ALK-negative anaplastic large cell lymphoma with *DUSP22* rearrangement has distinctive disease characteristics with better progression-free survival: a LYSA study

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SUPPLEMENTARY APPENDIX

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Supplementary Methods

Pathology review

Diagnostic histological slides were reviewed by at least two expert pathologists and the diagnoses were confirmed according to the criteria of the 2017 WHO classification of lymphoid neoplasms.[1] Immunohistochemistry results for expression of CD30, ALK1, T-cell antigens (CD2, CD3, CD4, CD5, CD7 and CD8), epithelial membrane antigen (EMA), and cytotoxic molecules (T-cell intracellular antigen-1 [TIA1]), Granzyme B and perforin) were systematically recorded. For clinical trial patients, central pathology review had been performed at the time of inclusion with scoring of immunohistochemical results. For other TENOMIC cases the information was obtained by reviewing the existing slides, performing additional stainings using routinely validated protocols, or retrieving the information from the pathology reports. Immunostains were scored as negative, <50% positive, and >50% positive. In the analyses, all positive cases (<50% and >50%) were aggregated.

For the specific purpose of this study, immunohistochemistry for phospho-STAT3^{Tyr705} (pSTAT3) was carried out on a subset of cases, using antibody clone D3A7 (Cell Signaling Technology, Danvers, MA; dilution 1:50) on automated immunostainers (BenchMark XT, Ventana Medical systems, Tucson, AZ; or Bond-III, Leica Biosystems, Nussloch, Germany). The cutoff for positivity was set at ≥20% positive tumor nuclei, as previously published (*Luchtel RA, Dasari S, Oishi N, et al. Molecular profiling reveals immunogenic cues in anaplastic large cell lymphomas with DUSP22 rearrangements. Blood 2018;132(13):1386–1398*), and staining was considered non contributive in the absence of internal positive controls (endothelial cells).

Clinical data

Staging, frontline treatment including chemotherapy regimen and consolidative stem-cell transplantation (and salvage treatment when available) and follow-up data were collected from the clinical trial files and the treating physicians. Initial investigations included 18-fluorodeoxyglucose-positron emission tomography (PET) and/or computed tomography scans of the chest, abdomen, and pelvis; bone marrow biopsy; and biologic evaluation including lactate dehydrogenase, and beta-2-microglobulin levels. Patients were staged according to the Ann Arbor classification. The International Prognostic Index (IPI) score was calculated at diagnosis. Response to treatment, including complete response (CR), partial response (PR),

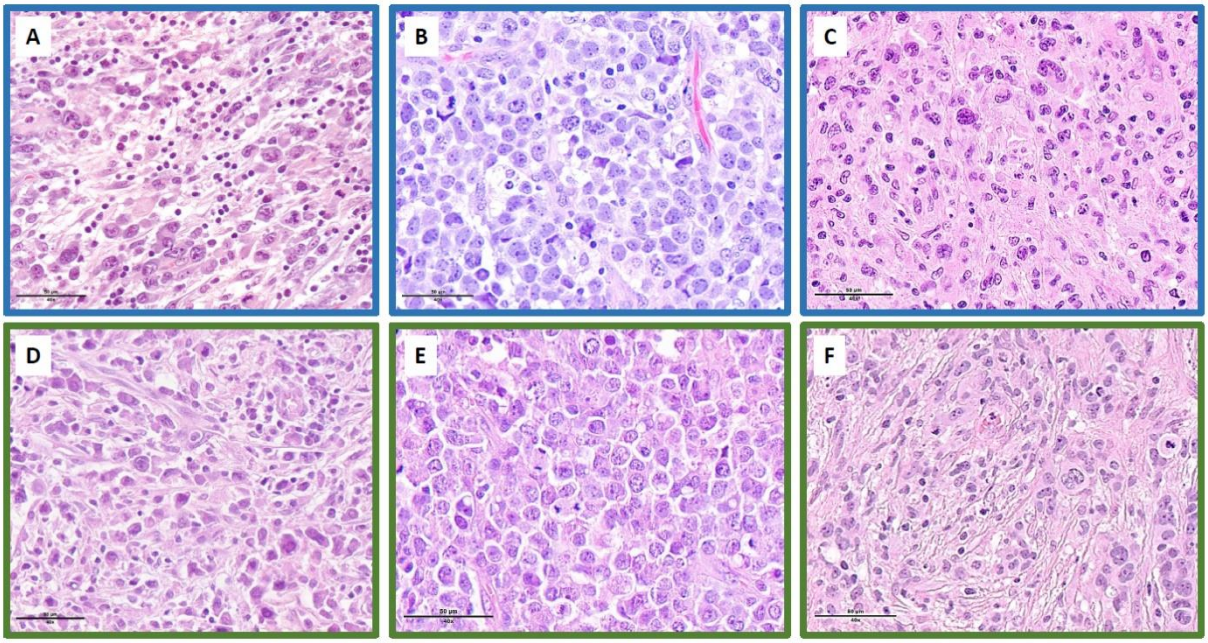
stable disease (SD), or progressive disease (PD), was assessed for evaluable patients. Objective response rate (ORR) was defined as the proportion of patients with a CR or PR to treatment. Response assessment was based on international response criteria, depending on the era (Cheson 1999, Cheson 2007 or Lugano). Regarding patients included in clinical trials, response was extracted from databases. For patients treated in routine care, response was retrieved from imaging and medical reports (collected by DS). For the current study, there was no central review of imaging.

Statistical analyses

Patient characteristics and response rates were compared using the $\chi 2$ test or Fisher's exact test when appropriate for qualitative data and the Student t test for quantitative data. Progression-free survival (PFS) was measured from the date of study entry for newly diagnosed patients included in clinical trials or the date of diagnosis for patients treated in routine care, until the date of the first event among progression, relapse or death from any cause, or the date of last contact for those who were progression-free. OS was measured from the same starting points, until death from any cause, or the date of last contact for those who were alive at the end of follow-up. OS2 was measured from the date of first progression or relapse, until death from any cause, or the date of last contact for those who were alive at the end of follow-up. Survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. PFS and OS at fixed time were estimated with 95% confidence intervals (95% CI). Median follow-up was estimated by the reverse Kaplan-Meier method. The associations between patient characteristics or treatment type and PFS or OS were analyzed by Cox proportional hazard models. Effect sizes of covariates were quantified by the hazard ratios (HR). Statistical tests were considered significant if two-sided *P* values were <0.05. All statistical analyses were performed using R v3.6 (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

Supplementary Figures

Figure S1. Morphologic spectrum and overlapping characteristics of *DUSP22*-R and *DUSP22*-NR ALK-negative ALCL.



Cases representative of the two genomic subgroups (A-C: *DUSP22*-NR; D-F: *DUSP22*-R) are illustrated. Cases A and D are characterized by prominent interstitial fibrosis, small background lymphocytes and large pleomorphic anaplastic cells. Cases B and E represent tumors with rather monomorphic large cells, less conspicuous nucleoli and without prominent anaplastic features. Cases C and F both contain many hallmark cells and doughnut-type cells. All photomicrographs are from routinely HE (hematoxylin-Eosin) stained sections and were taken at original x400 magnification.

Figure S2. Survival of the 84 *TP63*-NR patients treated with curative intent front-line anthracycline-based chemotherapy according to inclusion in first-line clinical trials. (A) Progression-free survival and (B) overall survival.

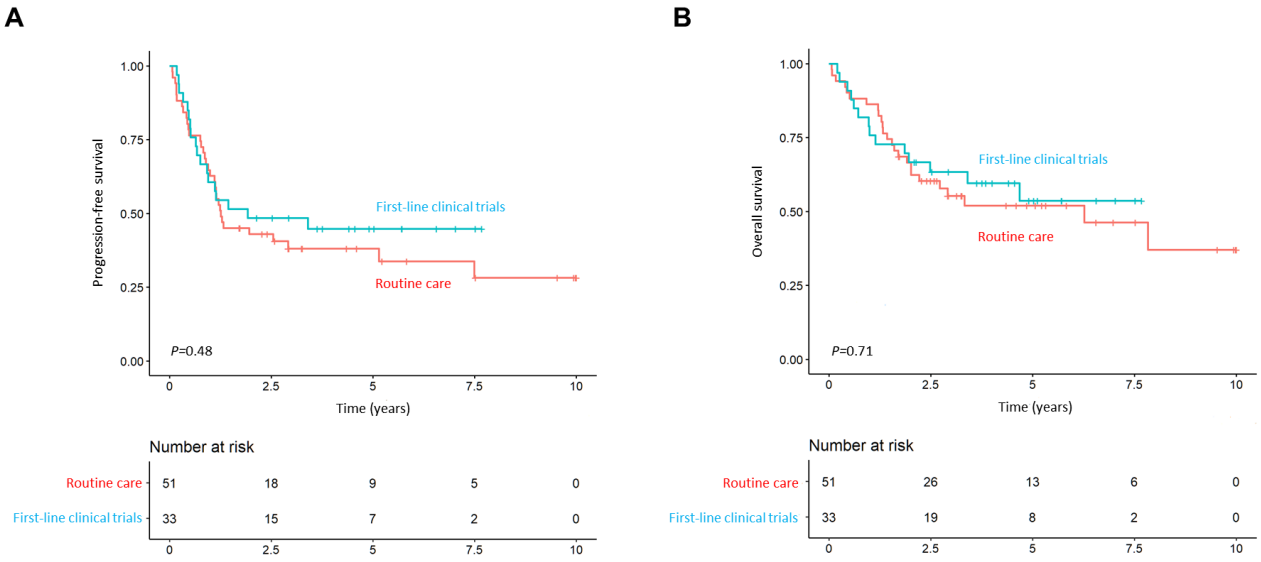
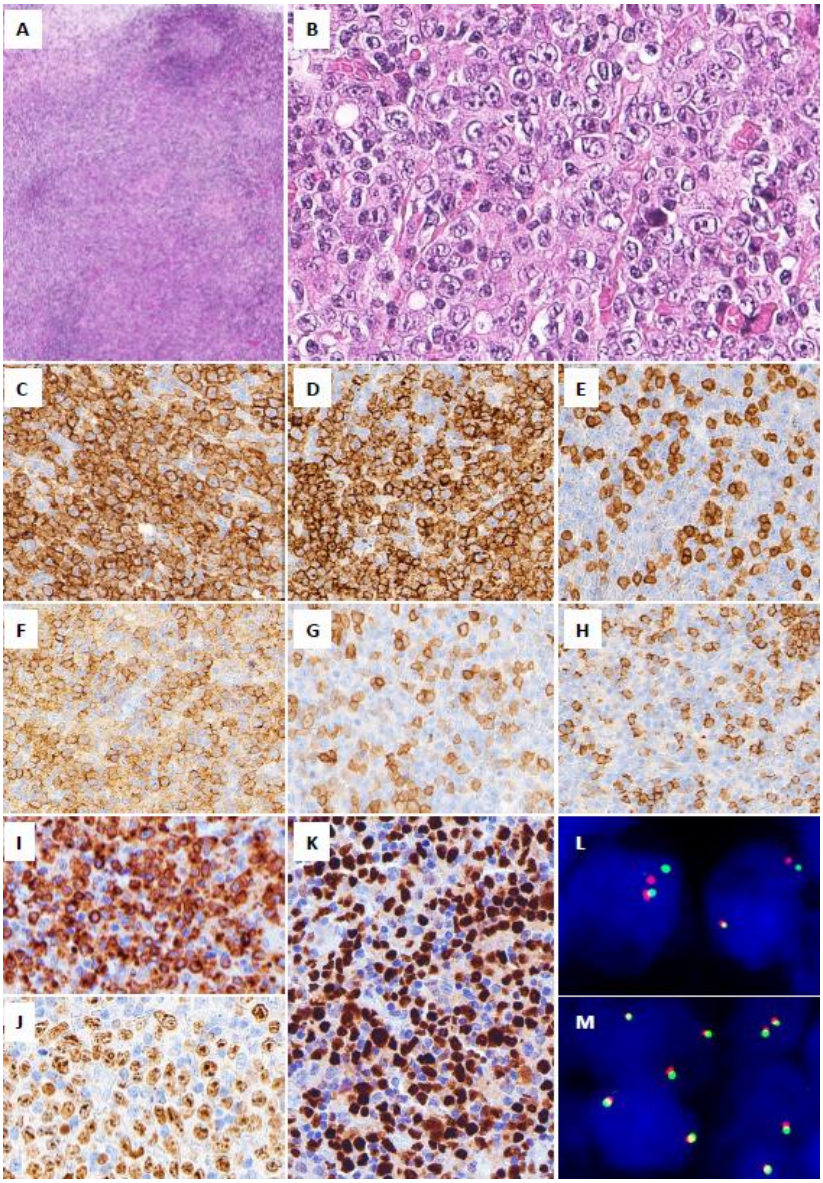


Figure S3. ALK-negative ALCL with *TP63* rearrangement.



(A-B) The tumor effaces the lymph node architecture, is associated with fibrosis and comprises cohesive sheets of rather monomorphic large atypical lymphoid cells with oval to irregular nuclei, multiple nucleoli, and moderately abundant cytoplasm (hematoxylin and eosin, original magnifications x100 and x400); (C-J) on immunohistochemical stains the neoplastic cells are strongly CD30+ (C), CD2+ (D), CD3- (E), CD4+ (F), CD5- (G), CD8- (H), with strong expression of perforin (I) and a high Ki67 proliferation index (J) (all immunoperoxidase, original magnification x400); (K-L) p63 was strongly positive by immunohistochemistry (K) (immunoperoxidase, x400) and break-apart FISH assay showed a rearrangement of the *TP63* locus (L) ; (M) *DUSP22* FISH assay showed a normal hybridization pattern (x630).

Supplementary Tables

Table S1. Patient and disease characteristics according to inclusion in first-line clinical trials.

Clinical features at diagnosis	All patients	Patients in routine care	Patients in first-line clinical trials	*P*
n	104	67	37	
Diagnosis era	2001-2020	2001-2020	2012-2017	
Age (years)				
Median (range)	60 (39-86)	61 (39-86)	59 (41-78)	
>60	53/104 (51%)	36/67 (54%)	17/37 (46%)	0.579
Male	77/104 (74%)	47/67 (70%)	30/37 (81%)	0.325
Performance status ≥ 2	37/103 (36%)	26/66 (39%)	11/37 (30%)	0.443
Staging at diagnosis				0.422
PET	84/100 (84%)	51/63 (81%)	33/37 (89%)	
CT	16/100 (16%)	12/63 (19%)	4/37 (11%)	
Ann Arbor stage (1-2 *vs* 3-4)				1
1	8/104 (8%)	7/67 (10%)	1/37 (3%)	
2	21/104 (20%)	12/67 (18%)	9/37 (24%)	
3	20/104 (19%)	14/67 (21%)	6/37 (16%)	
4	55/104 (53%)	34/67 (51%)	21/37 (57%)	
Involved site (any)				
Bone	22/103 (21%)	14/66 (21%)	8/37 (22%)	1
Liver	17/103 (17%)	12/66 (18%)	5/37 (14%)	0.737
Bone marrow	13/103 (13%)	8/66 (12%)	5/37 (14%)	1
Lung	13/103 (13%)	9/66 (14%)	4/37 (11%)	0.916
Spleen	12/103 (12%)	8/66 (12%)	4/37 (11%)	1
Soft tissue	12/103 (12%)	9/66 (14%)	3/37 (8%)	0.604
Skin	10/103 (10%)	5/66 (8%)	5/37 (14%)	0.529
Gastrointestinal tract	7/103 (7%)	3/66 (5%)	4/37 (11%)	0.421
Parotid	4/103 (4%)	4/66 (6%)	0/37 (0%)	0.319
Nasopharynx	3/103 (3%)	0/66 (0%)	3/37 (8%)	0.082
Tonsil	2/103 (2%)	0/66 (0%)	2/37 (5%)	0.245
Sinus	2/103 (2%)	2/66 (3%)	0/37 (0%)	0.745
Thyroid	1/103 (1%)	1/66 (2%)	0/37 (0%)	1
Adrenal	1/103 (1%)	1/66 (2%)	0/37 (0%)	1
Blood	1/103 (1%)	1/66 (2%)	0/37 (0%)	1
Ascites	1/103 (1%)	1/66 (2%)	0/37 (0%)	1
Pleura	0/103 (0%)	0/66 (0%)	0/37 (0%)	---
Extranodal site >1	29/104 (28%)	20/67 (30%)	9/37 (24%)	0.709
Elevated lactate dehydrogenase	58/103 (56%)	39/66 (59%)	19/37 (51%)	0.580
Beta-2-microglobulin ≥ 3 mg/L	24/55 (44%)	12/25 (48%)	12/30 (40%)	0.747
IPI score				0.093
0-1	29/103 (28%)	19/66 (29%)	10/37 (27%)	
2	24/103 (23%)	12/66 (18%)	12/37 (32%)	
3	26/103 (25%)	15/66 (23%)	11/37 (30%)	
4-5	24/103 (23%)	20/66 (30%)	4/37 (11%)	
***DUSP22*-R**	47/104 (45%)	34/67 (51%)	13/37 (35%)	0.185
Primary therapy				**<0.001**
CHOP	45/104 (43%)	28/67 (42%)	17/37 (46%)	
CHOEP	24/104 (23%)	16/67 (24%)	8/37 (22%)	
Romidepsin-CHOP	10/104 (10%)	0/67 (0%)	10/37 (27%)	
BV-CH(E)P	6/104 (6%)	4/67 (6%)	2/37 (6%)	

Mini-CHOP	7/104 (7%)	7/67 (10%)	0/37 (0%)	
ACVBP	5/104 (5%)	5/67 (8%)	0/37 (0%)	
Non-curative care	7/104 (7%)	7/67 (10%)	0/37 (0%)	
Consolidative transplantation				0.749
AutoSCT	14/104 (13%)	10/67 (15%)	4/37 (11%)	
AlloSCT	5/104 (5%)	3/67 (4%)	2/37 (5%)	
Auto-minialloSCT tandem	1/104 (1%)	1/67 (1%)	0/37 (0%)	

ACVBP: doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone; ALCL: anaplastic large cell lymphoma; ALK: anaplastic lymphoma kinase; BV: brentuximab vedotin; CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisone; CHOEP: CHOP + etoposide; IPI: international prognostic index; FISH: fluorescence in situ hybridization; SCT: stem-cell transplantation.

Table S2. Patient and disease characteristics of the 84 *TP63*-NR patients treated with curative intent front-line anthracycline-based chemotherapy.

Clinical features at diagnosis	Patients	Triple-negative ALCL	*DUSP22*-R/*TP63*-NR ALK-negative ALCL	*P*
n	84	45	39	
Diagnosis era	2002-2020	2002-2020	2004-2019	
Age (years)				
Median (range)	60 (40-86)	63 (41-85)	59 (40-86)	
>60	43/84 (51%)	24/45 (53%)	19/39 (49%)	0.839
Male	64/84 (76%)	33/45 (73%)	31/39 (80%)	0.687
Performance status ≥ 2	29/83 (35%)	18/45 (40%)	11/38 (29%)	0.412
Staging at diagnosis				0.824
PET	69/82 (84%)	37/45 (82%)	32/37 (86.5%)	
CT	13/82 (16%)	8/45 (18%)	5/37 (13.5%)	
Ann Arbor stage (1-2 *vs* 3-4)				1
1	6/84 (7%)	2/45 (4%)	4/39 (10%)	
2	19/84 (23%)	11/45 (24%)	8/39 (21%)	
3	16/84 (19%)	12/45 (27%)	4/39 (10%)	
4	43/84 (51%)	20/45 (44%)	23/39 (59%)	
Involved site (any)				
Bone	17/84 (20%)	5/45 (11%)	12/39 (31%)	0.05
Liver	14/84 (17%)	6/45 (13%)	8/39 (21%)	0.557
Bone marrow	11/84 (13%)	5/45 (11%)	6/39 (15%)	0.799
Lung	10/84 (12%)	4/45 (9%)	6/39 (15%)	0.563
Spleen	11/84 (13%)	4/45 (9%)	7/39 (18%)	0.366
Soft tissue	11/84 (13%)	10/45 (22%)	1/39 (3%)	0.019
Skin	8/84 (10%)	2/45 (4%)	6/39 (15%)	0.183
Gastrointestinal tract	6/84 (7%)	4/45 (9%)	2/39 (5%)	0.808
Parotid	3/84 (4%)	1/45 (2%)	2/39 (5%)	0.899
Nasopharynx	3/84 (4%)	1/45 (2%)	2/39 (5%)	0.899
Tonsil	1/84 (1%)	0/45 (0%)	1/39 (3%)	0.943
Sinus	2/84 (2%)	1/45 (2%)	1/39 (3%)	1
Thyroid	1/84 (1%)	0/45 (0%)	1/39 (3%)	0.943
Adrenal	1/84 (1%)	0/45 (0%)	1/39 (3%)	0.943
Blood	0/84 (0%)	0/45 (0%)	0/39 (0%)	---
Ascites	1/84 (1%)	0/45 (0%)	1/39 (3%)	0.943
Pleura	0/84 (0%)	0/45 (0%)	0/39 (0%)	---
Extranodal site >1	22/84 (26%)	12/45 (27%)	10/39 (26%)	1
Elevated lactate dehydrogenase	43/83 (52%)	21/45 (47%)	22/38 (58%)	0.424
Beta-2-microglobulin ≥ 3 mg/L	21/49 (43%)	16/32 (50%)	5/17 (29%)	0.279
IPI score*				0.558
0-1	25/83 (30%)	11/45 (24%)	14/38 (37%)	
2	20/83 (24%)	13/45 (29%)	7/38 (18%)	
3	21/83 (25%)	12/45 (27%)	9/38 (24%)	
4-5	17/83 (20%)	9/45 (20%)	8/38 (21%)	
First-line clinical trial	33/84 (39%)	20/45 (44%)	13/39 (33%)	0.415
Primary therapy				0.189
CHOP	38/84 (45%)	20/45 (44%)	18/39 (46%)	
CHOEP	21/84 (25%)	10/45 (22%)	11/39 (28%)	
Romidepsin-CHOP	10/84 (12%)	9/45 (20%)	1/39 (3%)	
BV-CH(E)P	4/84 (5%)	2/45 (4%)	2/39 (5%)	
Mini-CHOP	7/84 (8%)	2/45 (4%)	5/39 (13%)	
ACVBP	4/84 (5%)	2/45 (4%)	2/39 (5%)	
Consolidative transplantation				0.336

AutoSCT	11/84 (13%)	3/45 (7%)	8/39 (21%)	
AlloSCT	3/84 (4%)	1/45 (2%)	2/39 (5%)	
Auto-minialloSCT tandem	1/84 (1%)	1/45 (2%)	0/39 (0%)	

ACVBP: doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone; ALCL: anaplastic large cell lymphoma; ALK: anaplastic lymphoma kinase; BV: brentuximab vedotin; CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisone; CHOEP: CHOP + etoposide; IPI: international prognostic index; FISH: fluorescence in situ hybridization; SCT: stem-cell transplantation.

*The IPI score in 3 classes (0-1 *versus* 2-3 *versus* 4-5) also was not significantly different between the 2 groups.

Table S3. Immunophenotypic characteristics of 84 tumors from patients treated with curative intent front-line anthracycline-based chemotherapy.

	All patients (n=84)	**Triple-negative (n=45)**	***DUSP22*-R ALCL (n=39)**	***P***
CD30	84/84	45/45	39/39	1
ALK	0/84	0/45	0/39	1
T-cell antigens				
CD3	39/84 (46%)	15/45 (33%)	24/39 (62%)	**0.02**
CD5	27/78 (35%)	12/42 (29%)	15/36 (42%)	0.2
CD2	54/72 (75%)	25/39 (64%)	29/33 (88%)	**0.03**
CD7	10/61 (16%)	6/32 (19%)	4/29 (14%)	0.7
CD4	57/79 (72%)	30/40 (75%)	27/39 (69%)	0.6
CD8	11/72 (15%)	6/35 (17%)	5/37 (14%)	0.8
CD4+ CD8-	47/71 (66%)	22/34 (65%)	25/37 (68%)	0.8
CD4- CD8-	13/71 (18%)	6/34 (18%)	7/37 (19%)	1
CD4- CD8+	8/71 (11%)	4/34 (12%)	4/37 (11%)	1
CD4+ CD8+	3/71 (4%)	2/34 (6%)	1/37 (3%)	0.6
EMA	33/71 (46%)	29/38 (76%)	4/33 (12%)	**<0.0001**
Cytotoxic markers				
TIA1	19/66 (29%)	15/35 (43%)	4/31 (13%)	**0.01**
Granzyme B	21/77 (27%)	17/40 (43%)	4/37 (11%)	**0.002**
Perforin	23/62 (37%)	20/33 (61%)	3/29 (10%)	**<0.0001**
Cytotoxic profile*	36/63 (57%)	30/37 (81%)	6/26 (23%)	**<0.0001**
pSTAT3	19/39 (49%)	17/21 (81%)	2/18 (11%)	**<0.0001**

*Taking into consideration only fully conclusive cases, either negative for the three cytotoxic molecules analyzed, or positive for at least one of them.

Table S4. Response to treatment.

	Patients (n=84)	Triple-negative ALCL (n=45)	*DUSP22*-R/*TP63*-NR ALK-negative ALCL (n=39)	*P*
CR	56 (66.7%)	25 (55.6%)	31 (79.5%)	0.147
PR	7 (8.3%)	4 (8.9%)	3 (7.7%)	
SD	2 (2.4%)	1 (2.2%)	1 (2.6%)	
PD	15 (17.9%)	12 (26.7%)	3 (7.7%)	
NE	4 (4.8%)	3 (6.7%)	1 (2.6%)	

CR: complete response; NE: not evaluable; PD: progressive disease; PR: partial response; SD: stable disease.

Table S5. Univariate analysis of the impact of clinical and laboratory features on progression-free survival and overall survival.

Parameter	n with available data	PFS		OS	
		P	HR (95% CI)	*P*	HR (95% CI)
Male sex	84	0.56	1.221 (0.626 - 2.381)	0.67	0.857 (0.417 - 1.761)
Age >60	84	0.32	1.327 (0.759 - 2.319)	0.54	1.220 (0.646 - 2.304)
Performance status ≥ 2	83	**<0.001**	**2.645 (1.503 - 4.657)**	**<0.001**	**3.199 (1.694 - 6.040)**
Ann Arbor stage III-IV	84	0.54	1.207 (0.660 - 2.206)	0.90	1.047 (0.529 - 2.073)
No. of extranodal sites >1	84	0.23	1.446 (0.791 - 2.646)	0.13	1.666 (0.853 - 3.251)
Elevated lactate dehydrogenase	83	0.81	1.070 (0.614 - 1.865)	0.33	1.364 (0.724 - 2.572)
IPI score*	83	0.2		0.51	
2			1.609 (0.722 - 3.586)		1.419 (0.561 - 3.589)
3			2.158 (1.008 - 4.620)		1.733 (0.715 - 4.201)
4-5			1.984 (0.871 - 4.521)		2.344 (0.945 - 5.815)
Beta-2-microglobulin ≥ 3 mg/L	49	**0.045**	**2.115 (1 - 4.472)**	**0.007**	**3.207 (1.319 - 7.797)**
DUSP22-R	84	**0.001**	**0.391 (0.219 - 0.700)**	0.067	0.547 (0.284 - 1.053)
First-line clinical trials	84	0.48	0.953 (0.547 - 1.661)	0.71	1.078 (0.565 - 2.054)
CD3+	84	0.65	1.133 (0.654 - 1.964)	0.22	1.482 (0.788 - 2.788)
CD5+	78	0.52	0.815 (0.439 - 1.511)	0.33	1.400 (0.705 - 2.780)
CD2+	72	0.60	0.832 (0.419 - 1.652)	0.37	1.499 (0.618 - 3.638)
CD7+	61	0.27	1.595 (0.696 - 3.658)	0.052	2.337 (0.971 - 5.628)
CD4+	79	0.54	1.226 (0.636 - 2.364)	0.15	1.818 (0.791 - 4.177)
CD8+	72	0.98	1.015 (0.450 - 2.287)	0.48	0.687 (0.241 - 1.959)
EMA+	71	0.088	1.699 (0.918 - 3.144)	0.28	1.463 (0.729 - 2.936)
TIA1+	66	0.49	1.278 (0.635 - 2.571)	0.79	1.120 (0.495 - 2.535)
Granzyme B+	77	**0.021**	**2.025 (1.100 - 3.728)**	**0.016**	**2.299 (1.144 - 4.617)**
Perforin+	62	**<0.001**	**3.022 (1.565 - 5.836)**	**0.014**	**2.501 (1.177 - 5.312)**
Cytotoxic profile**	63	**0.01**	**2.367 (1.231 - 4,553)**	0.08	1,913 (0,927 - 3,949)

CI: Confidence interval; HR: Hazard ratio; IPI: International Prognostic Index; OS: Overall survival; PFS: Progression-free survival.

* The IPI score in 3 classes (0-1 *versus* 2-3 *versus* 4-5) or in 2 classes (0-2 *versus* 3-5; or 0-3 *versus* 4-5) also had no significant prognostic impact in PFS and OS.

** Taking into consideration only fully conclusive cases, either negative for the three cytotoxic molecules analyzed, or positive for at least one of them.

TENOMIC consortium members

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