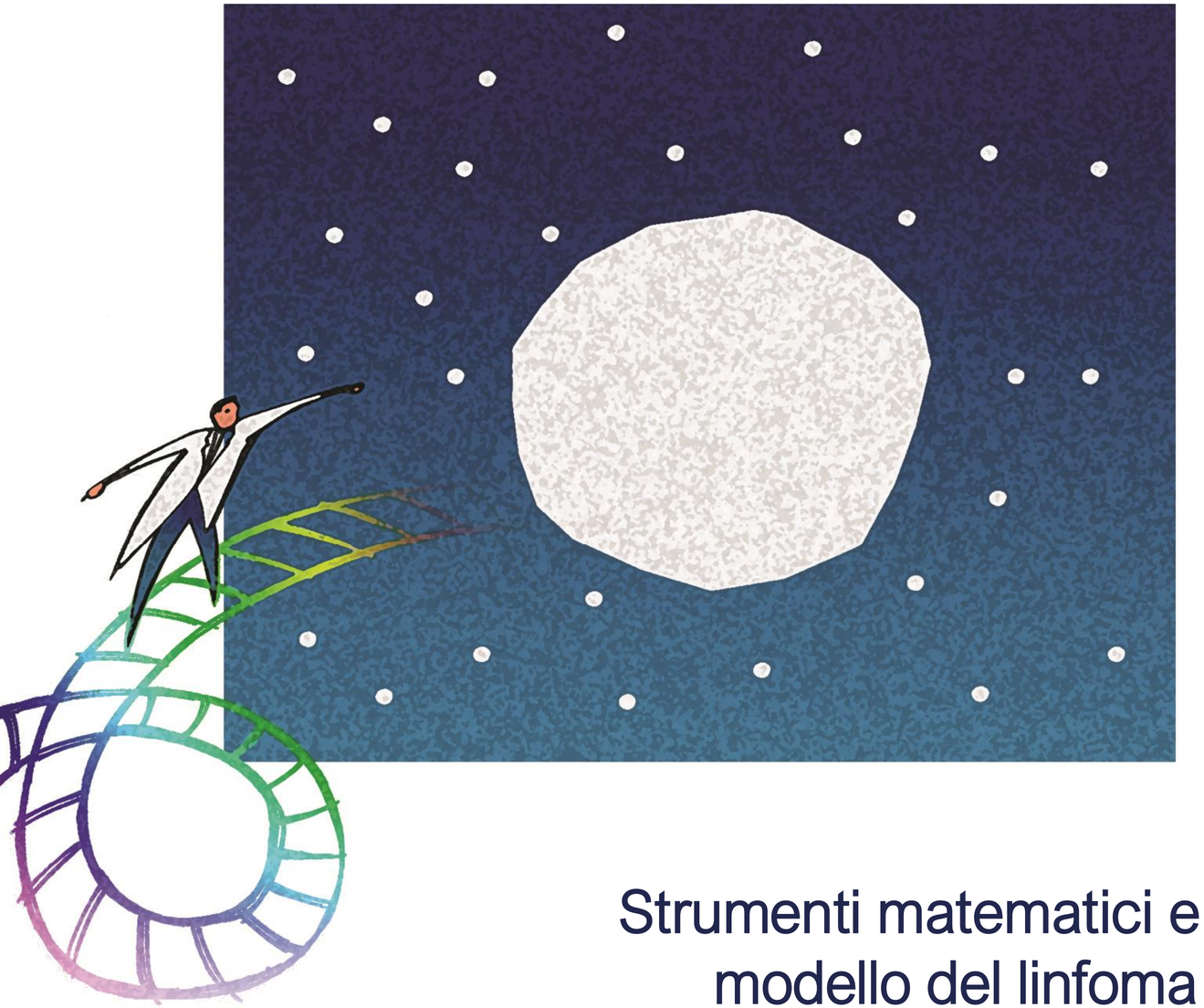


The young side of
LYMPHOMA

gli under 40 a confronto

Pescara, Auditorium Petruzzi
11-12 ottobre 2024

Strumenti matematici e biologia dei linfomi: il modello del linfoma della zona grigia

Grazia Gargano

Department of Mathematics, University of Bari Aldo Moro
Hematology and Cell Therapy Unit, IRCCS Istituto Tumori “Giovanni Paolo II”, Bari

Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Math behind the medicine

blood

BRIEF REPORT | FEBRUARY 20, 2014

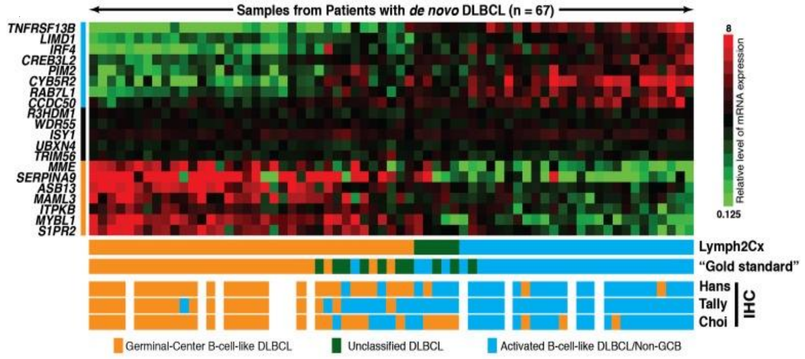
Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue

Brief Report

David W. Scott, George W. Wright, P. Mickey Williams, Chih-Jian Lih, William Walsh, Elaine S. Jaffe, Andreas Rosenwald, Elias Campo, Wing C. Chan, Joseph M. Connors, Erlend B. Smeland, Anja Mottok, Rita M. Braziel, German Ott, Jan Delabie, Raymond R. Tubbs, James R. Cook, Dennis D. Weisenburger, Timothy C. Greiner, Betty J. Glinsmann-Gibson, Kai Fu, Louis M. Staudt, Randy D. Gascoyne, Lisa M. Rimsza

Key Points

- A 20-gene gene expression-based assay accurately and robustly assigns COO subtypes of DLBCL using formalin-fixed paraffin-embedded tissue.



blood

BRIEF REPORT | NOVEMBER 29, 2018

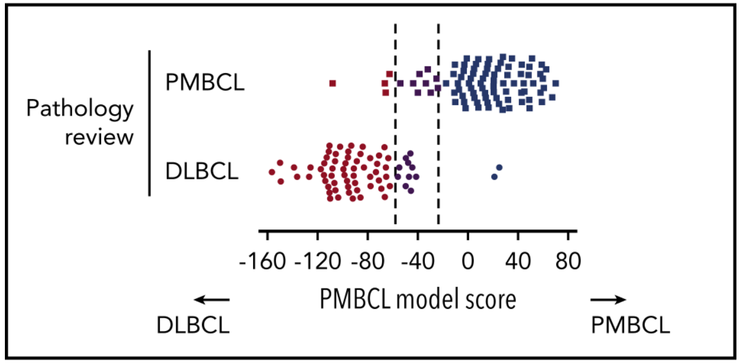
Molecular classification of primary mediastinal large B-cell lymphoma using routinely available tissue specimens

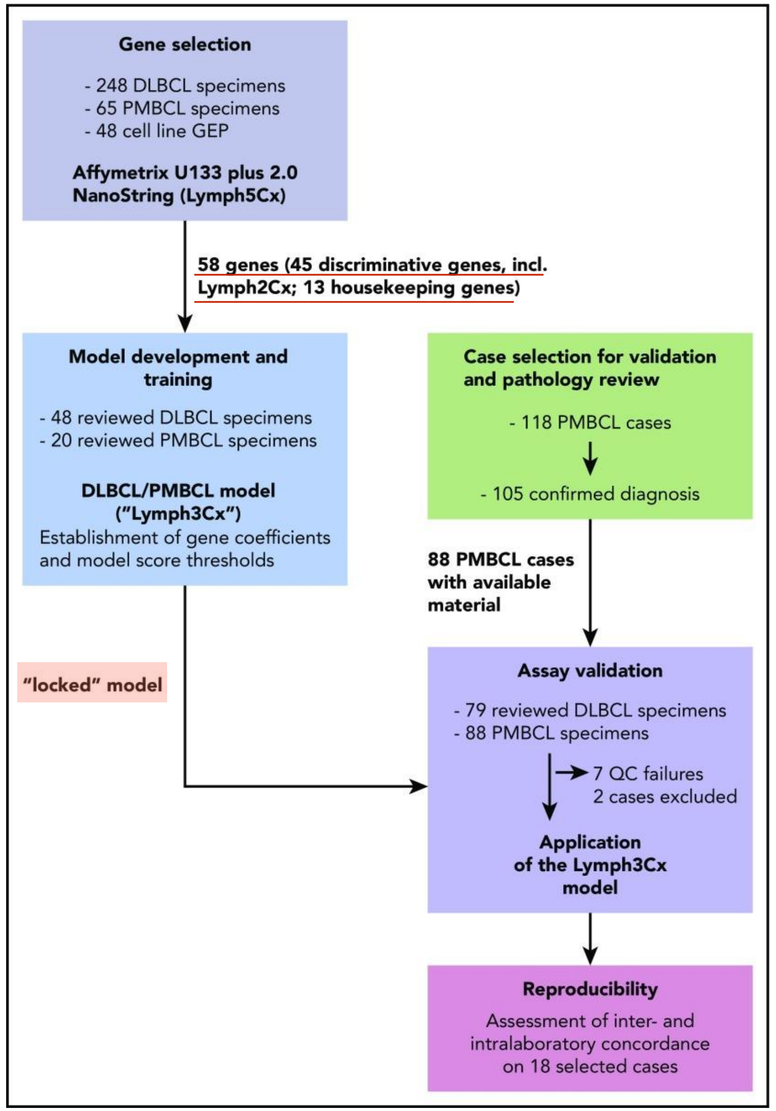
Brief Report

Anja Mottok, George Wright, Andreas Rosenwald, German Ott, Colleen Ramsower, Elias Campo, Rita M. Braziel, Jan Delabie, Dennis D. Weisenburger, Joo Y. Song, Wing C. Chan, James R. Cook, Kai Fu, Tim Greiner, Erlend Smeland, Harald Holte, Kerry J. Savage, Betty J. Glinsmann-Gibson, Randy D. Gascoyne, Louis M. Staudt, Elaine S. Jaffe, Joseph M. Connors, David W. Scott, Christian Steidl, Lisa M. Rimsza

Key Points

- A 58-gene expression-based assay aids in the molecular distinction of PMBCL and DLBCL using archival tissue biopsy specimens.





Mottok A. *et al*, Blood 2018

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)
(19) World Intellectual Property Organization
International Bureau
(43) International Publication Date
20 December 2018 (20.12.2018)
WIPO | PCT
(10) International Publication Number
WO 2018/231589 A1

BRIEF SUMMARY OF THE INVENTION

[0007] In an embodiment, the present invention provides a method for classifying the lymphoma type of a sample, which method comprises providing a formalin-fixed and paraffin-embedded (FFPE) lymphoma sample from the subject, isolating RNA from the sample, obtaining gene expression data from the RNA, wherein the gene expression data comprises signal values that represent expression levels for each gene of Table 1, and determining a predictor score from the gene expression data, wherein the tumor predictors score is calculated by

$$S = \sum_{k=0}^{58} a_i x_i$$

wherein α_i is the model coefficient value for gene i, as listed in Table 1, column D for determining whether the sample is PMBCL or DLBCL and as listed in Table 1 column E for determining whether a sample is ABC DLBCL or GCB DLBCL, and X_i is the $\log_2$ transformed expression signal value for gene i; and when the coefficient values in column D of Table 1 are used, classifying the lymphoma as DLBCL when S is less than -57.95, PMBCL when S is greater than -23.57, or uncertain DLBCL/PMBCL when S is

between -57.95 and -23.57; and when the coefficient values in column E are used, classifying the lymphoma as GCB DLBCL when S is less than 798.5, ABC DLBCL when S is greater than 1324.5, or uncertain ABC/GCB DLBCL when S is between 798.5 and 1324.5.

Basic research

TRANSALTIONAL MEDICINE

Clinical practice

Multidisciplinary research team

haematologica
Open access journal of the Ferrata-Storti Foundation, a non-profit organization

LETTERS TO THE EDITOR

A targeted gene signature stratifying mediastinal gray zone lymphoma into classical HL-like or PMBL-like subtypes

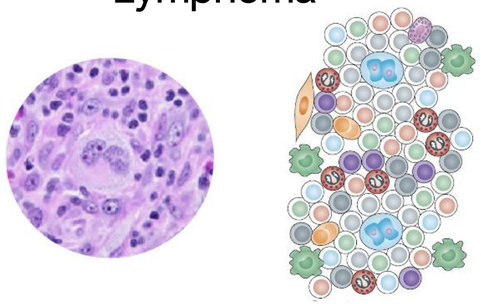
Grazia Gargano, Maria Carmela Vegliante, Flavia Esposito, Susanna A. Pappagallo, Elena Sabattini, Claudio Agostinelli, Stefano A. Pileri, Valentina Tabanelli, Maurilio Ponzoni, Luisa Lorenzi, Fabio Facchetti, Arianna Di Napoli, Marco Lucioni, Marco Paulli, Lorenzo Leoncini, Stefano Lazzi, Stefano Ascani, Giuseppina Opinto, Gian Maria Zaccaria, Giacomo Volpe, Paolo Mondelli, Antonella Bucci, Laura Selicato, Antonio Negri, Giacomo Loseto, Felice Clemente, Anna Scattone, Alfredo F. Zito, Luca Nassi, Nicoletta Del Buono, Attilio Guarini, Sabino Ciavarella

ILLUSTRATION: JENNA LUECKE

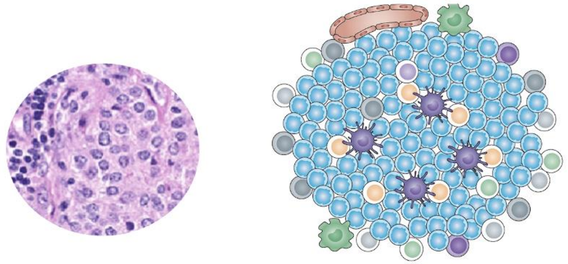
Mediastinal Gray Zone Lymphoma (MGZL)

MGZL is a very rare and aggressive lymphoma that is present as bulky mass in the mediastinum.

Classical Hodgkin Lymphoma



Primary Mediastinal B-cell Lymphoma



MGZL

CD20-/+CD30+CD15+ OCT2-BOB.1-

CD20+CD30+/-CD15- OCT2+BOB.1+

B-Cell lymphoma with overlapping morphological and/or immunophenotypic features between CHL and PMBL

Alaggio R. *et al*., Nature 2022
Campo E. *et al.*, Blood 2022

Uncertain pathological and clinical aspects

- **Challenging diagnosis** (> 60% to be reclassified)

- **Treatment heterogeneity**:

  - **CHL-like regimen**: doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD);

  - **PMBL-like treatment**: cyclophosphamide, doxorubicin, oncovin, and prednisone (CHOP) +/− rituximab;

  - EFS of patients treated with dose-intensive regimens was better than patients treated with a less intensive regimen.

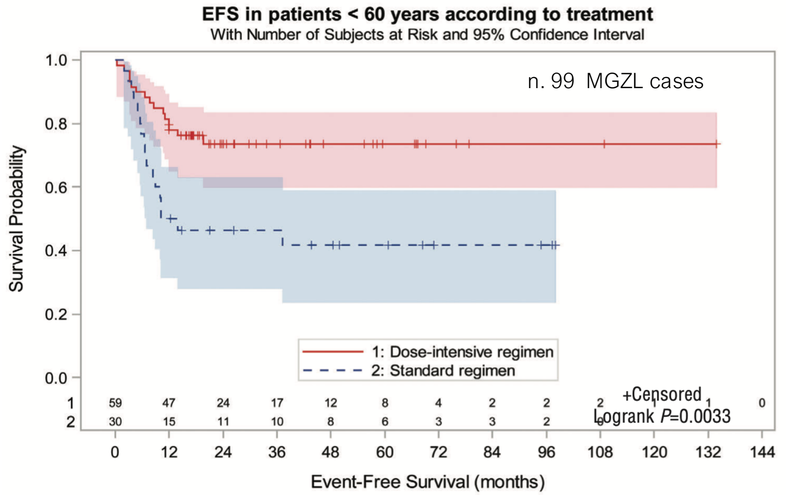
REGULAR ARTICLE

blood advances

Clinicopathologic consensus study of gray zone lymphoma with features intermediate between DLBCL and classical HL

Monika Pilichowska,[1] Stefania Pittaluga,[2] Judith A. Ferry,[3] Jessica Hemminger,[4] Hong Chang,[5,6] Jennifer A. Kanakry,[5] Laurie H. Sehn,[7] Tatyana Feldman,[8] Jeremy S. Abra
Timothy S. Fenske,[13] Jonathan W.
Randy D. Gascoyne,[7] Elaine S. Jaf

Gray zone lymphoma (GZL) is described as sharing features with classical Hodgkin lymphoma (cHL) and diffuse large B-cell lymphoma (DLBCL). However, there remains complexity in establishing diagnosis, delineating prognosis, and determining optimum therapy. Sixty-eight cases diagnosed as GZL across 15 North American academic centers were evaluated by central pathology review to achieve consensus. Of these, only 26 (38%) were confirmed as GZL. Morphology was critical to GZL consensus diagnosis (eg, tumor cell richness); immunohisto-



Sarkozy C. *et al.,* Haematologica 2017

Transcriptomic boundaries of MGZL

REGULAR ARTICLE

blood advances

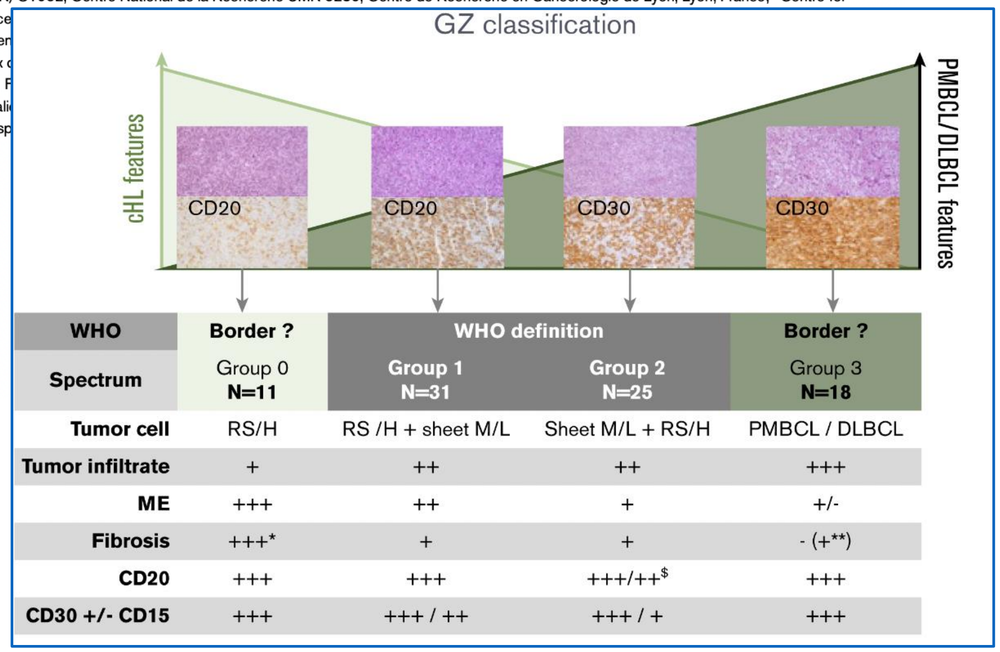
Gene expression profiling of gray zone lymphoma

Clémentine Sarkozy,[1,2] Lauren Chong,[2] Katsuyoshi Takata,[2] Elizabeth A. Chavez,[2] Tomoko Miyata-Takata,[2] Gerben Duns,[2] Adèle Telenius,[2] Merrill Boyle,[2] Graham W. Slack,[2] Camille Laurent,[3] Pedro Farinha,[2] Thierry J. Molina,[4] Christiane Copie-Bergman,[5] Diane Damotte,[6] Gilles A. Salles,[1,7] Anja Mottok,[8] Kerry J. Savage,[2] David W. Scott,[2] Alexandra Traverse-Glehen,[1,9,*] and Christian Steidl[2,*]

[1]INSERM Unité Mixte de Recherche (UMR)-S1052, Centre National de la Recherche UMR 5286, Centre de Recherche en Cancérologie de Lyon, Lyon, France; [2]Centre for Lymphoid Cancer, British Columbia Cance
INSERM U.1037, Centre de Recherche en
Descartes, Assistance Publique–Hôpitaux d
University, UMR-S 955, INSERM, Créteil, F
[7]Hospices Civils de Lyon, Centre Hospitali
Medical Center, Ulm, Germany; and [9]Hosp

Key Points

- Macrophage infiltration is a key feature of the tumor microenvironment across the GZL spectrum.

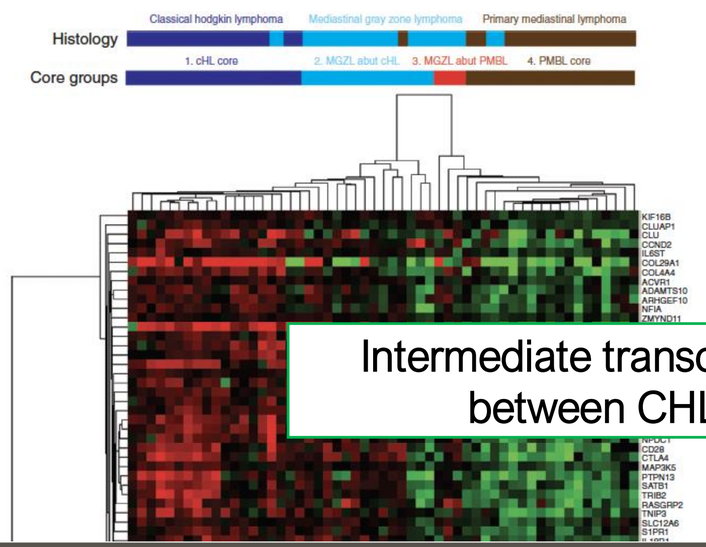


WHO	Border ?	WHO definition		Border ?
Spectrum	Group 0 N=11	Group 1 N=31	Group 2 N=25	Group 3 N=18
Tumor cell	RS/H	RS /H + sheet M/L	Sheet M/L + RS/H	PMBCL / DLBCL
Tumor infiltrate	+	++	++	+++
ME	+++	++	+	+/-
Fibrosis	+++*	+	+	- (+**)
CD20	+++	+++	+++/++$	+++
CD30 +/- CD15	+++	+++ / ++	+++ / +	+++

RESEARCH BRIEF

Gene Expression Profiling of Mediastinal Gray Zone Lymphoma and Its Relationship to Primary Mediastinal B-cell Lymphoma and Classical Hodgkin Lymphoma

Stefania Pittaluga[1], Alina Nicolae[1], George W. Wright[2], Christopher Melani[3], Mark Roschewski[3], Seth Steinberg[4], DaWei Huang[3], Louis M. Staudt[3], Elaine S. Jaffe[1], and Wyndham H. Wilson[3]



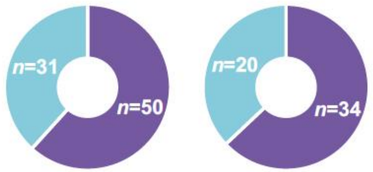
Intermediate transcriptional program between CHL and PMBL

Dissecting MGZL biology through a new mathematical approach

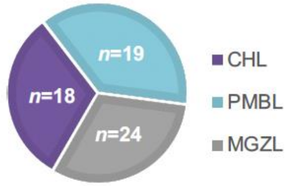
Discovery cohort

84 CHL 51 PMBL
profiled by microarrays

TRAINING TESTING



Real-life cases

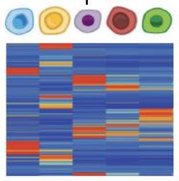


FFPE tumor specimens

Deconvolution of tumor and TME expression profiles

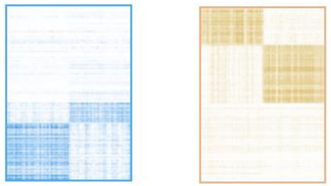
Bulk transcriptomic profiles of the training cohort

Custom signature matrix



CIBERSORTx

Gene expression purification of tumor and TME components



TUMOR TME

Gene signature extraction

NMF & GENE SELECTION

Identification of a distinctive gene signature for CHL and PMBL

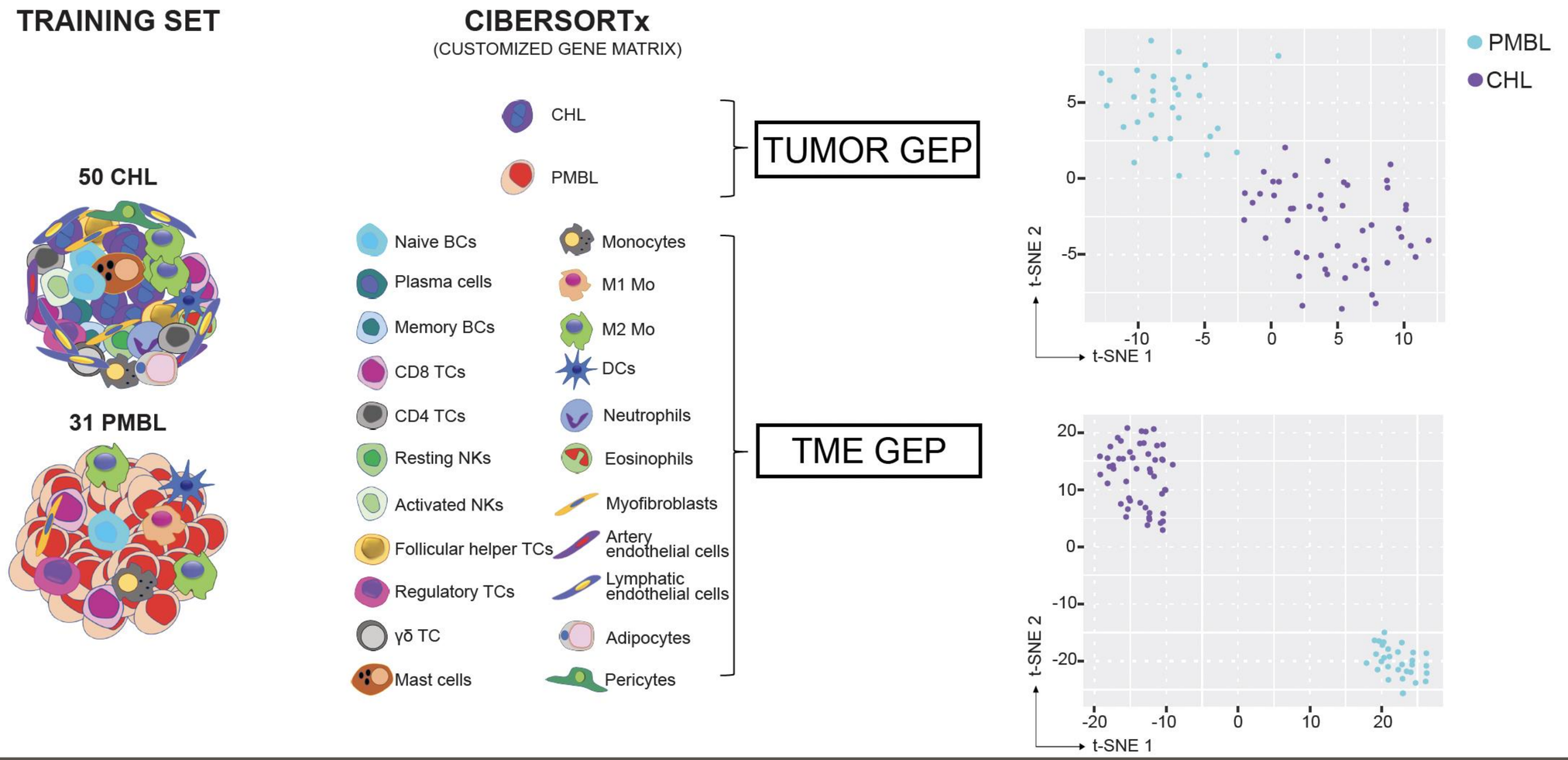
Validation of the gene signature in the testing cohort

Transcriptional stratification of MGZL within the CHL/PMBL spectrum

Real-life cohort profiled by NanoString Technology

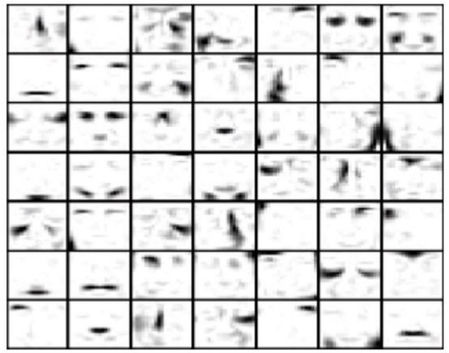
Identification of a signature to distinguish CHL and PMBL

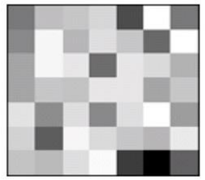
Schematic overview of CIBERSORTx application and its results on the training set



Nonnegative Matrix Factorization - A Short Introduction

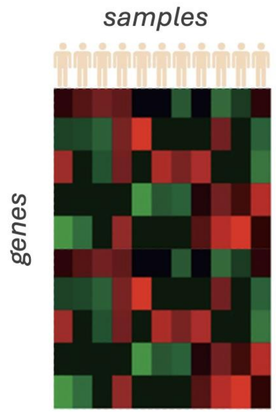
$$\underbrace{X(:,j)}_{j\text{th facial image}} \approx \sum_{k=1}^{r} \underbrace{W(:,k)}_{\text{facial features}} \underbrace{H(k,j)}_{\substack{\text{importance of features} \\ \text{in } j\text{th image}}} = \underbrace{WH(:,j)}_{\substack{\text{approximation} \\ \text{of } j\text{th image}}} .$$

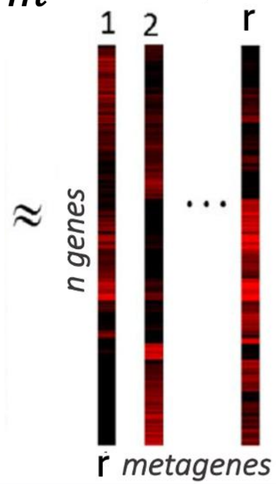


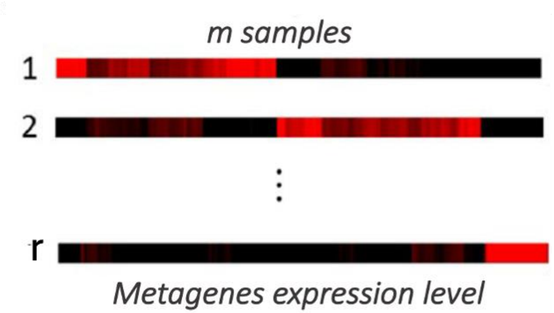


Gene expression data matrix

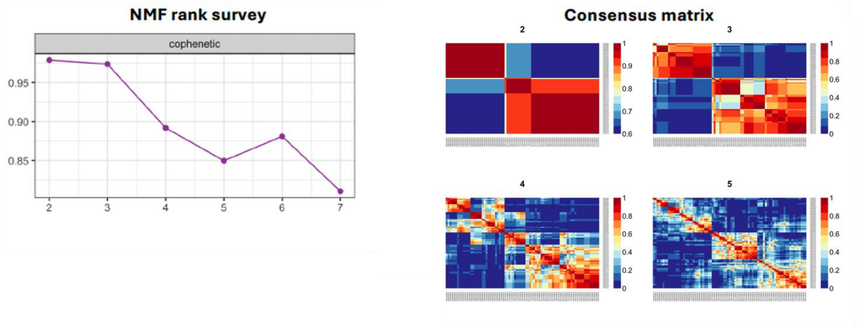
$\mathbf{X} \in \mathbb{R}^{n \times m}, n \gg m$

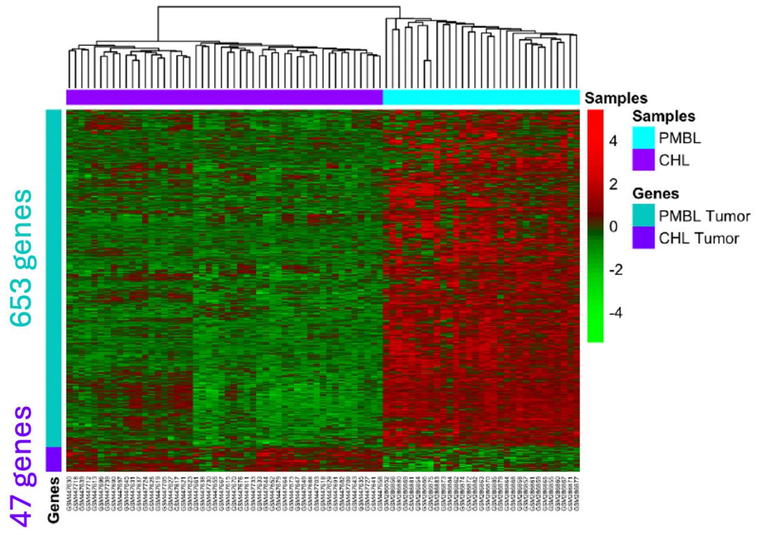




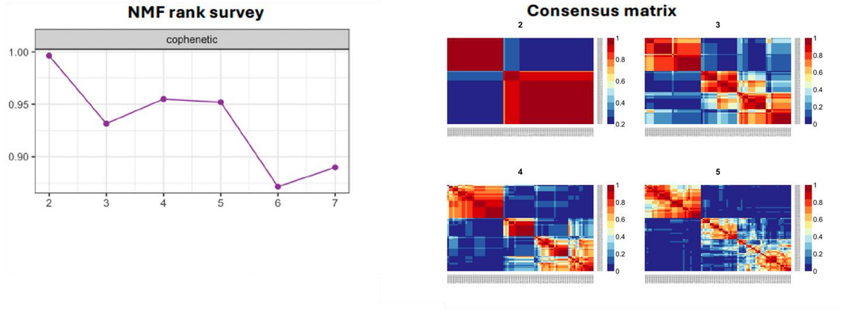


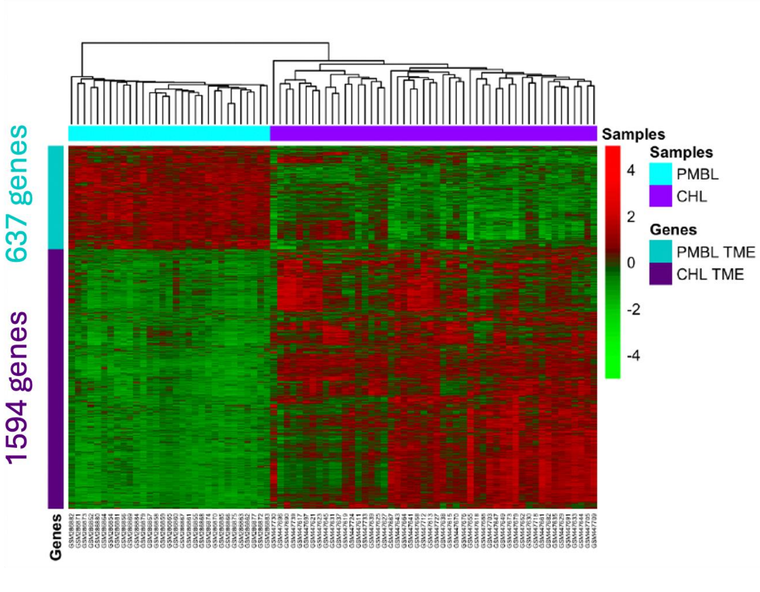
NMF-based Gene Signature Extraction





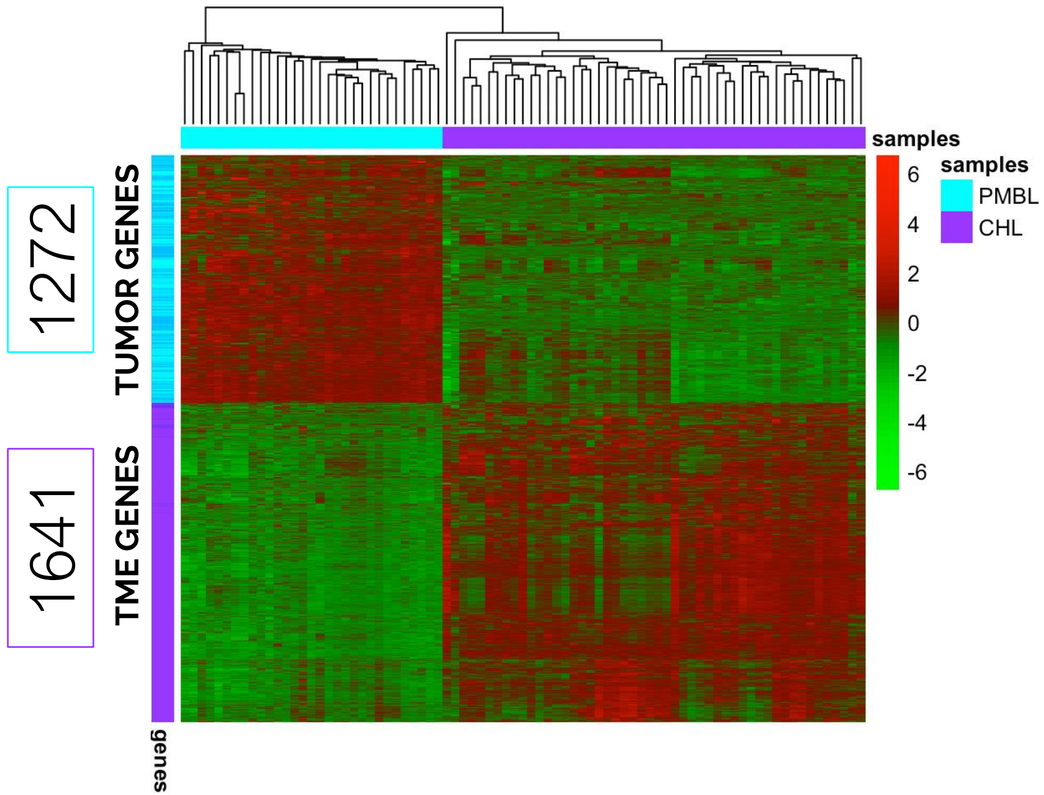
TUMOR COMPONENT





TUMOR MICROENVIRONMENT (TME) COMPONENT

NMF-based gene signature performance on the training set



- ✓ Genes related to tumor and TME components
- ✓ 2913-gene signature
- ✓ Efficient CHL/PMBL segregation

Reduction of genes number to potentiate the final **translational value** of the signature

Ensemble of Filter-based Feature Selection Methods

Relief feature score

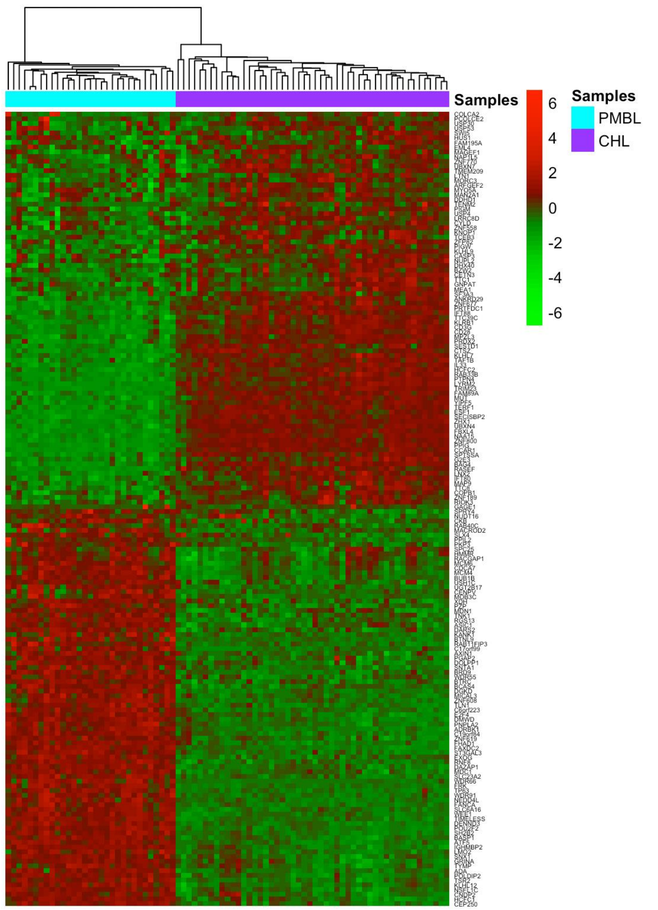
Laplacian score

geometric mean

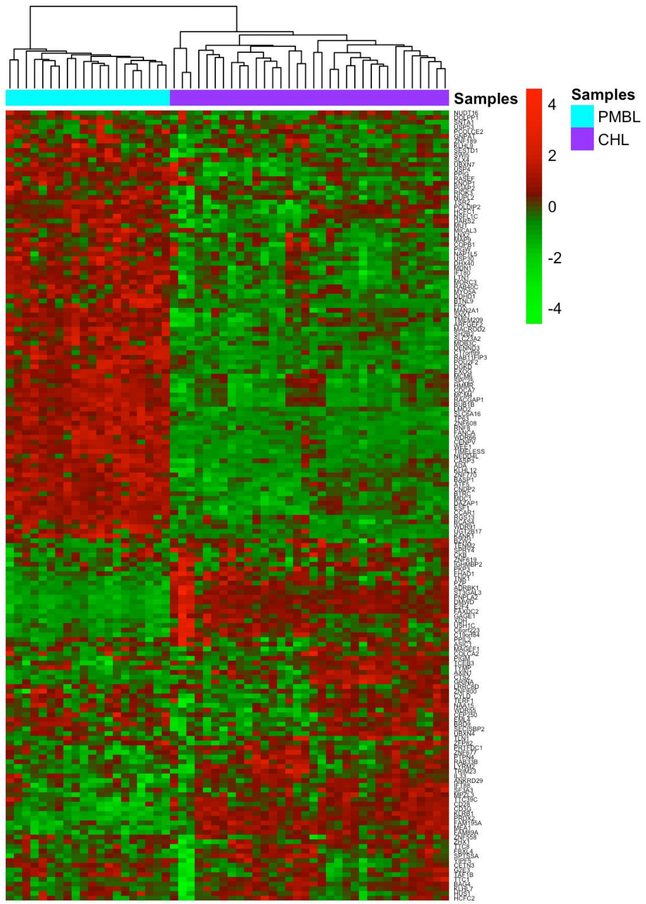
168-gene signature

Performance of a 168-gene signature on training and testing sets

TRAINING SET
50 CHL + 81 PMBL



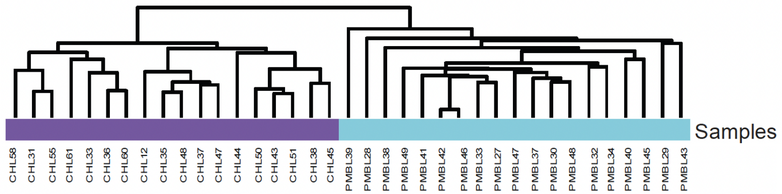
TESTING SET
34 CHL + 20 PMBL



The 168-gene signature produces a successful clustering of CHL and PMBL cases on both training and testing sets.

Validation of NanoString-Based 168-Gene Signature and MGZL Assignment

VALIDATION SET
18 CHL + 19 PMBL



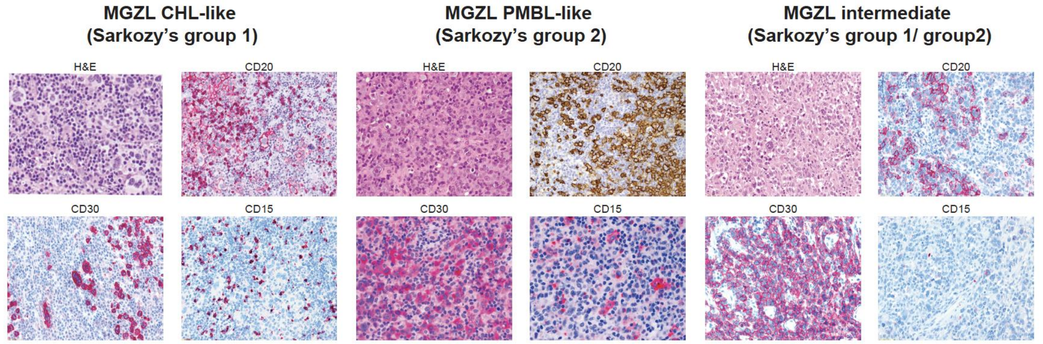
39 GZL specimens

IN-PANEL PATHOLOGY REVISION

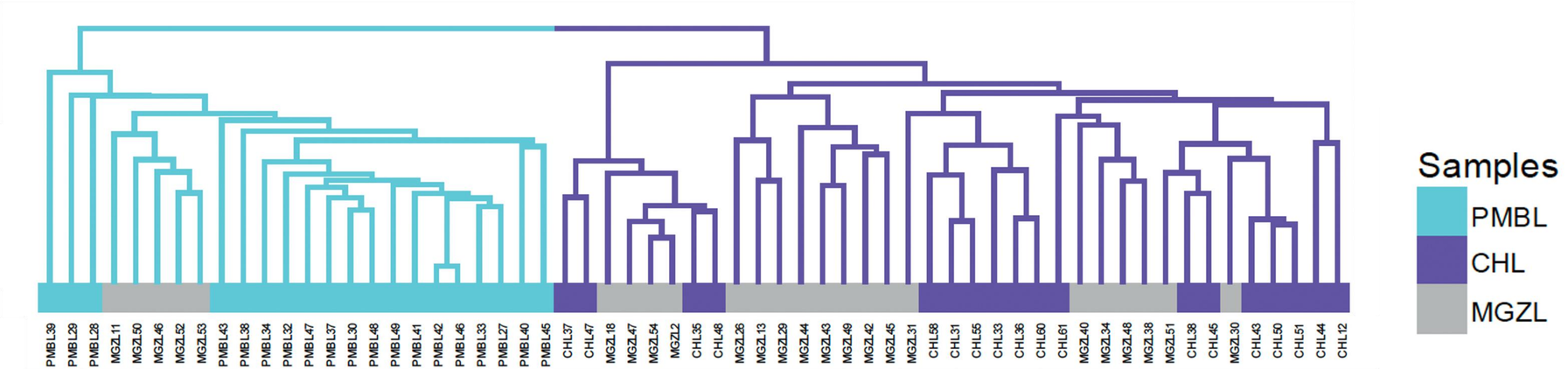
28 MGZL

QUALITY CONTROL

24 MGZL



$$\mathbf{X}_{val} \in \mathbb{R}_{+}^{168\times 61}$$



	CHL-cluster (N=10)	PMBL-cluster (N=4)
Morphology		
CHL-like	4 (40.0%)	1 (25.0%)
Intermediate	1 (10.0%)	1 (25.0%)
PMBL-like	5 (50.0%)	2 (50.0%)
Sarkozy's groups		
group0	2 (20.0%)	0 (0%)
group1	3 (30.0%)	1 (25.0%)
group1/group2	2 (20.0%)	1 (25.0%)
group2	2 (20.0%)	2 (50.0%)
group2/group3	1 (10.0%)	0 (0%)
Stage		
1	0 (0%)	1 (25.0%)
2	8 (80.0%)	1 (25.0%)
3	0 (0%)	1 (25.0%)
4	2 (20.0%)	1 (25.0%)
First-line therapy		
EPOCH	7 (70.0%)	2 (50.0%)
CHOP-like	2 (20.0%)	1 (25.0%)
MACOPB	1 (10.0%)	0 (0%)
ABVD	0 (0%)	1 (25.0%)
Therapy response rate		
CR	6 (60.0%)	2 (50.0%)
PD	2 (20.0%)	1 (25.0%)
PR	1 (10.0%)	1 (25.0%)
SD	1 (10.0%)	0 (0%)
PFS (months)		
Median [Min, Max]	34.3 [2.23, 106]	55.0 [2.93, 62.0]
Age		
Median [Min, Max]	43.5 [17.0, 52.0]	30.5 [25.0, 37.0]

Case ID	Cytoarchitecture and morphology	Sarkozy'sgroups	GEP cluster	Stage	Terapia	Outcome
MGZL11	PMBL-like	group2	PMBL-cluster			
MGZL13	CHL-like	group2	CHL-cluster			
MGZL18	Intermediate	group1/group2	CHL-cluster			
MGZL2	Intermediate	group1/group2	CHL-cluster			
MGZL26	Intermediate	group1/group2	CHL-cluster			
MGZL29	PMBL-like	group2	CHL-cluster			
MGZL30	Intermediate	group1/group2	CHL-cluster			
MGZL31	CHL-like	group1	CHL-cluster			
MGZL34	CHL-like	group1	CHL-cluster			
MGZL38	PMBL-like	group2/3	CHL-cluster			
MGZL45	CHL-like	group1	CHL-cluster	2	EPOCH, intensified, 6 cycles / Radiotherapy	PR
MGZL52	CHL-like	group1	PMBL-cluster	2	ABVD, standard, 2 cycles	PD
MGZL43	CHL-like	group0	CHL-cluster	2	EPOCH, intensified, 6 cycles / Radiotherapy	CR
MGZL44	PMBL-like	group1/group2	CHL-cluster	2	EPOCH, intensified, 6 cycles	CR
MGZL40	PMBL-like	group1/group2	CHL-cluster	4	CHOP-like, standard, 4 cycles	SD
MGZL46	Intermediate	group1/group2	PMBL-cluster	1	CHOP-like, standard, 6 cycles / Radiotherapy	CR
MGZL47	Intermediate	group1	CHL-cluster	2	CHOP-like, standard, 6 cycles / Radiotherapy	CR
MGZL48	CHL-like	group0	CHL-cluster	2	MACOPB, intensified, 12 cycles / Radiotherapy	CR
MGZL49	PMBL-like	group2	CHL-cluster	4	EPOCH, intensified, 6 cycles	CR
MGZL42	PMBL-like	group2/group3	CHL-cluster	2	EPOCH, intensified, 3 cycles	PD
MGZL51	CHL-like	group1	CHL-cluster	2	EPOCH, intensified, 6 cycles	CR
MGZL50	PMBL-like	group2	PMBL-cluster	4	EPOCH, intensified, 5 cycles / Radiotherapy	PR
MGZL53	PMBL-like	group2	PMBL-cluster	3	EPOCH, intensified, 6 cycles	CR
MGZL54	PMBL-like	group2	CHL-cluster	2	EPOCH, intensified, 2 cycles	PD

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Review

Mediastinal Gray-Zone Lymphoma: Still an Open Issue

Stefano Pileri [1,2,*], Valentina Tabanelli [1], Roberto Chiarle [1,3], Angelica Calleri [1], Federica Melle [1], Giovanna Motta [1], Maria Rosaria Sapienza [1], Elena Sabattini [4], Pier Luigi Zinzani [2,5] and Enrico Derenzini [6,7]

From what has been discussed above, MGZL, indeed, still represents a condition, to which the title of Luigi Pirandello's play "Six Characters in Search of an Author" can well apply. The rarity of the disease, the diagnostic difficulties, and the suboptimal response to most therapies underline the need for internationally shared guidelines. The complexity is further increased by the possibility that MGZL might not represent a single entity but rather a spectrum of diseases that require an individual tailoring of therapies. The 5th edition of the WHO Classification and ICC incorporate in their criteria an increased understanding of MGZL. Nonetheless, current studies are insufficient, and more in-depth molecular characterisation is needed to further understand the pathobiology of MGZL. In this respect, a significant contribution can be expected by the application of the new technologies and platforms allowing the molecular characterisation at the single cell level. It is likely that an improved understanding of genetic aberrations, microenvironmental characteristics, and cell-to-cell interactions in MGZL will lead to more effective targeted therapeutic approaches.

Conclusions and Future Directions

- Application of **computational and mathematic approach** that capitalize transcriptomic data for translational purposes;
- Development of a robust signature capable of distinguishing CHL from PMBL and placing MGZL within this spectrum based on selected genes related to **tumor** and **microenvironment**;
- Validation of **NanoString-based 168-gene signature** on formalin-fixed paraffin-embedded (FFPE) real-life cohort .

Development of combined histopathological/transcriptomic model of MGZL stratification

to be continued...

Thank you for your attention

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