LETTER TO THE EDITOR

A targeted gene signature stratifying mediastinal gray zone lymphoma into classical Hodgkin lymphoma-like or primary mediastinal B-cell lymphoma-like subtypes

Authors

Grazia Gargano,[1,2*] Maria Carmela Vegliante,[1*] Flavia Esposito,[2] Susanna A. Pappagallo,[1] Elena Sabattini,[3] Claudio Agostinelli,[3,4] Stefano A. Pileri,[5] Valentina Tabanelli,[5] Maurilio Ponzoni,[6] Luisa Lorenzi,[7] Fabio Facchetti,[7] Arianna Di Napoli,[8] Marco Lucioni,[9] Marco Paulli,[9] Lorenzo Leoncini,[10] Stefano Lazzi,[10] Stefano Ascani,[11] Giuseppina Opinto,[12] Gian Maria Zaccaria,[13] Giacomo Volpe,[1] Paolo Mondelli,[1] Antonella Bucci,[1] Laura Selicato,[2] Antonio Negri,[1] Giacomo Loseto,[1] Felice Clemente,[1] Anna Scattone,[14] Alfredo F. Zito,[14] Luca Nassi,[15] Nicoletta Del Buono,[2] Attilio Guarini[1] and Sabino Ciavarella[1]

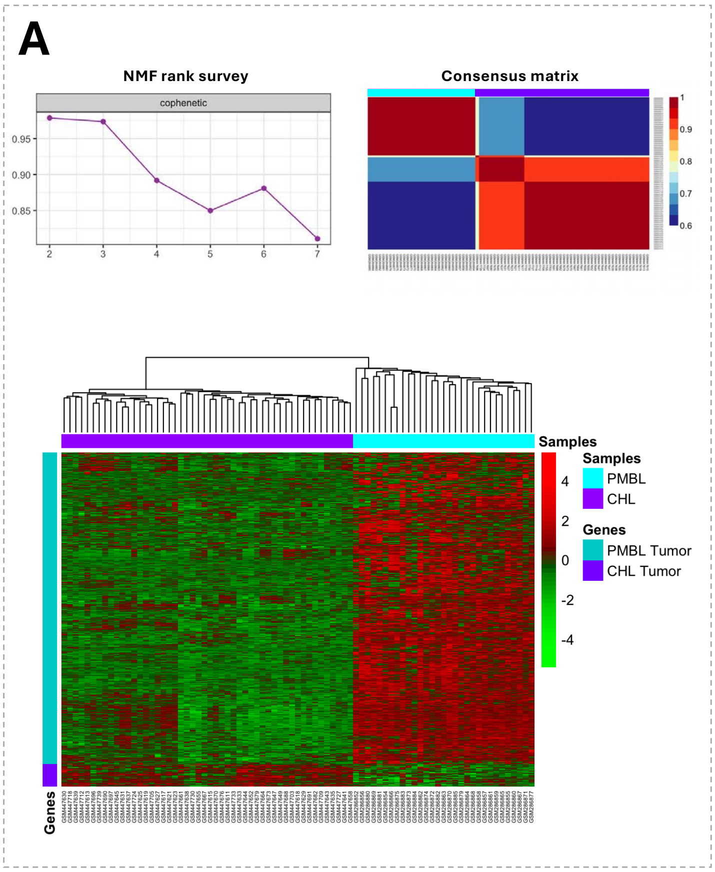
[1]Hematology and Cell Therapy Unit, IRCCS Istituto Tumori 'Giovanni Paolo II', Bari; [2]Department of Mathematics, Aldo Moro University of Bari, Bari; [3]Hematopathology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna; [4]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna; [5]Division of Hematopathology, European Institute of Oncology IRCCS, Milan; [6]Ateneo Vita-Salute San Raffaele Milan and Pathology Unit, IRCCS San Raffaele Scientific Institute, Milan; [7]Pathology Unit, Department of Molecular and Translational Medicine, University of Brescia, Brescia; [8]Department of Clinical and Molecular Medicine, Sant'Andrea University Hospital, Sapienza University of Rome, Rome; [9]Department of Molecular Medicine, University of Pavia/Foundation IRCCS Policlinico San Matteo, Pavia; [10]Section of Pathology, Department of Medical Biotechnology, University of Siena, Siena; [11]Pathology Unit, Azienda Ospedaliera Santa Maria di Terni, University of Perugia, Terni; [12]Clinical Pathology and Microbiology Unit, Bonomo Hospital, Andria; [13]Department of Electrical and Information Engineering (DEI), Polytechnic University of Bari, Bari; [14]Department of Pathology, IRCCS Istituto Tumori 'Giovanni Paolo II', Bari and [15]Department of Hematology, Careggi Hospital and University of Florence, Florence, Italy

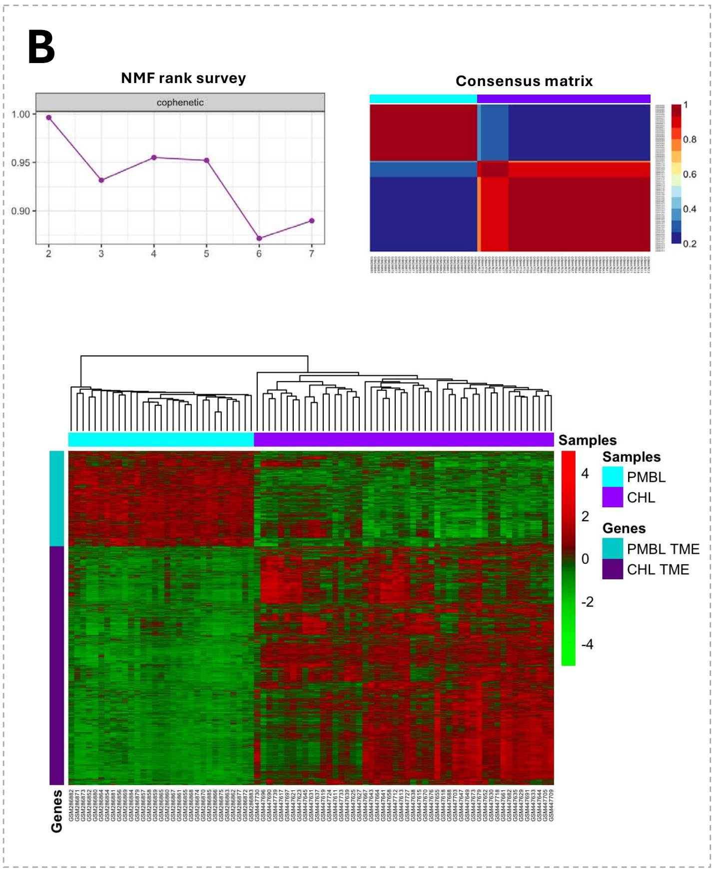
*GG and MCV contributed equally as first authors.

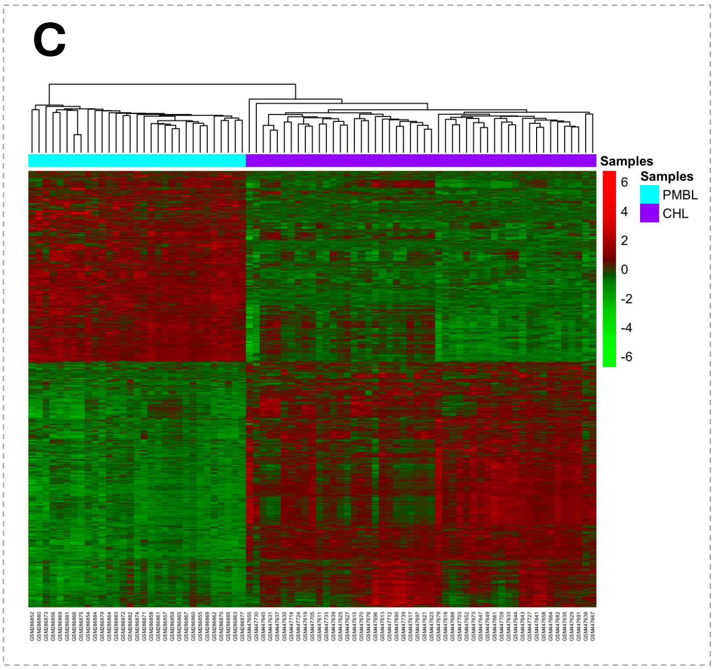
Correspondence:
S. CIAVARELLA - s.ciavarella@oncologico.bari.it

https://doi.org/10.3324/haematol.2024.285266

Supplementary Data







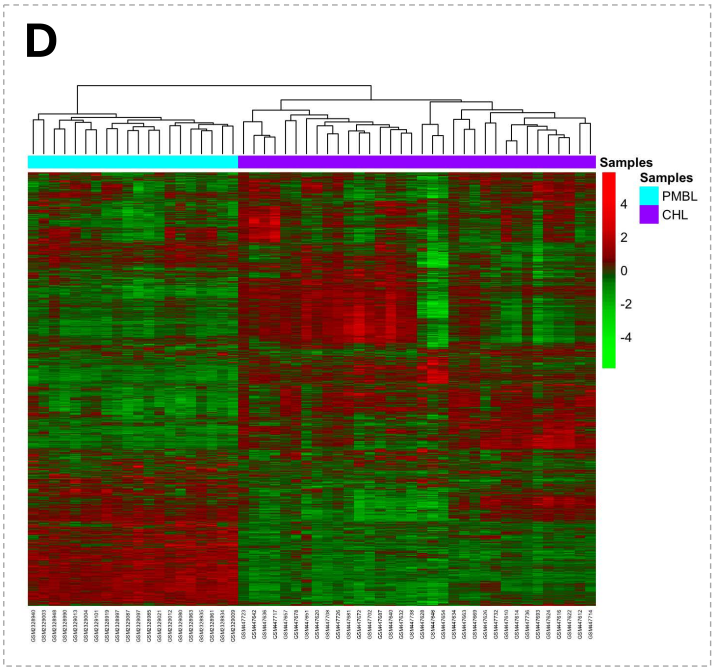
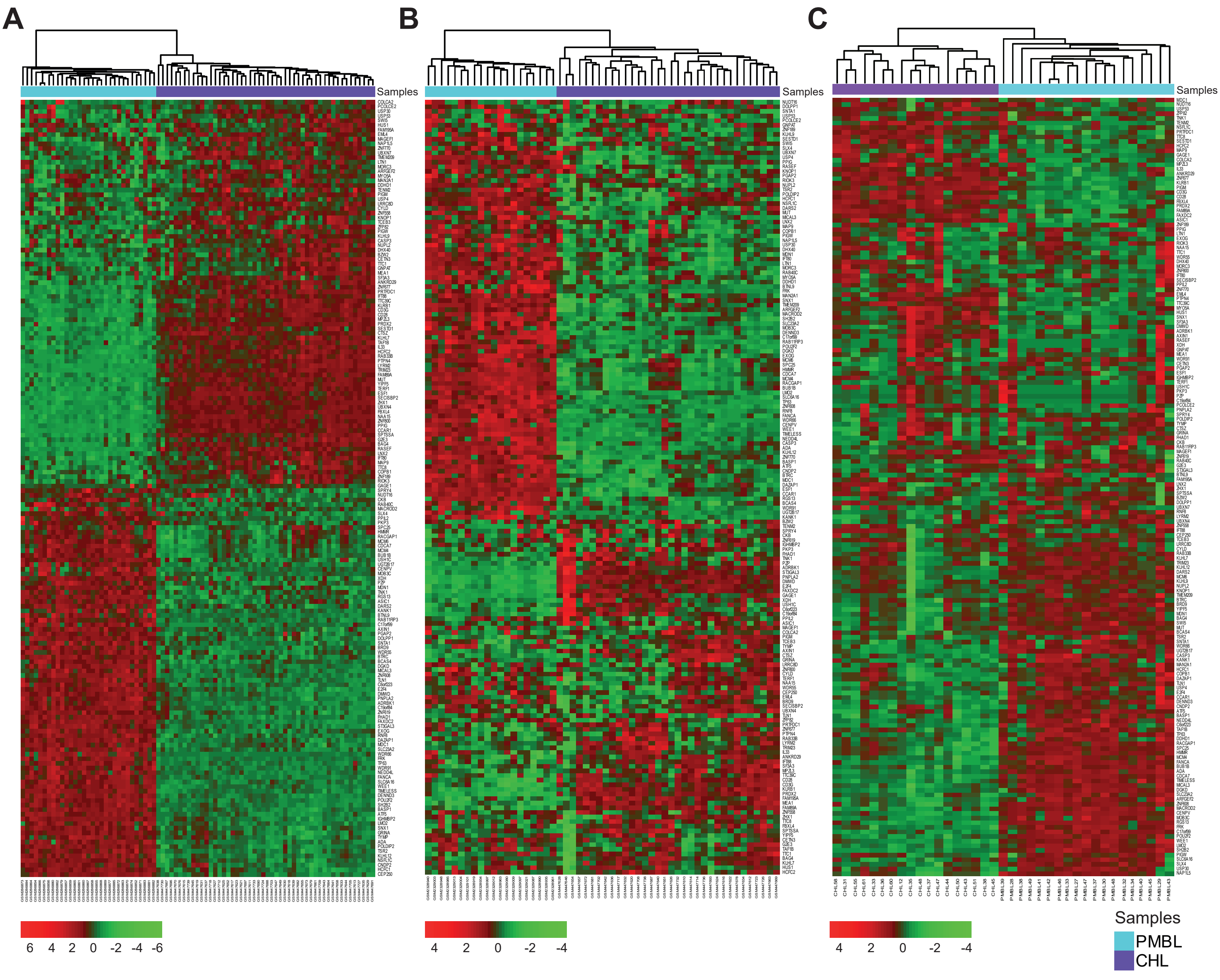


Figure S1. NMF application and performance of 2,913-gene signature in the training and testing sets.

A. Optimal rank determination by NMF for tumor GEP derived from CIBERSORTx: CCC against different rank *r* in the range [2,7] and consensus matrix for optimal rank *r* = 2 (upper panel). Heatmap representing the expressions of 700 tumor-related genes on the training cohort (CCC = 0.92, bottom panel). **B.** Optimal rank determination by NMF for TME GEP: CCC against different rank *r* in the range [2,7] and consensus matrix for optimal rank *r* = 2 (upper panel). Heatmap representing the expressions of 2,231 TME-associated genes on the training cohort (CCC = 0.88, bottom panel). NMF was performed by using the R package *NMF* (version 0.24.0). Hierarchical clustering analyses were performed using average linkage with Pearson correlation distance metric according to the highest CCC (*cluster* R-package, version 2.1.3); clustered heatmaps were drawn using the R package *pheatmap* (version 1.0.12). **C.** Heatmap showing clustering results of the overall 2,913 tumor/TME related genes distinctive of CHL and PMBL in the training set (CCC = 0.90). **D.** Heatmap depicting clustering results of 2,913-gene signature in the testing set (CCC = 0.90). NMF, nonnegative matrix factorization; GEP, gene expression profiles; CCC, cophenetic correlation coefficient; TME, tumor microenvironment. MGZL, mediastinal gray zone lymphoma; CHL, classical Hodgkin lymphoma; PMBL, primary mediastinal B-cell lymphoma.


A
B
C
Samples
Samples
Samples
6 4 2 0 -2 -4 -6
4 2 0 -2 -4
4 2 0 -2 -4
Samples
PMBL
CHL

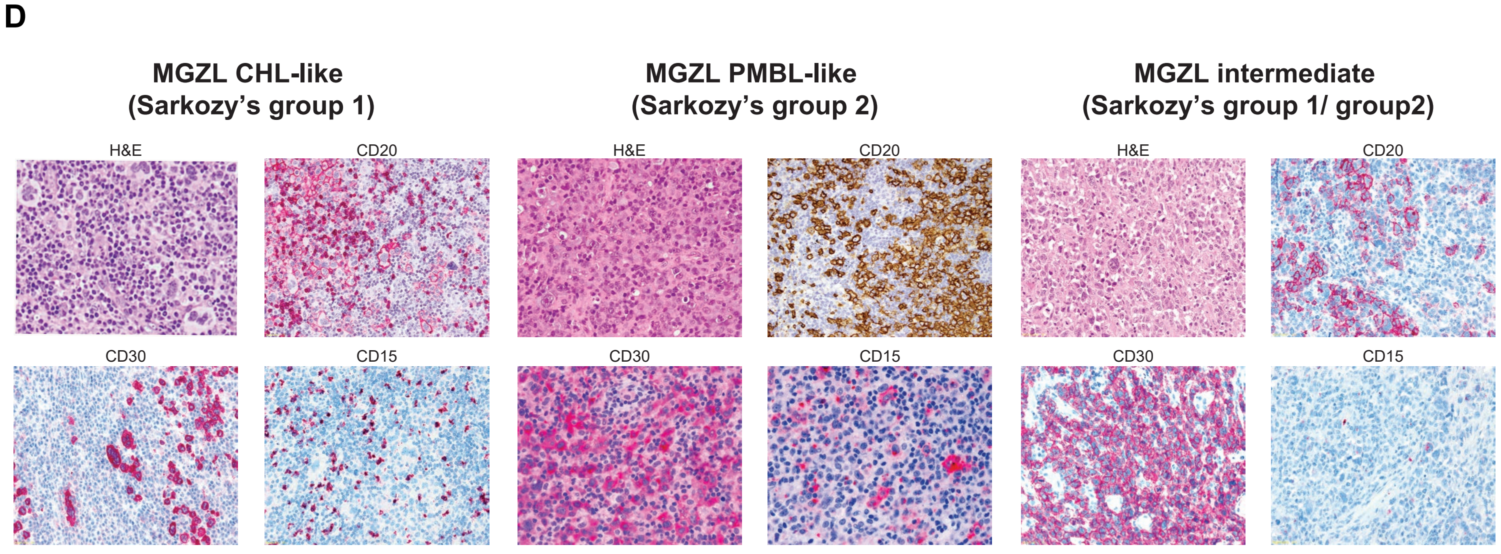

D
MGZL CHL-like
(Sarkozy's group 1)
MGZL PMBL-like
(Sarkozy's group 2)
MGZL intermediate
(Sarkozy's group 1/ group2)
H&E
CD20
CD30
CD15
H&E
CD20
CD30
CD15
H&E
CD20
CD30
CD15

Figure S2. Clustering analysis of the 168-gene signature in different cohorts and immunohistochemical staining of representative MGZL.

A-B-C. Heatmaps showing the unsupervised clustering of samples in the training set (*n* =81, CCC = 0.98), in the independent testing cohort (*n*= 54, CCC = 0.93), and in the real-life cohort (n =37, CCC = 0.87) based on the expression of the 168-gene signature. **D.** IHC images of H&E, CD20, CD30 and CD15 staining from three MGZL representatives of CHL-like, PMBL-like and intermediate morphology respectively, labeled also according to Sarkozy's classification. Magnification at 20X. CCC, cophenetic correlation coefficient; IHC, immunohistochemistry; H&E, hematoxylin and eosin; MGZL, mediastinal gray zone lymphoma; CHL, classical Hodgkin lymphoma; PMBL, primary mediastinal B-cell lymphoma.

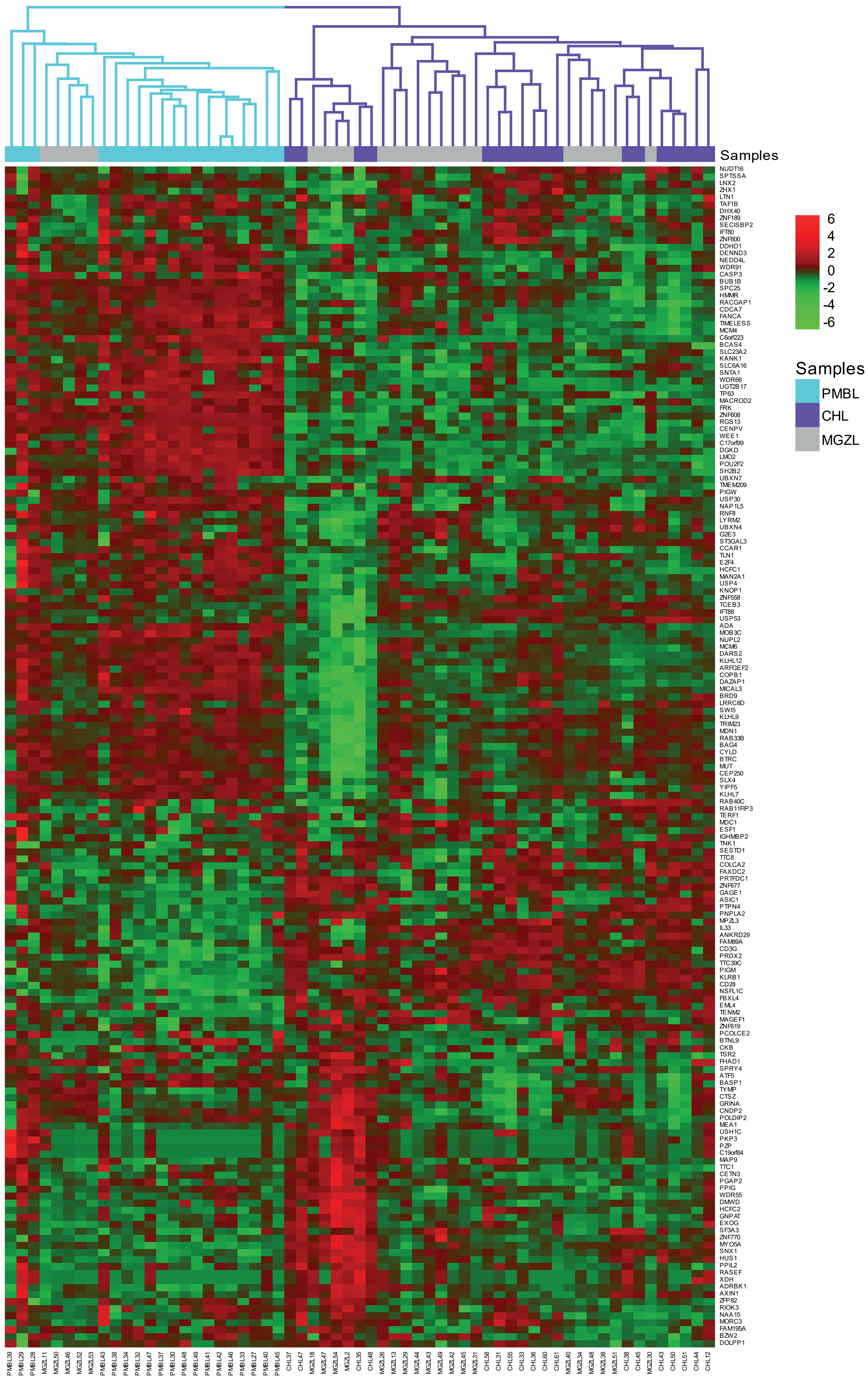

Samples
6
4
2
0
-2
-4
-6
Samples
PMBL
CHL
MGZL
NUDT16
SPTSSA
LNX2
ZHX1
LTN1
TAF1B
DHX40
ZNF189
SECISBP2
IFT80
ZNF800
DDHD1
DENND3
NEDD4L
WDR91
CASP3
BUB1B
SPC25
HMMR
RACGAP1
CDCA7
FANCA
TIMELESS
MCM4
C6orf223
BCAS4
SLC23A2
KANK1
SLC6A16
SNTA1
WDR66
UGT2B17
TP63
MACROD2
FRK
ZNF608
RGS13
CENPV
WEE1
C17orf99
DGKD
LMO2
POU2F2
SH2B2
UBXN7
TMEM209
PIGW
USP30
NAP1L5
RNF8
LYRM2
UBXN4
G2E3
ST3GAL3
CCAR1
TLN1
E2F4
HCFC1
MAN2A1
USP4
KNOP1
ZNF558
TCEB3
IFT88
USP53
ADA
MOB3C
NUPL2
MCM6
DARS2
KLHL12
ARFGEF2
COPB1
DAZAP1
MICAL3
BRD9
LRRC8D
SWI5
KLHL9
TRIM23
MDN1
RAB33B
BAG4
CYLD
BTRC
MUT
CEP250
SLX4
YIPF5
KLHL7
RAB40C
RAB11FIP3
TERF1
MDC1
ESF1
IGHMBP2
TNK1
SESTD1
TTC8
COLCA2
FAXDC2
PRTFDC1
ZNF677
GAGE1
ASIC1
PTPN4
PNPLA2
MPZL3
IL33
ANKRD29
FAM89A
CD3G
PRDX2
TTC39C
PIGM
KLRB1
CD28
NSFL1C
FBXL4
EML4
TENM2
MAGEF1
ZNF619
PCOLCE2
BTNL9
CKB
TSR2
FHAD1
SPRY4
ATF5
BASP1
TYMP
CTSZ
GRINA
CNDP2
POLDIP2
MEA1
USH1C
PKP3
PZP
C19orf84
MAP9
TTC1
CETN3
PGAP2
PPIG
WDR55
DMWD
HCFC2
GNPAT
EXOG
SF3A3
ZNF770
MYO5A
SNX1
HUS1
PPIL2
RASEF
XDH
ADRBK1
AXIN1
ZFP82
RIOK3
NAA15
MORC3
FAM195A
BZW2
DOLPP1
PMBL39
PMBL29
PMBL28
MGZL11
MGZL50
MGZL46
MGZL52
MGZL53
PMBL43
PMBL38
PMBL34
PMBL32
PMBL47
PMBL37
PMBL30
PMBL48
PMBL49
PMBL41
PMBL42
PMBL46
PMBL33
PMBL27
PMBL40
PMBL45
CHL37
CHL47
MGZL18
MGZL47
MGZL54
MGZL2
CHL35
CHL48
MGZL26
MGZL13
MGZL29
MGZL44
MGZL43
MGZL49
MGZL42
MGZL45
MGZL31
CHL58
CHL31
CHL55
CHL33
CHL36
CHL60
CHL61
MGZL40
MGZL34
MGZL48
MGZL38
MGZL51
CHL38
CHL45
MGZL30
CHL43
CHL50
CHL51
CHL44
CHL12

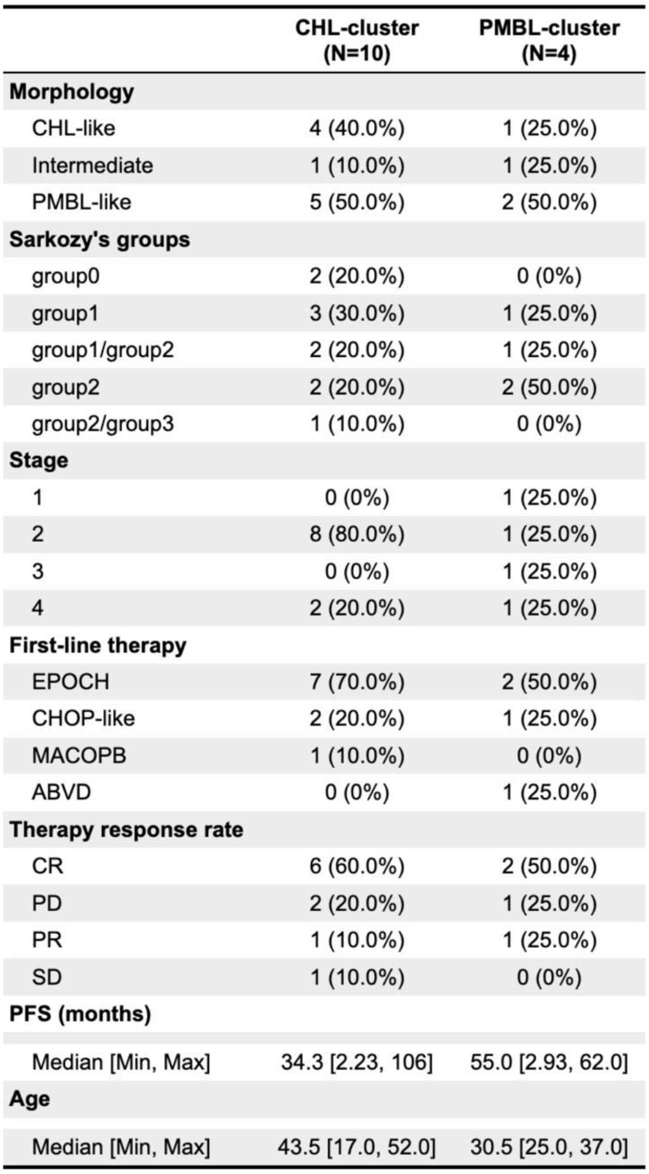
Figure S3. Transcriptional assignment of MGZL.

Heatmap showing the clustering analysis of 18 CHL, 19 PMBL, and 24 MGZL based on the 168-gene signature expression (CCC = 0.80). The two main clusters, respectively including all PMBL and all CHL samples, also incorporated the MGZL samples based on their transcriptional proximity to PMBL or CHL. CHL, classical Hodgkin lymphoma; PMBL, primary mediastinal B-cell lymphoma; MGZL, mediastinal gray zone lymphoma; CCC, cophenetic correlation coefficient.

Table S1. Morphologic and immunohistochemical features of MGZL cases and GEP cluster assignment.

Case ID	Age	Sex	Mediastinal involvment	EBV	Tumor cells (%)	Cytoarchitecture and morphology	Sarkozy's groups	Reed-Sternberg cells (%)	Degree of inflammatory background	Degree of fibrosis	Necrosis	CD15 (%, intensity)	CD20 (%, intensity)	CD30 (%, intensity)	CD79A (%, intensity)	PAX5 (%, intensity)	OCT-2 (%, intensity)	BOB-1 (%, intensity)	CD45 (%, intensity)	GEP CLUSTER
MGZL34	71	M	yes	neg	30	CHL-like	group1	>10%	low	moderate	absent	25-50, moderate	75-100, strong	75-100, strong	75-100, moderate	75-100, moderate	75-100, strong	na	na	CHL-like
MGZL13	77	F	yes	neg	60	CHL-like	group2	<10%	low	moderate	present	50-75, moderate	negative	75-100, strong	negative	75-100, moderate	negative	negative	negative	CHL-like
MGZL31	63	F	yes	neg	60	CHL-like	group1	>10%	moderate	moderate	absent	rare, moderate	25-50, weak	75-100, strong	50-75, moderate	75-100, strong	negative	25-50, moderate	na	CHL-like
MGZL43	40	M	yes	neg	20	CHL-like	group0	>10%	high	moderate	absent	negative	75-100, strong	75-100, strong	75-100, strong	75-100, moderate	75-100, moderate	75-100, moderate	negative	CHL-like
MGZL48	30	M	yes	neg	40	CHL-like	group0	>10%	moderate	high, with thick bands	absent	75-100, moderate	75-100, strong	50-75, strong	75-100, strong	75-100, strong	75-100, moderate	75-100, moderate	negative	CHL-like
MGZL51	52	F	yes	neg	30	CHL-like	group1	>10%	high	high, with thick bands	absent	negative	75-100, strong	50-75, moderate	75-100, strong	75-100, strong	75-100, strong	50-75, weak	negative	CHL-like
MGZL45	46	M	yes	neg	55	CHL-like	group1	>10%	moderate	moderate	absent	75-100, strong	75-100, strong	75-100, moderate	50-75, moderate	75-100, moderate	25-50, weak	negative	negative	CHL-like
MGZL52	37	M	yes	neg	60	CHL-like	group1	>10%	moderate	absent	absent	negative	75-100, strong	75-100, moderate	75-100, moderate	75-100, moderate- strong	75-100, strong	75-100, moderate	negative	PMBL-like
MGZL47	45	M	yes	neg	20	Intermediate	group1	>10%	moderate	moderate	absent	negative	75-100, moderate	75-100, strong	50-75, strong	75-100, strong	75-100, moderate	25-50	25-50, strong	CHL-like
MGZL18	55	M	yes	neg	50	Intermediate	group1/group2	>10%	na	moderate	absent	25-50, strong	75-100, strong	75-100, moderate	75-100, moderate	75-100, moderate	75-100, strong	75-100, strong	na	CHL-like
MGZL2	29	F	yes	neg	60	Intermediate	group1/group2	<10%	low	high	absent	negative	50-75, strong	50-75, strong	75-100	50-75, strong	75-100	75-100	50-75	CHL-like
MGZL26	37	F	yes	neg	70	Intermediate	group1/group2	>10%	low	moderate	absent	rare, moderate	25-50, moderate	75-100, strong	50-75, moderate	75-100, strong	negative	na	negative	CHL-like
MGZL46	28	F	yes	neg	45	Intermediate	group1/group2	>10%	moderate	moderate	absent	negative	75-100, strong	70, variable strong	75-100, strong	75-100, strong	75-100, strong	75-100, strong	50-75	PMBL-like
MGZL30	52	F	yes	neg	85	Intermediate	group1/group2	>10%	moderate	moderate	absent	50-75, strong	75-100, strong	75-100, strong	75-100, strong	75-100, strong	75-100, moderate	75-100, strong	negative	CHL-like
MGZL38	25	M	yes	neg	50	PMBL-like	group2/group3	<10%	moderate	high, w/o thick bands	present	negative	negative	75-100, strong	negative	50-75, weak to moderate	50-75, weak	negative	75-100	CHL-like
MGZL40	42	M	yes	neg	na	PMBL-like	group1/group2	<10%	low/moderate	high, w/o thick bands	absent	50-75, strong	75-100, strong	75-100, strong	negative	75-100, weak	50-75, strong	negative	50-75, strong	CHL-like
MGZL49	51	M	yes	neg	80	PMBL-like	group2	<10%	moderate	high, with thick bands	absent	rare	rare, weak	75-100, strong	50-75, moderate	75-100, moderate	rare, strong	negative	negative	CHL-like
MGZL54	31	M	yes	neg	70	PMBL-like	group2	<10%	low	low	present	75-100, strong	75-100, strong	75-100, strong	negative	75-100, moderate	75-100	75-100	negative	CHL-like
MGZL11	41	M	yes	neg	80	PMBL-like	group2	>10%	low	low	absent	50-75, moderate	25-50, strong	50-75, moderate	75-100	25-50, strong	25-50, strong	na	25-50	PMBL-like
MGZL42	17	M	yes	neg	70	PMBL-like	group2/group3	>10%	low	moderate	present	25-50, moderate	25-50, weak	75-100, moderate	negative	75-100, weak	rare, weak	negative	negative	CHL-like
MGZL50	33	M	yes	neg	50	PMBL-like	group2	>10%	low	moderate	present	rare	75-100, strong	75-100, moderate	25-50	75-100, strong	75-100, strong	75-100, strong	negative	PMBL-like
MGZL29	69	M	yes	neg	90	PMBL-like	group2	>10%	absent	absent	absent	rare, moderate	negative	75-100, strong	negative	75-100, moderate	negative	50-75, moderate	negative	CHL-like
MGZL53	25	M	yes	neg	50	PMBL-like	group2	<10%	moderate	absent	absent	negative	75-100, strong	50-75, weak-moderate	50-75,weak	75-100, strong	25-50, moderate	75-100	75-100	PMBL-like
MGZL44	50	F	yes	neg	na	PMBL-like	group1/group2	<10%	moderate	high, with thick bands	absent	rare	75-100, strong	75-100, strong	50-75, moderate	75-100, moderate	75-100, strong	75-100, moderate	negative	CHL-like

Table S2. Clinical characteristic and outcome of a subset of 14 MGZL patients



	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]

CHL, classical Hodgkin lymphoma; PMBL, primary mediastinal B-cell lymphoma; CR, complete response; PD, partial disease; PR, partial response; SD, stable disease; PFS, progression-free survival.