the world

1	Magic Leap
2	Nano-architecture
3	Car-to-car communication
4	Apple Pay
5	Virtual reality
6	Smart advisers
7	Advanced robotics
8	Brain organoids
9	Nuclear fusion
10	**Liquid Biopsy**

A simple blood test that can
detect cancer and monitor treatment
by analyzing tumor DNA
circulating in the blood.

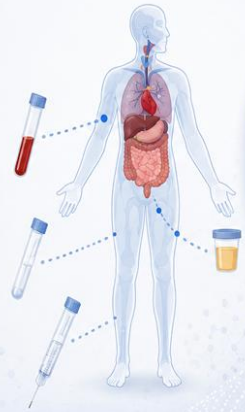
It was the beginning of a new era in oncology...

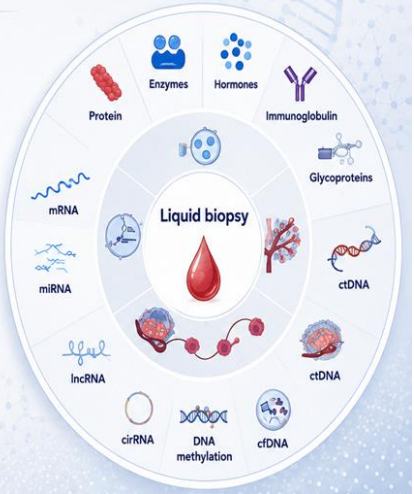
LIQUID BIOPSY

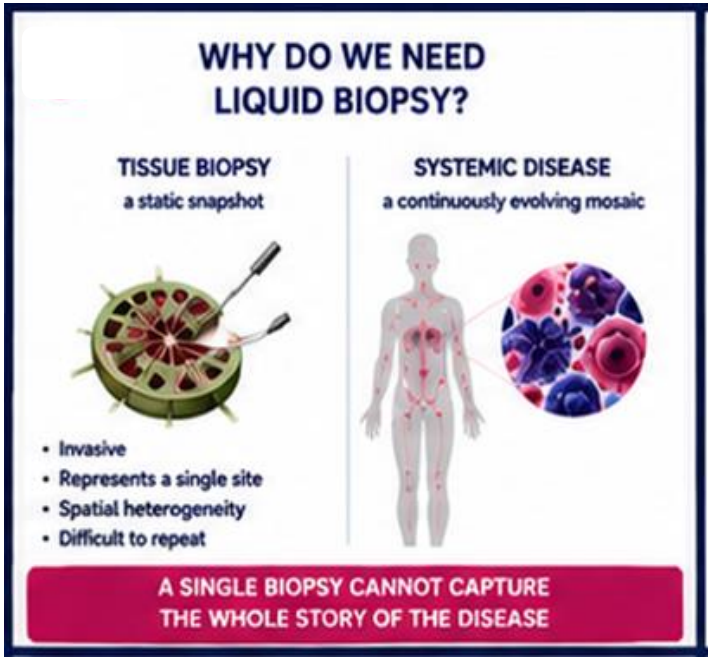
Non-invasive tests that detect tumor biomarkers that are shed into the body fluid from the tumor.

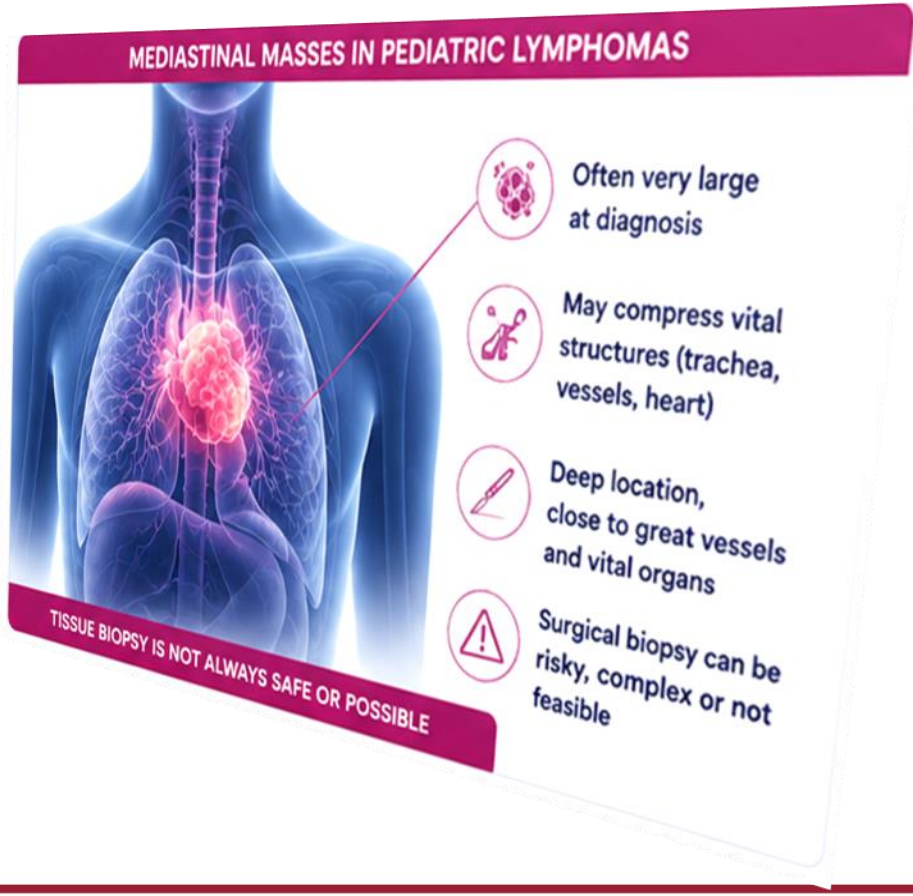
SAMPLES INCLUDED

- ✓ Blood
- ✓ Saliva
- ✓ Urine
- ✓ CSF

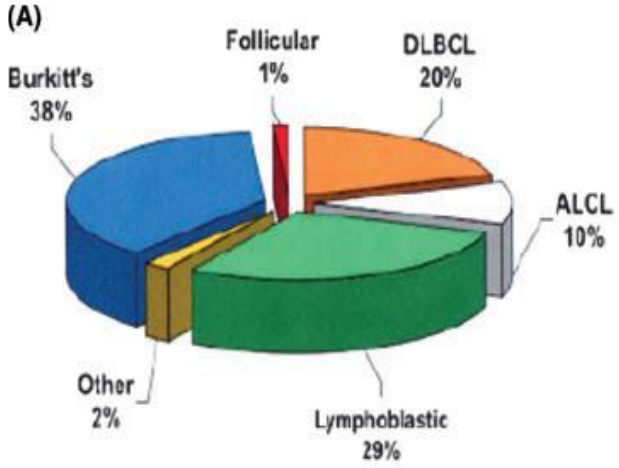




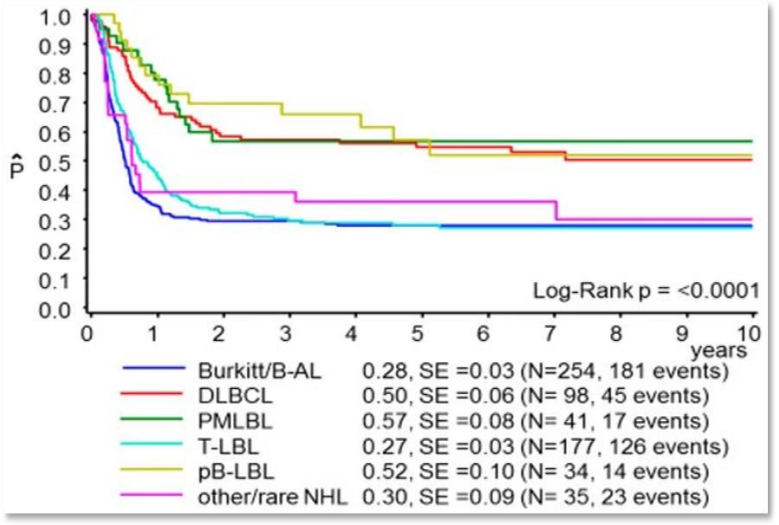

WHY DO WE NEED LIQUID BIOPSY?
TISSUE BIOPSY
a static snapshot
SYSTEMIC DISEASE
a continuously evolving mosaic
• Invasive
• Represents a single site
• Spatial heterogeneity
• Difficult to repeat
A SINGLE BIOPSY CANNOT CAPTURE THE WHOLE STORY OF THE DISEASE


MEDIASTINAL MASSES IN PEDIATRIC LYMPHOMAS
Often very large at diagnosis
May compress vital structures (trachea, vessels, heart)
Deep location, close to great vessels and vital organs
Surgical biopsy can be risky, complex or not feasible
TISSUE BIOPSY IS NOT ALWAYS SAFE OR POSSIBLE

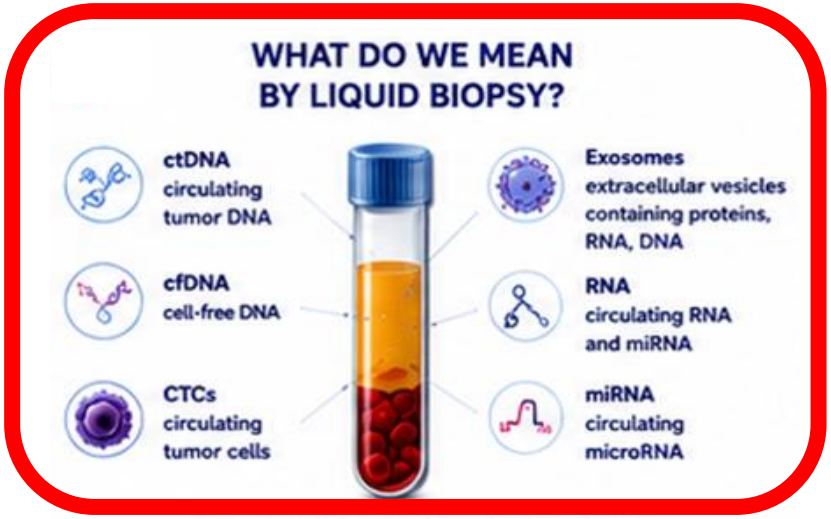
Non-Hodgkin Lymphomas: Heterogeneous Diseases with Poor Prognosis After Relapse



Survival in refractory/relapsed pediatric NHL



Burkhardt B, *et al.* Cancers (**2022**)



ALCL: THE BEST MODEL

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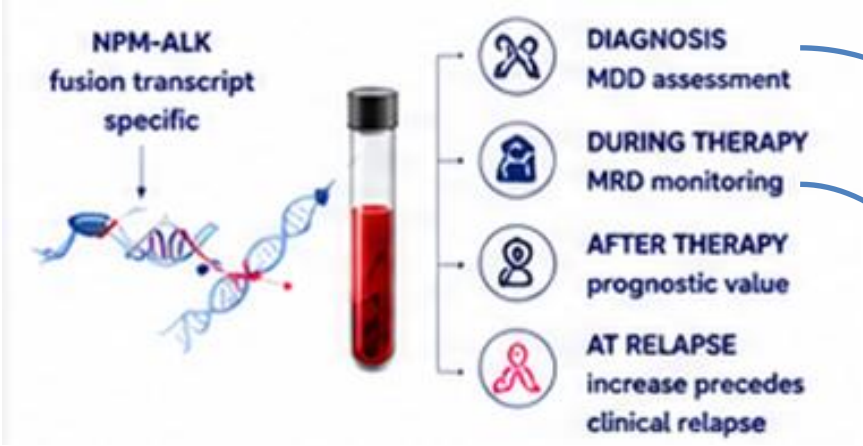
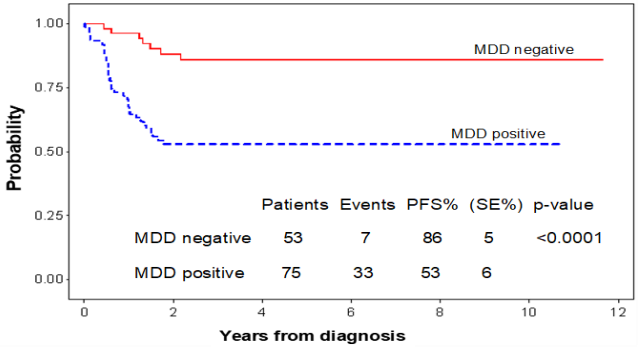
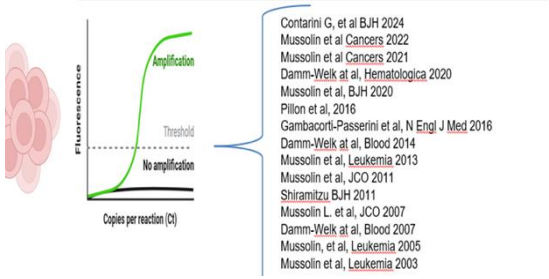
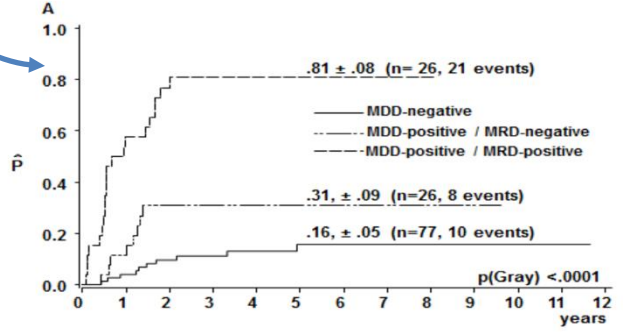






Contarini G, et al BJH 2024
Mussolin et al Cancers 2022
Mussolin et al Cancers 2021
Damm-Welk at al, Hematologica 2020
Mussolin et al, BJH 2020
Pillon et al, 2016
Gambacorti-Passerini et al, N Engl J Med 2016
Damm-Welk at al, Blood 2014
Mussolin et al, Leukemia 2013
Mussolin et al, JCO 2011
Shiramitzu BJH 2011
Mussolin L. et al, JCO 2007
Damm-Welk at al, Blood 2007
Mussolin, et al, Leukemia 2005
Mussolin et al, Leukemia 2003



Article

Prognostic Factors in Childhood Anaplastic Large Cell Lymphoma: Long Term Results of the International ALCL99 Trial

420 patients

Cox-Regression

Variable	relative risk of relapse	95% CI	P
MDD + low ALK a.t.	4.9	2.4-9.8	<0.0001
Histology not common	3.7	1.54-8.7	0.003
Clinical risk factors	1.1	0.28-4.33	0.9
MRD	6.0	2.01-17.92	0.001

eicnhl
European Inter-Group for Childhood Non-Hodgkin Lymphoma

ASSOCIAZIONE ITALIANA DI EMATOLOGIA ED ONCOLOGIA PEDIATRICA
A.I.E.O.P.

AIEOP "ALCL 99"

International protocol for the treatment of childhood anaplastic large cell lymphoma

Based on ..tumor biopsy

ALCL 2024

International cooperative study for patients with anaplastic large cell lymphoma (ALCL)

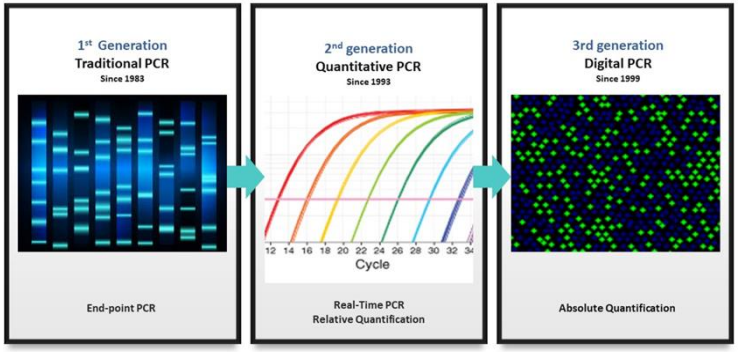
Epidemiology, Biology and Outcome of ALCL in children and adolescents

and

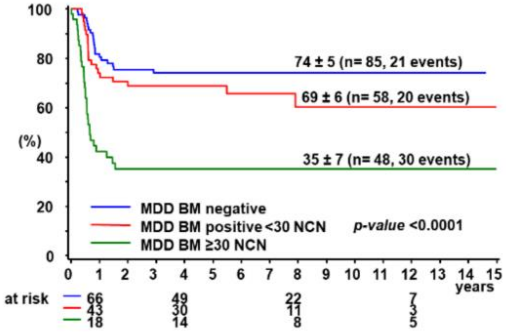
Randomized trial on the efficacy of vinblastine monotherapy for children, adolescents and adults with standard risk (SR) ALK-positive ALCL

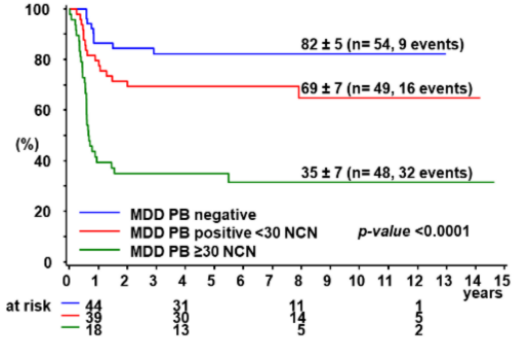
Based on ..liquid biopsy

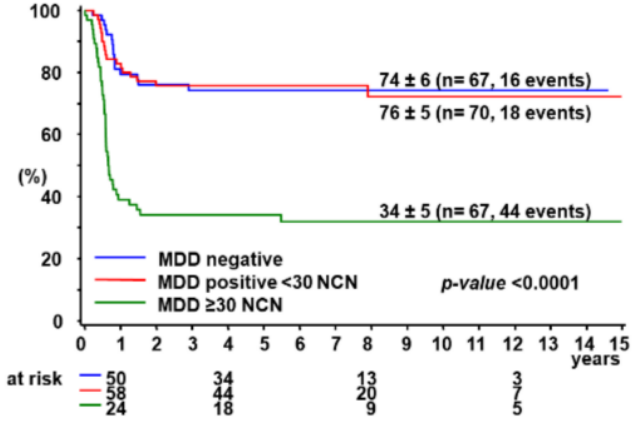
Damm-Welk et *al Front Biosc* 2015 ; Damm-Welk, Mussolin et al , *Blood* 2014; Mussolin et al , *leukemia* 2013; Mussolin et al , *leukemia* 2009



Minimal disseminated diseased ≥ 30 NCN in BM and or PB identifies patients at higher risk of treatment failure



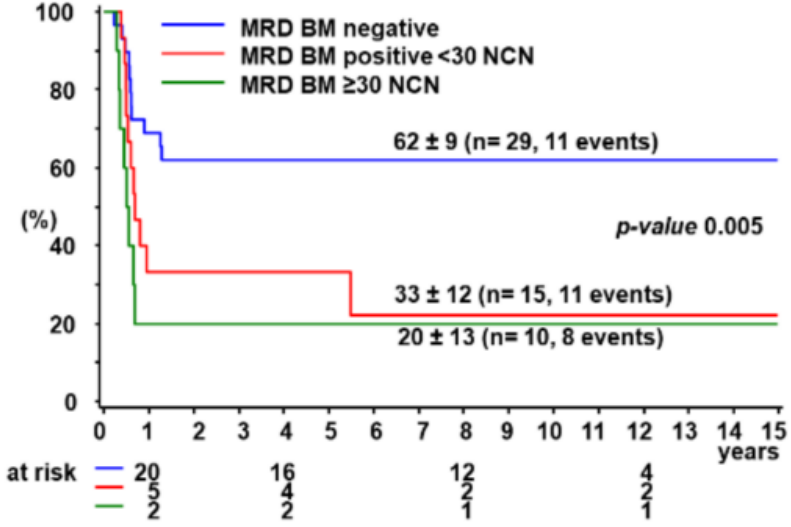


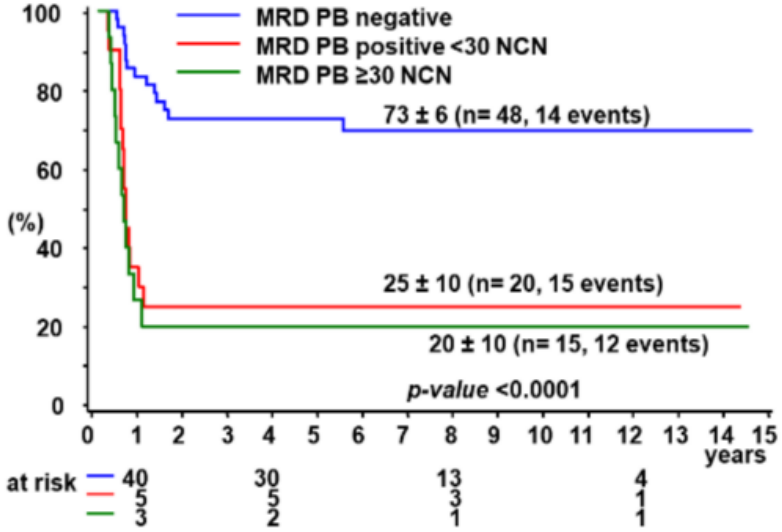


BM and/or PB

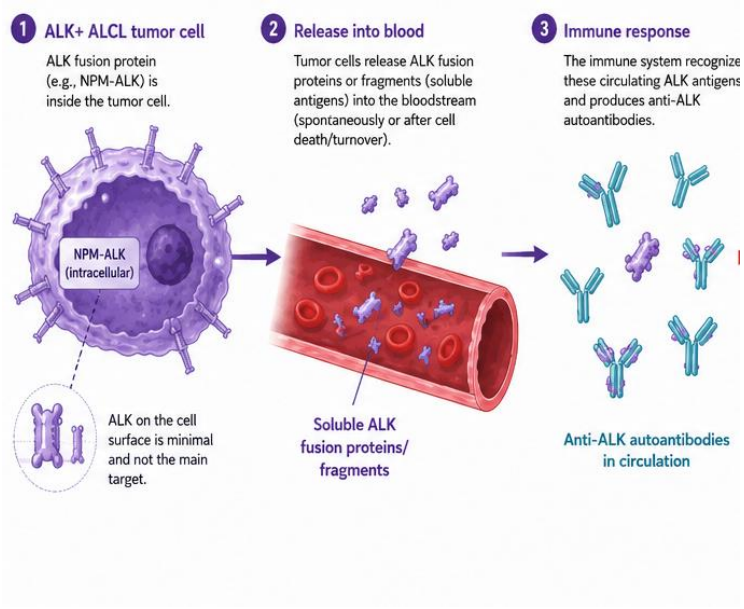
MDD quantification improves stratification!

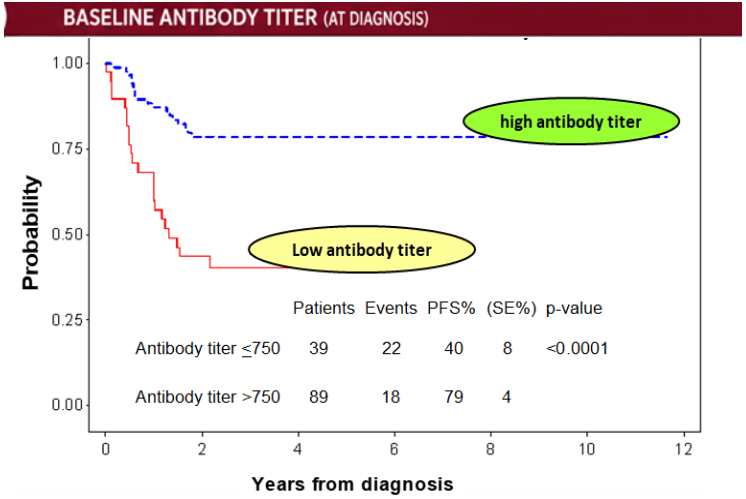
Any level of minimal residual diseased in BM and or PB identifies patients at higher risk of treatment failure

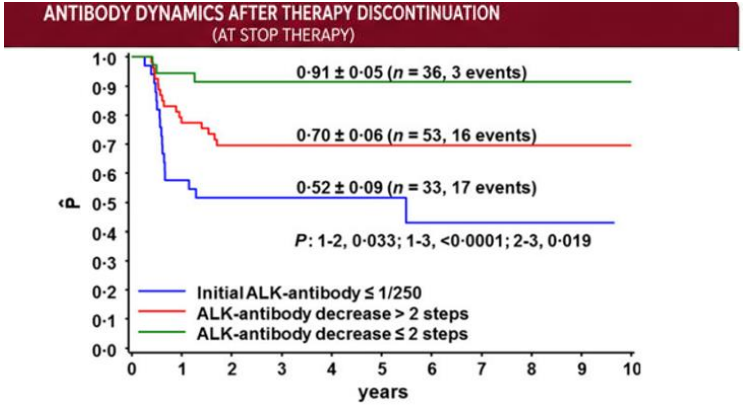




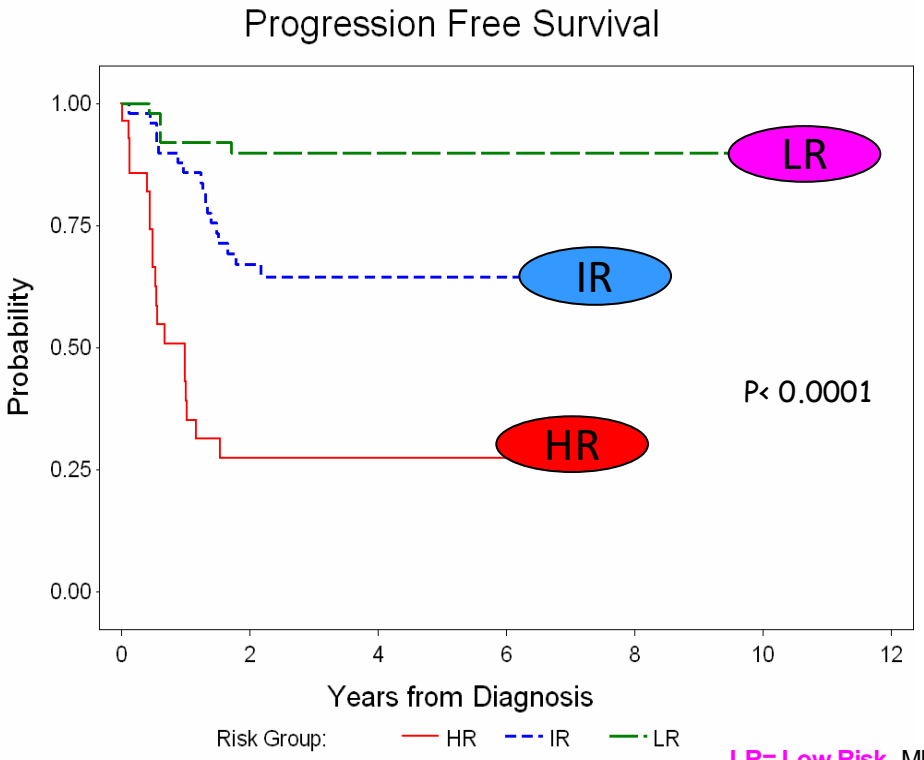
Anti-ALK Antibody Titers as a Prognostic Biomarker

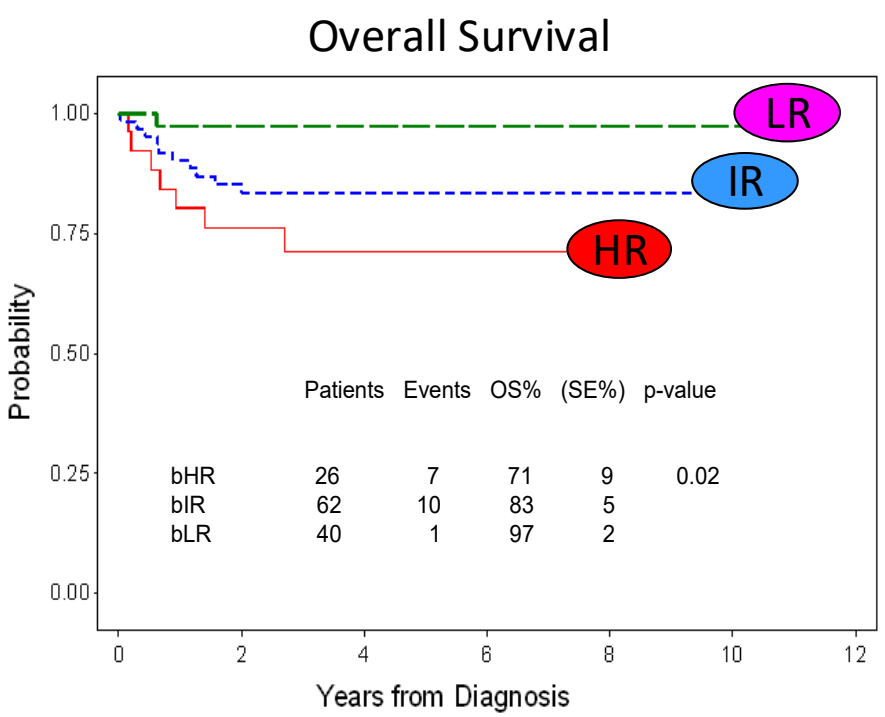






Mussolin L et al, Leukemia 2013, Mussolin L et al, BJH 2018





LR= Low Risk, MDD neg and high antibody titer anti ALK

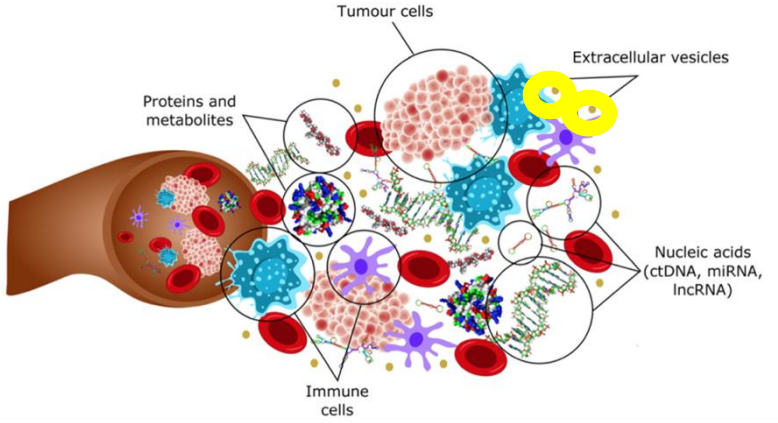
IR= Intermediate Risk, MDD pos and high antibody titer or MDD neg and low antibody titer

HR= High Risk, MDD pos and low antibody titer anti ALK

Table 1. Main characteristics of the study population: prognostic analysis of the clinical and molecular features

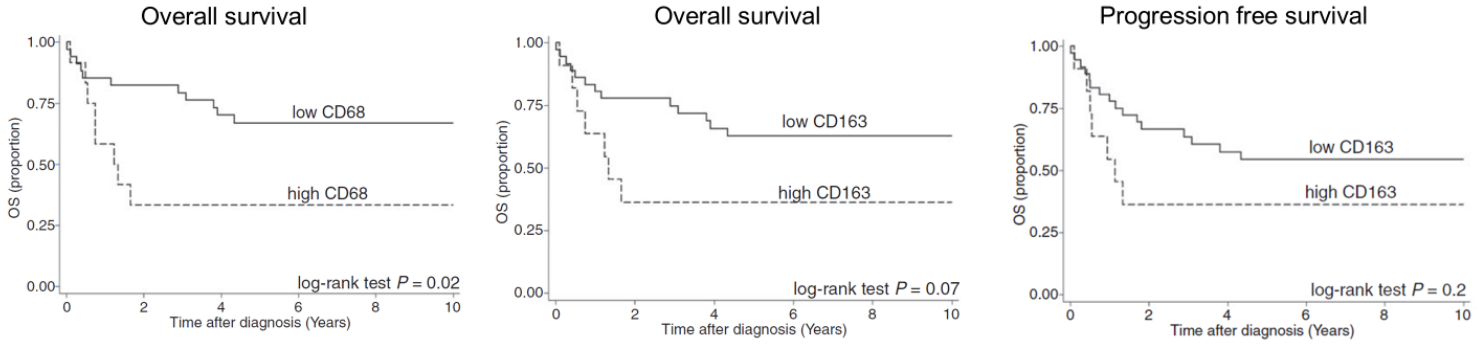
Characteristic	No. of patients	No. of events	5-year PFS		Univariate P-value	HR	95% CI	Multivariate P-value	HR	95% CI
			%	s.e. %						
Sex										
Male	85	26	68	5	0.93					
Female	43	14	67	7						
Median age (years)										
⩽11.0	64	19	69	6	0.65					
>11.0	64	21	67	6						
BM morphological involvement										
No	117	33	71	4	0.0004	3.9	1.7–9.0	0.06		
Yes	11	7	22	14						
CNS involvement[a]										
No	119	36	69	4	0.53					
Yes	5	2	60	22						
Common type histology[a]										
Yes	60	12	80	5	0.0003	3.4	1.7–6.9	0.006	2.70	1.31–5.6
No	42	22	46	8						
CD3[a]										
Negative	63	19	70	6	0.30					
Positive	28	11	59	9						
MDD										
Negative	53	7	87	5	<0.0001	4.5	2.0–10.2	0.58		
Positive	75	33	54	6						

Mussolin L et al , Cancer 2020



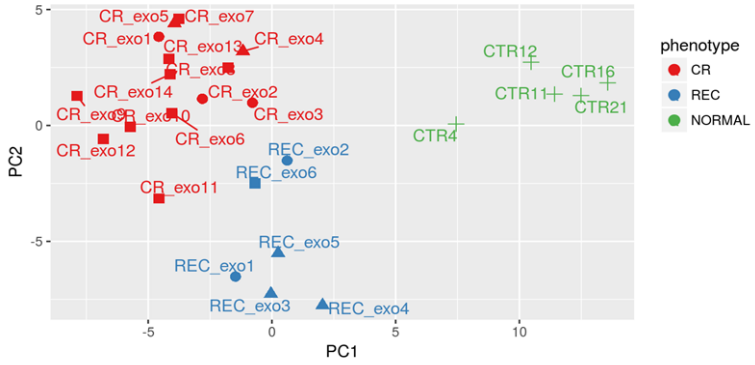
Molecular Cancer, 2018

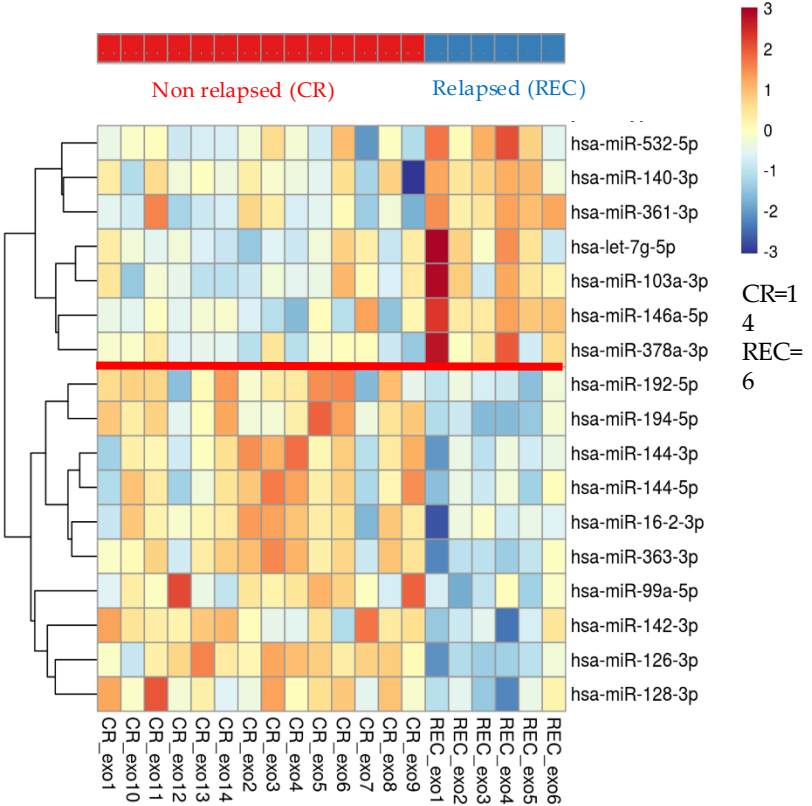
High intratumoral macrophage content correlates with worse prognosis in **adult ALCL**



Overall survival (OS) and progression free survival for ALCL (38 ALK-, 14 ALK+) according to CD68 and CD163 expression.
Pedersen, et al., DOI: 10.1111/his.12407

Relapsed patients have a peculiar EVs signature compared to complete remission ones in ALCL





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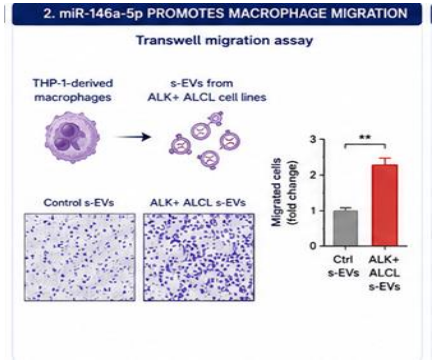
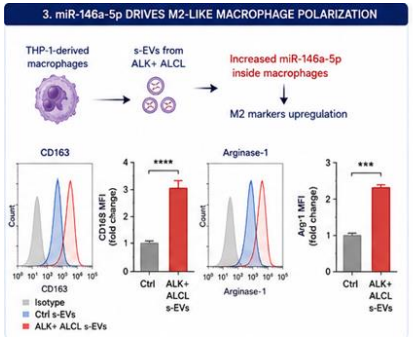
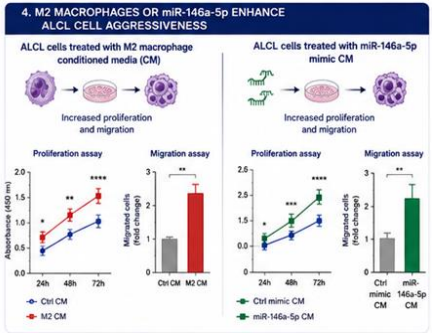
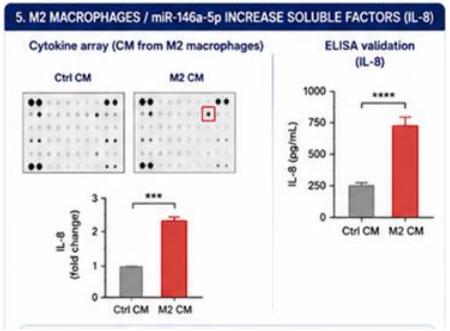
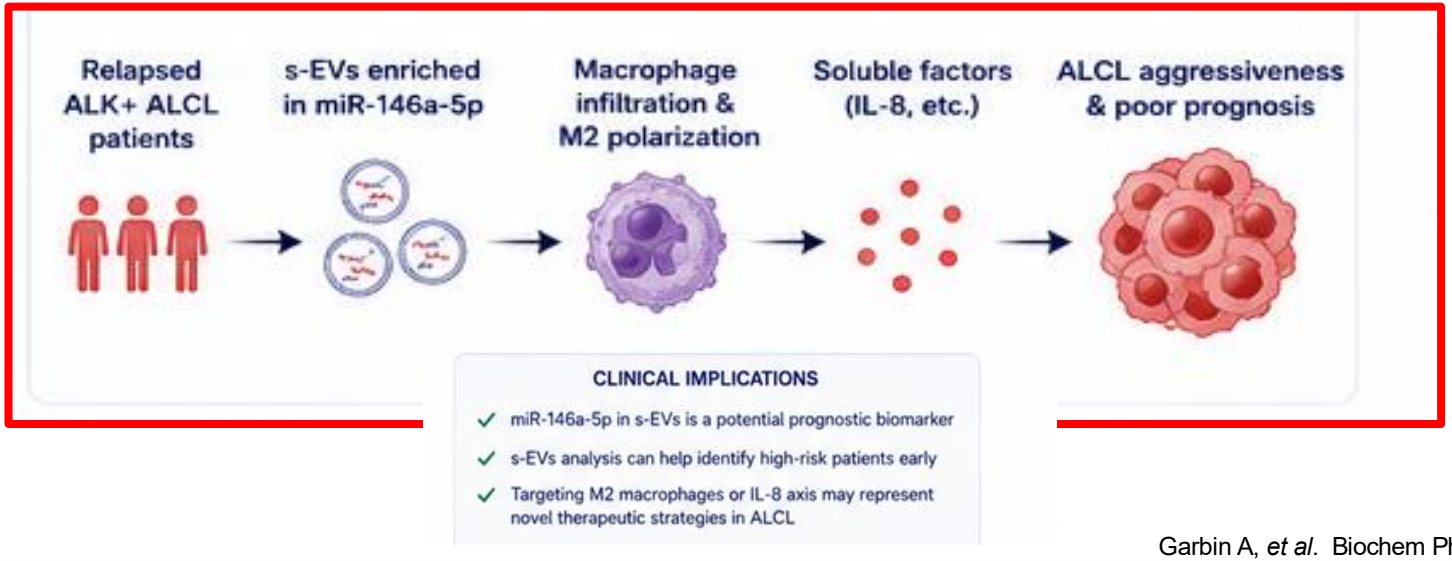
REC=6





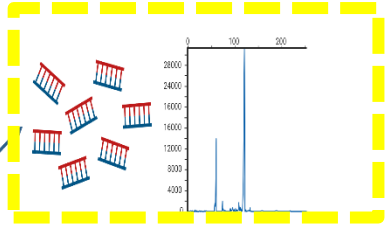




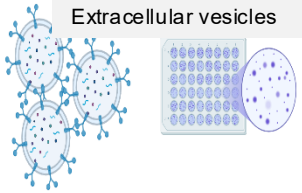


Garbin A, *et al*. Biochem Pharmacol 2023

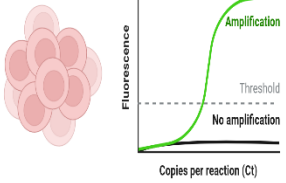
Cell free DNA- cell tumor DNA

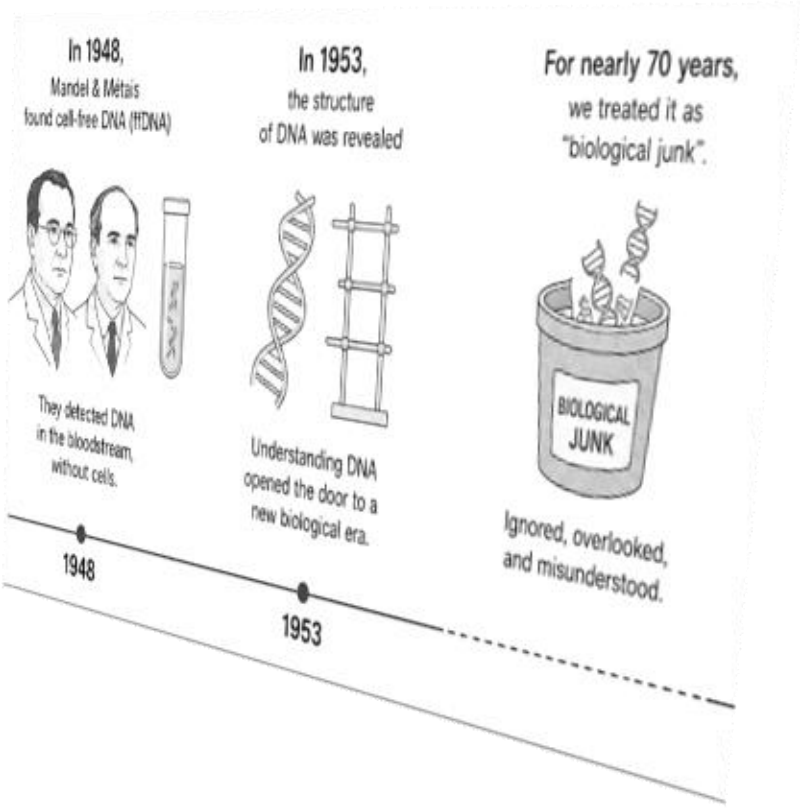


Extracellular vesicles

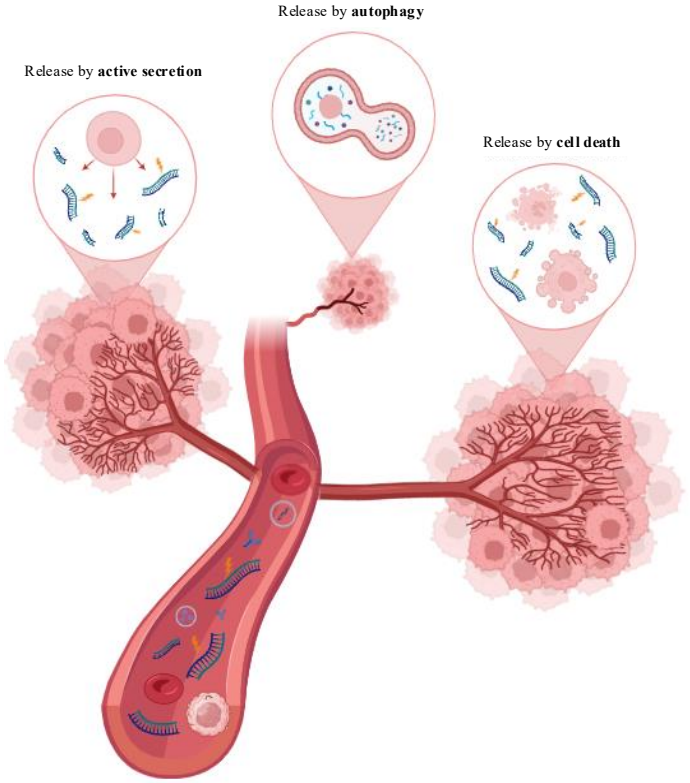


Minimal Disseminated Disease – Minimal Residual Disease

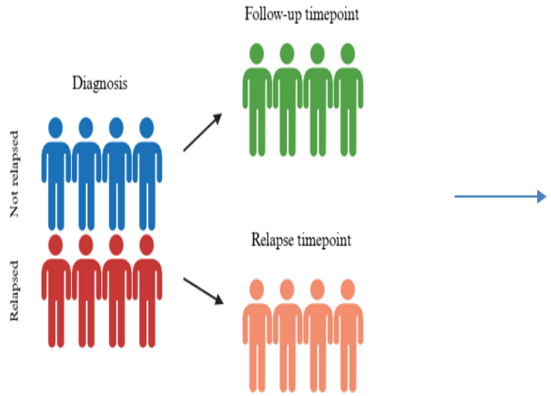


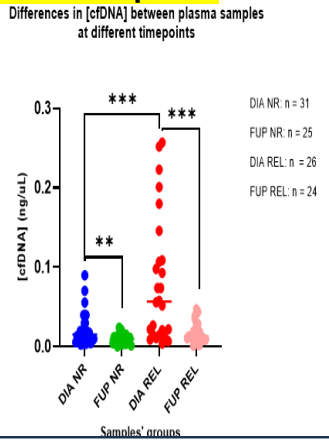


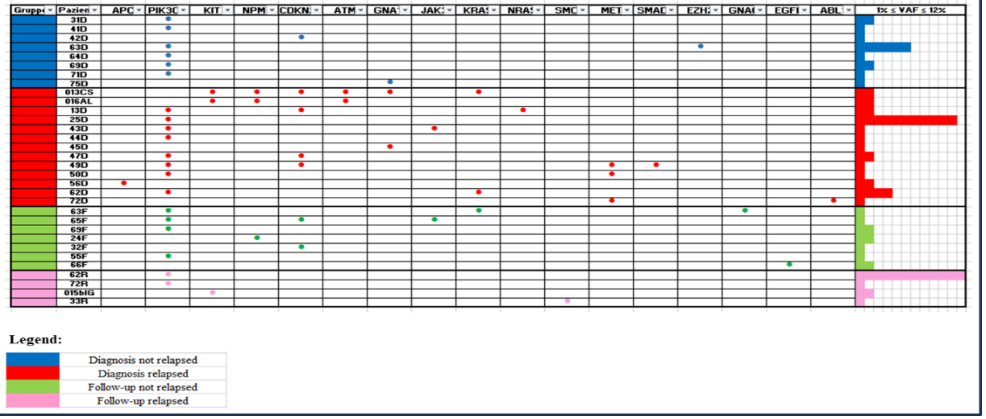
Mechanisms of release of cfDNA into the bloodstream

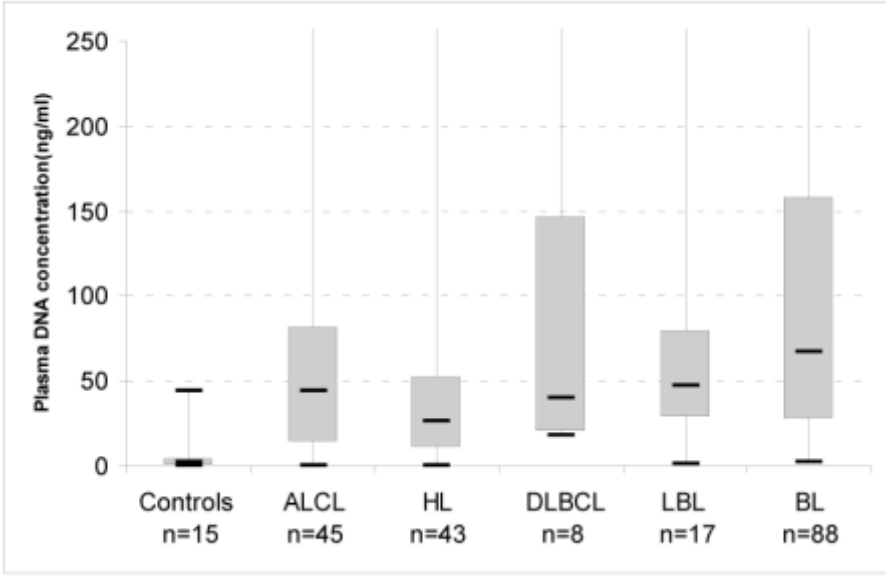


106 ALCL plasma samples









nature medicine

Article

https://doi.org/10.1038/s41591-026-04291-z

Liquid biopsy for the diagnosis of EBV-positive Burkitt's lymphoma in endemic areas

Received: 4 June 2025

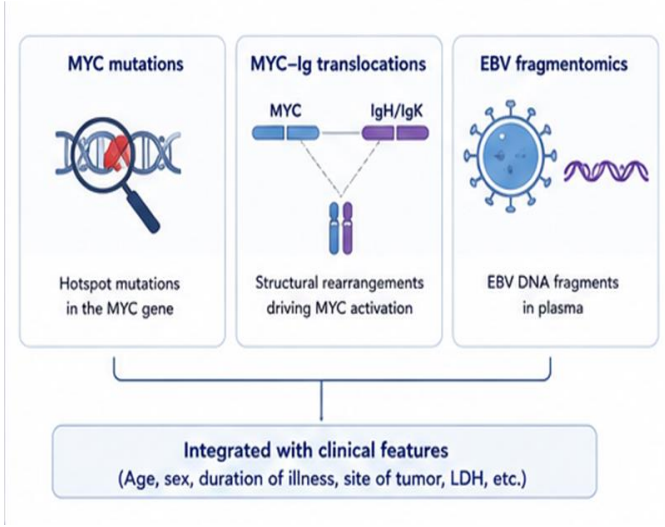
Accepted: 13 February 2026

Published online: 19 March 2026

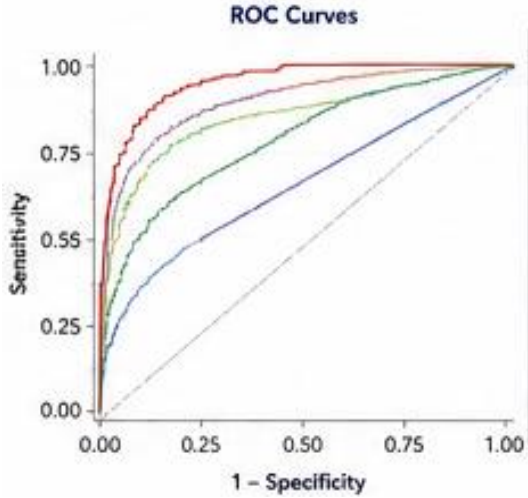
Table 2 | BL diagnostic models

Model name	Variables
Clinical model	Age, sex, duration of presenting illness, site of tumor and LDH
EBV quantitative model	*EBER1*, *EBER2*, *EBNA2*, EBV_{max} (maximum EBV copies per cell)
EBV quantitative + clinical model	Age, sex, duration of presenting illness, site of tumor, LDH, *EBER1*, *EBER2*, *EBNA2*, EBV_{max}
EBV model	*EBER1*, *EBER2*, *EBNA2*, EBV_{max}, EBV size ratio, EBVP, EBV entropy
Liquid biopsy model	CtDNA, median VAF, *MYC* intron 1 mutations, *MYC* exon 2 mutations, *EBER1*, *EBER2*, *EBNA2*, EBV_{max}, EBV size ratio, EBP, EBV entropy, autosomal entropy and *MYC*–Ig translocation
Comprehensive model	Age, sex, duration of presenting illness, site of tumor, LDH, ctDNA, median VAF, *MYC* intron 1 mutations, *MYC* exon 2 mutations, *EBER1*, *EBER2*, *EBNA2*, EBV_{max}, EBV size ratio, EBVP, EBV entropy, autosomal entropy and *MYC*–Ig translocation

Circulating Tumor DNA Markers in Plasma



Model Performance (Training Cohort, n = 212)

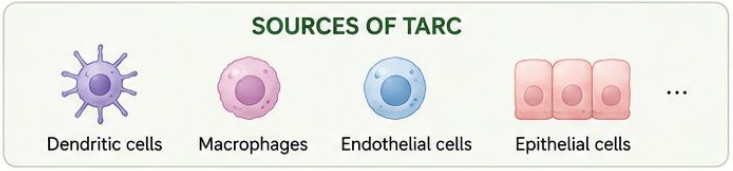


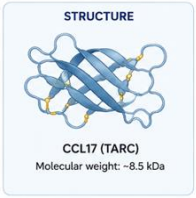
Model	AUC (95% CI)
Clinical model	0.73 (0.66 – 0.80)
EBV quantitative model	0.86 (0.80 – 0.91)
EBV quant. + clinical	0.88 (0.83 – 0.93)
EBV / MYC model	0.90 (0.85 – 0.95)
Liquid biopsy model (ctDNA)	0.92 (0.87 – 0.96)
Comprehensive model	**0.95 (0.90 – 0.98)**

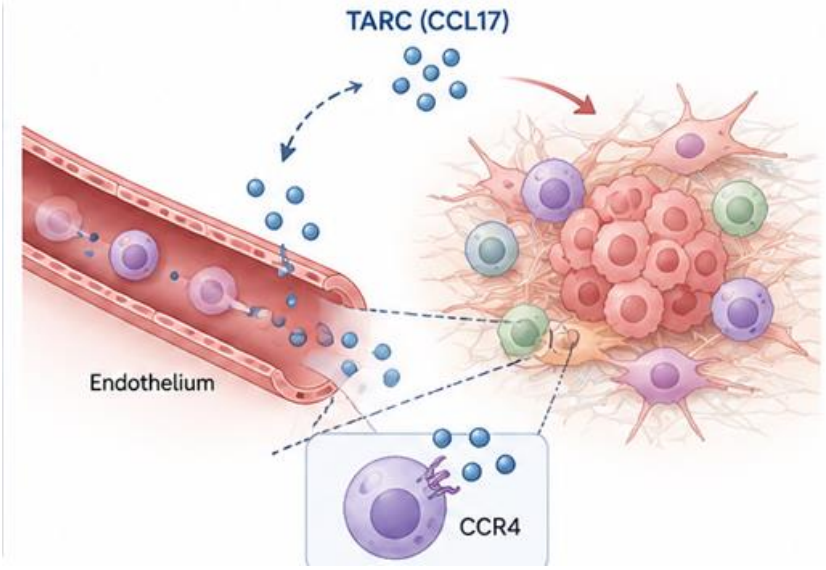
TARC (CCL17)

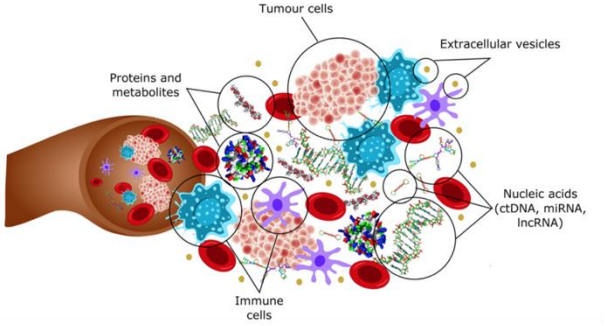
Structure, Role and Clinical Relevance

TARC is a CC chemokine involved in immune cell trafficking, inflammation and cancer.









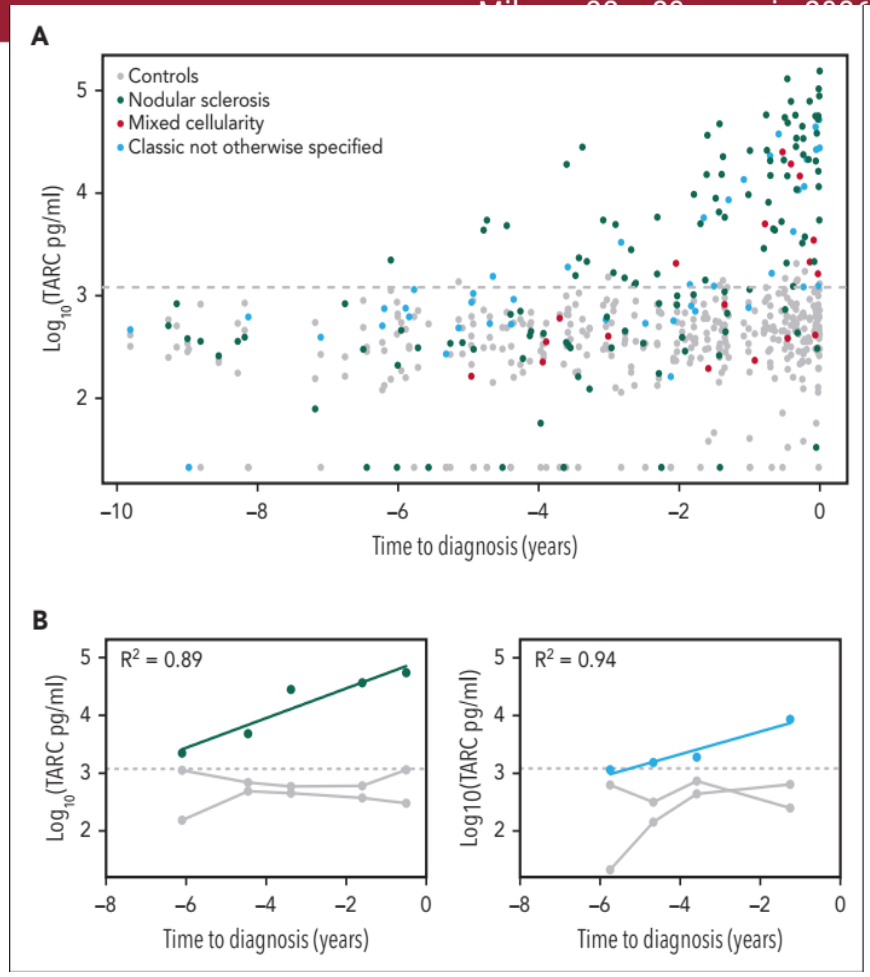
Molecular Cancer, 2018

blood
Letters to *Blood*

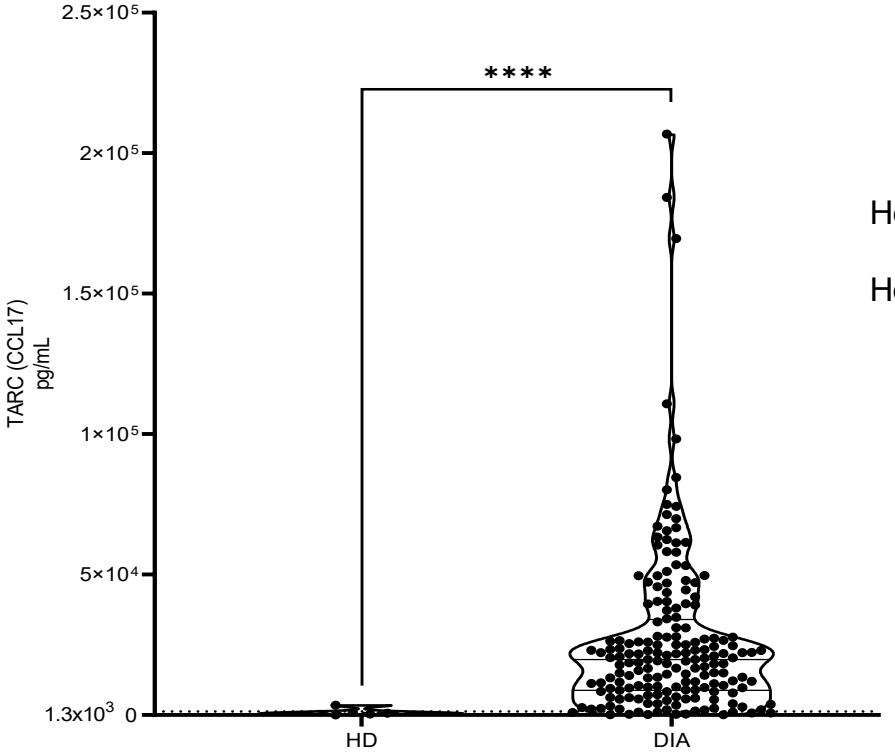
TO THE EDITOR:

Elevated serum TARC levels precede classic Hodgkin lymphoma diagnosis by several years

Arjan Diepstra,[1] Ilja M. Nolte,[2] Anke van den Berg,[1] Larry I. Magpantay,[3] Otoniel Martínez-Maza,[3] and Lynn I. Levin[4]



AIEOP cohort (176 pts) - TARC level at diagnosis



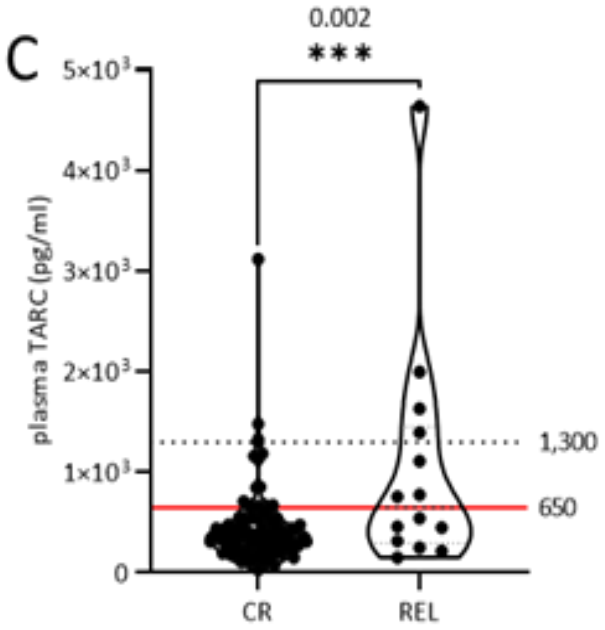
Unpaired t-test, two-tailed, p <0.0001

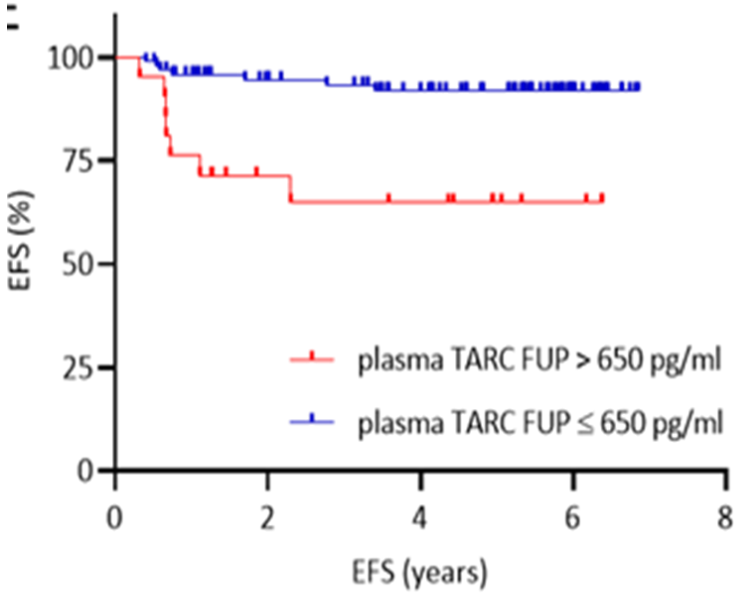
Healthy donors (HD): median value 685 pg/mL

Hodgkin Lymphoma cases: median value 19.700 pg/mL

paper in submission..

Prognostic Value of TARC After 2 Chemotherapy Cycles





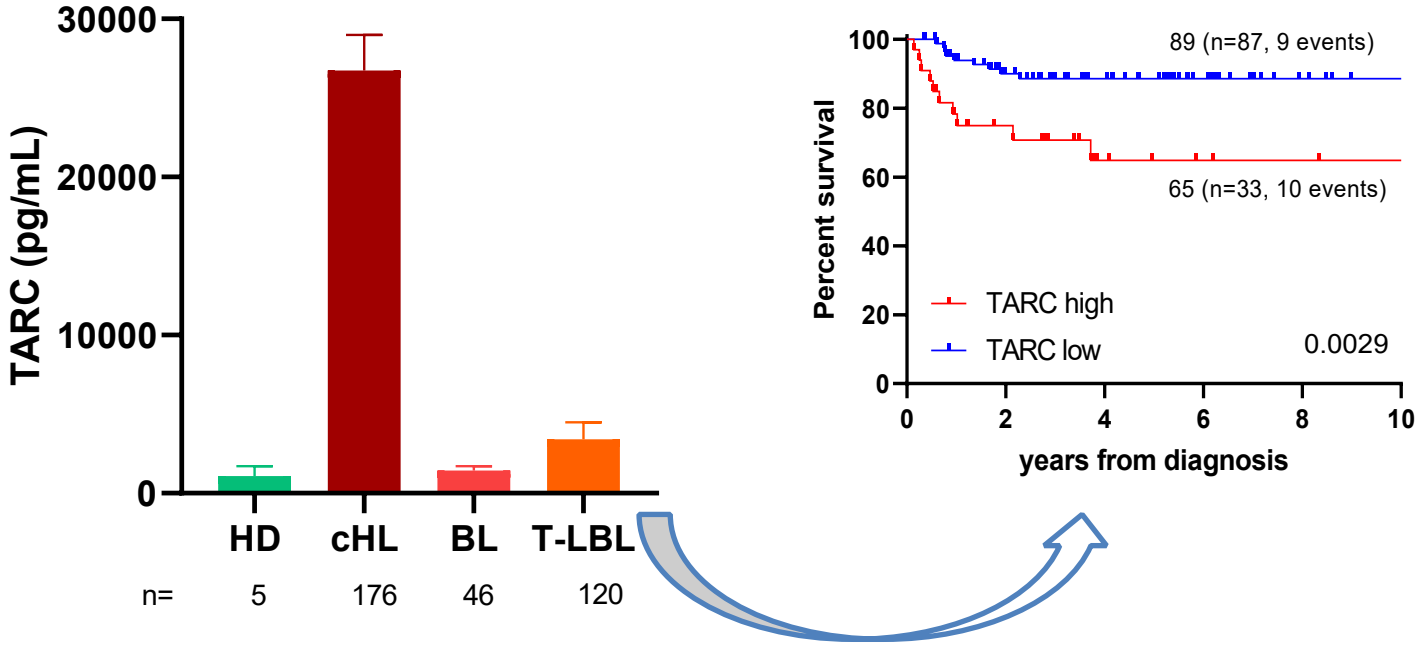
Characteristics		Number of patients	Events	5 years EFS %	SE%	p-value
Cutoff 650	<650	95	7	92	3	**0,0005**
	>650	21	7	65	11	

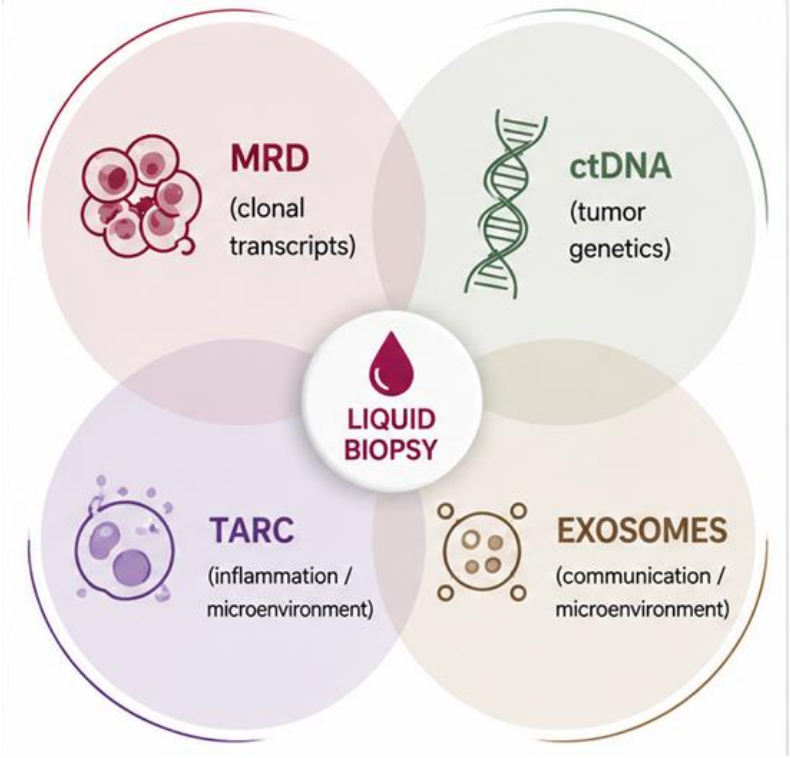
Multivariate Analysis: TARC After 2 Cycles as Main Prognostic Factor

Characteristics		Patients	Events	Univariate EFS (%)	SE	p-value	Multivariate p-value	HR (95% CI)
TARC at II CT (*15)	< 757.4 pg/ml	125	7	94	2	<0.0001	0.0004	6.1 (2.2-16.5)
	≥ 757.4 pg/ml	28	10	59	10			
Bulky disease (≥ 200 ml)	No	110	6	92	3	0.0006	0.015	4.1 (1.3-12.8)
	Yes	58	14	75	6			
Symptoms	A	101	6	93	3	0.0024	ns	
	B	67	14	78	5			
Treatment level	1+2	101 (26+75)	8	91	3	0.0366	ns	
	3	67	12	81	5			
ERA-PET (*4)	AR	118	10	90	3	0.0146	ns	
	IR	46	10	78	6			

paper in submission..

Plasma TARC levels in pediatric Hodgkin and non-Hodgkin Lymphomas at diagnosis





Searching for a Shared Biomarker....

- Primary Mediastinal Large B-Cell Lymphoma (PMBCL)
- Diffuse Large B-Cell Lymphoma (DLBCL)
- Gray Zone Lymphoma (GZL)
- Peripheral T-cell lymphomas (PTCL)

biopsia liquida “metabolica”

THANKS TO

Lab: **Molecular Diagnostics of NHLs**

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AIEOP Associazione Italiana Ematologia Oncologia Pediatrica

eicnhl European Inter-Group for Childhood Non-Hodgkin Lymphoma

CRO Aviano

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Valli del Re

Egesta Lopci

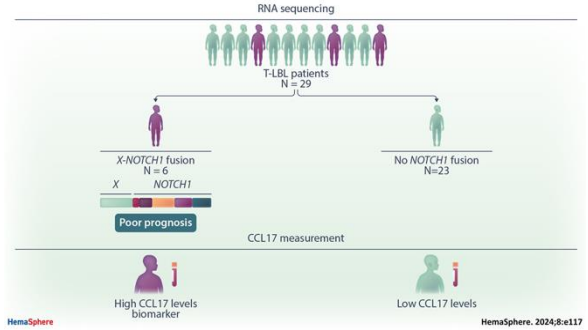
Fondazione Cassa di Risparmio di Padova e Rovigo

AIRC

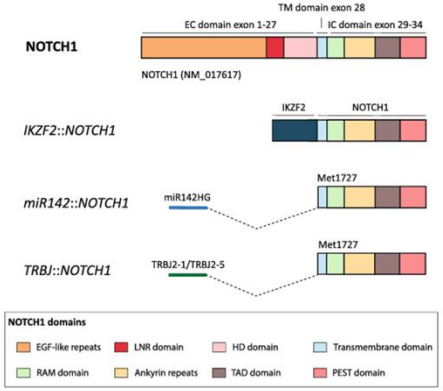
MUR PRIN

Marie Curie Actions

TARC levels and NOTCH1 gene fusions in pediatric T-cell Lymphoblastic Lymphoma



Kroeze et al, *HemaSphere* 2024



- N° of T-LBL cases analyzed = **26**