DUSP22-rearranged anaplastic lymphomas are characterized by specific morphological features and a lack of cytotoxic and JAK/STAT surrogate markers

ALK-negative anaplastic large cell lymphoma (ALK-negative ALCL) is a heterogeneous disease with very disparate outcomes. Molecular studies have identified chromosomal rearrangements involving the *DUSP22-IRF4* locus on 6p25.3 (*DUSP22* rearrangements) as a favorable prognostic factor, associated with complete remission after first treatment thereby suggesting that this subgroup of patients may not gain additional benefit from autologous stem cell transplantation in first remission.[1-3] Recognition of these cases is critical, and we therefore aimed to study in greater detail the histological and immunophenotypic features of *DUSP22*-rearranged ALK-negative ALCLs.

After approval by the Institutional Review Board of the Hospital Universitario Marqués de Valdecilla and the Fundación Jiménez Díaz, Spain, we collected 91 cases with a diagnosis of systemic or primary cutaneous ALCL made at the participating institutions. Clinical data were retrieved and cases were reviewed by 3 independent pathologists (AO, SMRP, and MAP) using hematoxylin & eosin stains. Immunohistochemistry was performed using a panel of antibodies against ALK, CD3, CD4, CD8, granzyme B, MUM1, perforin, P-STAT3 (D3A7, 1/400 Cell Signaling), TIA1, P-STAT5, TCR-βF1, P63, STAT3 (*Online Supplementary Appendix*). Of 91 evaluated cases, 18 were primary cutaneous ALCLs (pcALCLs) and 73 cases were systemic ALCLs (19 were ALK-positive ALCLs). ALK-positive cases were not further considered for the study. Only 31 cases were eligible for further study due to tissue scarcity, including 22 ALK-negative ALCL and 9 pcALCLs. Fluorescence *in situ* hybridization (FISH) analyses were performed on these cases using an IRF4-DUSP22 (6p25.3) break-apart probe (KBI-10613; Kreatech, Leica, Spain) following standard procedures.[4,5] Cytotoxic markers, pSTAT3, p63 and MUM1 expression were evaluated as described in the *Online Supplementary Appendix*. Associations of genetic and immunohistochemical subgroups with overall survival (OS) and progression-free survival were assessed using Kaplan-Meier curves. Differences between genetic subgroups in patients' characteristics, tumor phenotype and other clinical factors were assessed using the χ^2 test and Wilcoxon rank-sum test, as appropriate.

Of the 31 cases tested for p63 rearrangements, 1 case (1 out of 31, 3.2%) was positive, 26 were negative (26 out of 31, 83.8%), and 3 showed gains of p63 (3 out of 31, 9.7%). One case (1 out of 22, 4.5%) had *DUSP22* gains, and another case had *DUSP22* amplification. Twenty-five cases (25 out of 31, 80.6%) were classified as triple-negative ALCLs, and 6 cases had *DUSP22* rearrangements, including 4 ALK-negative ALCLs (4 out of 22, 18.2%) and 2 pcALCLs (2 out of 9, 22.2%), representing the study cohort.

Demographic and clinical characteristics of *DUSP22*-rearranged cases are shown in Table 1. The 6 patients were aged 39-65 years at presentation (mean, 56 years), with a predominance of males (2M:1F). In one of the pcALCL cases (case 5), the lesions were restricted to a single body area (the cheek); the site location was not available for case 6. Systemic *DUSP22*-rearranged cases exhibited a high clinical stage at presentation, with low Eastern Cooperative Oncology Group performance status, International Prognostic Index and Prognostic Index for T-cell lymphomas. One patient had bone marrow involvement at diagnosis and high lactate dehydrogenase levels. Two patients received CHOP-based treatment regimens, and another received radiotherapy. All 3 patients achieved complete remission according to the available clinical information. Only the patient receiving radiotherapy as front-line treatment relapsed nine months after initial treatment. None of them underwent stem cell transplantation. After a median follow up of 55 months, all 4 patients with systemic *DUSP22*-rearranged ALCL were alive without disease. Patients with pcALCL were treated by excision, and there was no recurrence or progression during follow up (Table 1). Median follow-up time from diagnosis for systemic ALCL patients who were still alive was 43 months (range, 3-126 months).

Consistent with the results of previous studies, patients with ALK-negative ALCL had a poorer outcome than patients with ALK-positive ALCL [3-year (y) OS: 52%, 95% Confidence Interval (CI): 36-68% *vs.* 80%, 95%CI: 60-100%; log-rank, *P*=0.156]. Patients with systemic *DUSP22*-rearranged ALCL showed better OS rates than the triple-negative ALCL genetic subtype (3-y OS: 100% *vs.* 28%, 96%CI: 4-72%; log-rank, *P*=0.05, for triple-negative patients) and similar to ALK-positive ALCL patients (3-y OS: 80%, 96%CI: 60-100%; log-rank, *P*=0.422) (Figure 1).

As previously described,[6] *DUSP22*-rearranged ALCLs showed unusual histological features that were consis-

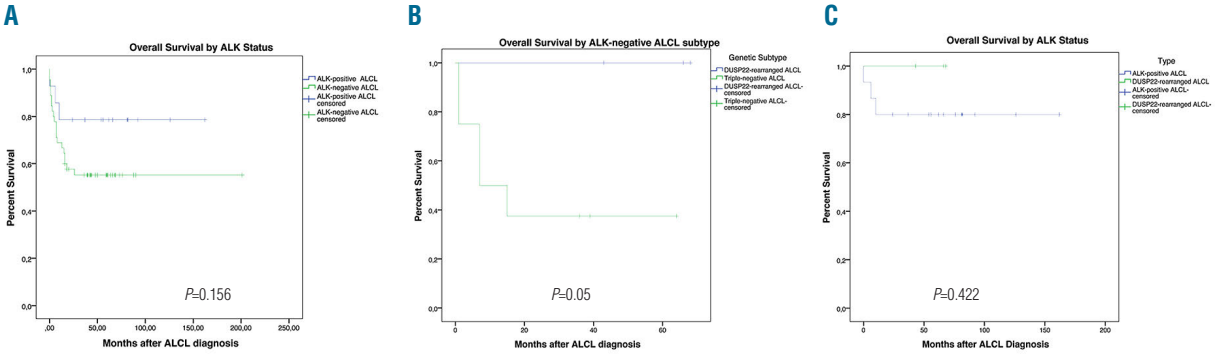

Figure 1. Outcome in patients with anaplastic large cell lymphoma (ALCL) based on genetic subtype. (A) Overall survival (OS) rates in patients with ALCL, stratified by ALK status. (B) OS rates in patients with systemic ALK-negative ALCL, stratified by rearrangements. (C) OS rates in patients with ALK-positive ALCL and DUSP22-rearranged ALCL.

Table 1. Clinical, histological, immunophenotypic, and genetic features of 6 patients with *DUSP22*-rearranged anaplastic large cell lymphoma.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Clinical presentation						
Age at diagnosis	65	71	50	39	73	39
Gender	M	F	M	M	F	M
Site	Lymph node	Lymph node	Lymph node	Lymph	Skin (right cheek)	Skin
Ann-Arbor Stage	III	IV	–	III	–	–
ECOG Status	0-1	0-1	–	–	–	–
IPI	Low-intermediate	–	–	Low	–	–
PIT	Group 1 (PIT=0)	–	–	–	–	–
Extranodal involvement	Pleural effusion	Skin	–	Tonsil	–	–
Bone marrow involvement	Absent	Present	Absent	Absent	Absent	Absent
Histological features	Hallmark cells	Hallmark cells	Hallmark cells	Hallmark cells	Hallmark cells	Hallmark cells
Cell morphology	Doughnut cells	Doughnut cells	Doughnut cells	Doughnut cells	Doughnut cells	Doughnut cells
Pattern	Sheet-like growth pattern	Sheet-like growth pattern	Sheet-like growth pattern	Sheet-like growth pattern	Biphasic pattern (dermal nodule and pagetoid reticulosis-like epidermal infiltrate)	Biphasic pattern (dermal nodule and pagetoid reticulosis-like epidermal infiltrate)
Background	Inflamatory infiltrate absent. Macrophages with tingible bodies. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.
Pathological diagnosis	Systemic ALK-negative ALCL	Systemic ALK-negative ALCL	Systemic ALK-negative ALCL	Systemic ALK-negative ALCL	pcALCL	pcALCL
Immunophenotype						
ALK	Negative	Negative	Negative	Negative	Negative	Negative
CD3	Positive	Positive	Positive	Positive	Negative	Positive
CD30	Positive	Positive	Positive	Positive	Positive	Positive
TCR F1	Positive	–	Positive	Positive	Positive	Positive
TIA-1	Negative (10%)	Negative (15%)	Negative (25%)	Negative (5%)	Negative (10%)	Negative (5%)
Granzyme B	Negative (1%)	Negative (0%)	Negative (15%)	Negative (5%)	Negative (1%)	Negative (1%)
Perforin	Negative (1%)	Negative (5%)	Negative (5%)	Negative (0%)	Negative (0%)	Negative (0%)
MUM1	Positive (95%)	Positive (85%)	Positive (100%)	Positive (75%)	Positive (95%)	Negative (0%)
p63	Negative (15%)	Positive (100%)	Positive (85%)	Negative (0%)	–	Negative (0%)
P-STAT1	Negative (<1%)	–	Negative (<1%)	Negative (<1%)	–	Negative (<1%)
P-STAT3	Negative (0%)	–	Negative (15%)	Negative (7%)	Negative (2%)	Negative (10%)
P-STAT5	Negative (0%)	–	Negative (2%)	Negative (2%)	–	Negative (2%)
STAT3	–	Negative (15%)	–	–	–	–
Cytotoxic phenotype	Absent	Absent	Absent	Absent	Absent	Absent
Follow up						
Treatment	CHOP	RT	–	CHOEP	Excision	Excision
Treatment response	CR	CR	CR	CR	–	–
Recurrence/progression	No	Yes (skin)	No	No	No	No
SCT	No	No	No	No	No	No
Status at last follow up	NED	NED	NED	NED	NED	NED
Months since onset	68	66	43	7	1423	12
Months disease free	68	9	43	7	1423	12

ALCL: anaplastic large cell lymphoma; pcALCL: primary cutaneous ALCL; M: male; F: female; CHOP: cyclophosphamide + hydroxydaunorubicin + vincristine prednisone; RT: radiotherapy; CHOEP: cyclophosphamide + hydroxydaunorubicin + vincristine + etoposide + prednisone; RT: radiotherapy; SCT: stem cell transplantation; NED: no evidence of disease; CR: complete remission.

tent among all cases. In the systemic cases, lymph node architecture was effaced, with neoplastic infiltration by intermediate cells that were smaller than those observed in triple-negative and ALK-positive ALCLs, with a sheet-like growth pattern, and a monomorphic appearance. Histopathological findings were consistent among all cases. Neoplastic cells exhibited prominent nucleoli and pseudo-inclusions in the so-called "doughnut" cells, although they were not specific to this group. Hallmark cells, mitotic figures and apoptotic bodies were abundant. Tumor cells were predominant, with no lympho-histiocytic or inflammatory background infiltrate. No sinusoidal involvement was observed, in contrast to the pattern commonly observed in ALK-positive ALCLs (Figure 2). Triple-negative ALCL cases had a more variable morphology, with the presence of hallmark cells and large pleomorphic and multinucleated cells.

The 2 pcALCL cases with *DUSP22* rearrangements had a biphasic pattern, as previously reported by our group.[7] A prominent dermal nodule with a dense lymphoid infiltrate and overlying ulceration was noted at low magnification. The neoplastic infiltrate was composed of medium-to-large atypical cells, with abundant finely granular cytoplasm, intermingled with abundant hallmark cells. A characteristic pagetoid reticulosis-like intraepidermal lymphocytosis pattern was also present, along with intraepidermal small atypical lymphocytes featuring hyperchromatic and irregular nuclei. Mitotic figures and apoptotic bodies were abundant within the dermal infiltrate. Eosinophils and neutrophils were absent (Figure 3).

Among *DUSP22*-rearranged cases, neoplastic cells were positive in all cases for at least one T-cell antigen (Table 1), CD3 and/or the T-cell receptor (TCR) β chain (TCRβF1), negative for ALK, and strongly and diffusely positive for CD30. TCRβF1 stain was not available in case 2, but CD3 was positive. Case 5 was CD3-negative but TCRβF1-positive. These markers accentuated the sheet-like growth pattern in the systemic cases, and the epidermotropic pagetoid reticulosis-like infiltrate in the primary cutaneous cases. All cases had a non-cytotoxic phenotype. TIA-1 was negative in all cases, being found in 5-25% of the tumoral cells. Granzyme B and perforin were also negative in all cases (<5% of tumoral cells). MUM1 was positive in 4 cases (median expression in 95% of tumoral cells, range: 75-100%), and only case 6 was completely negative. P63 expression was more variable, being positive in 2 out of 5 cases tested (85-100% of tumoral cells), and negative (<15% of tumoral cells) in 3

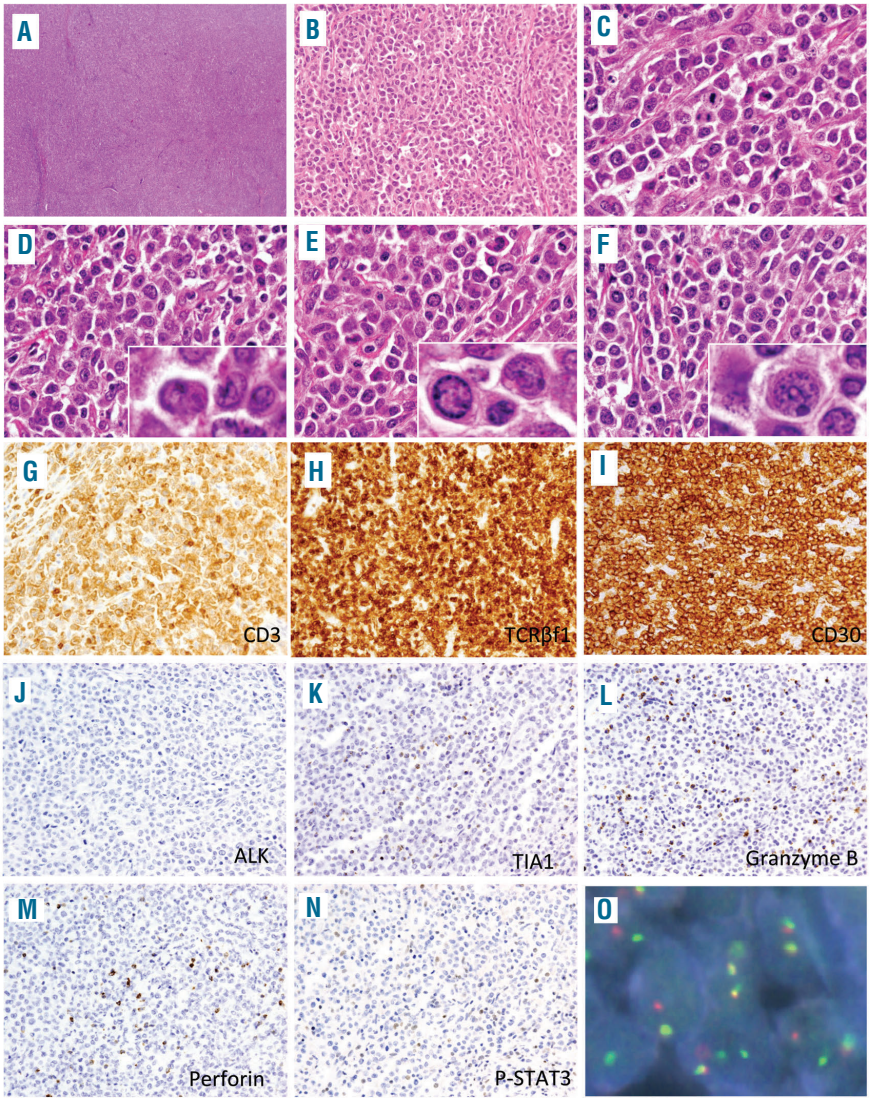

Figure 2. Histological and immunophenotypic features of systemic ALK-negative anaplastic large cell lymphoma with *DUSP22* rearrangement (case 1). (A) Low-power microscopic image of a lymph node with effaced architecture. (B) Sheets of medium-to-large neoplastic cells (C) with abundant hallmark cells, apoptotic cells and doughnut cells (inset), with an eosinophilic nuclear inclusion (D, E and F). Neoplastic cells were diffusely positive for CD3 (G), TCRβF1 (H), and CD30 (I), and negative for ALK (J), cytotoxic markers TIA-1 (K), granzyme B (L) and perforin (M), and for p-STAT3 (N). (O) FISH using a break-apart probe at the *DUSP22* locus shows a rearrangement, with one normal fusion signal and an abnormal split signal.

out of 5 cases. The three surrogate markers of the JAK/STAT pathway (phosphorylated STAT1, STAT3 and STAT5) were consistently negative in all 6 cases (expression in <20% of tumoral cells).

In this study, we report 6 cases of *DUSP22*-rearranged ALCL (systemic and cutaneous) with common histological features, with the presence of intermediate cells with a doughnut-like morphology, and abundant hallmark cells, apoptotic and mitotic figures, as previously reported.[6] In addition, both primary cutaneous cases exhibited a biphasic pattern,[7,8] which has also been described in lymphomatoid papulosis cases carrying the same translocation.[8]

Furthermore, our results support those recently pub-

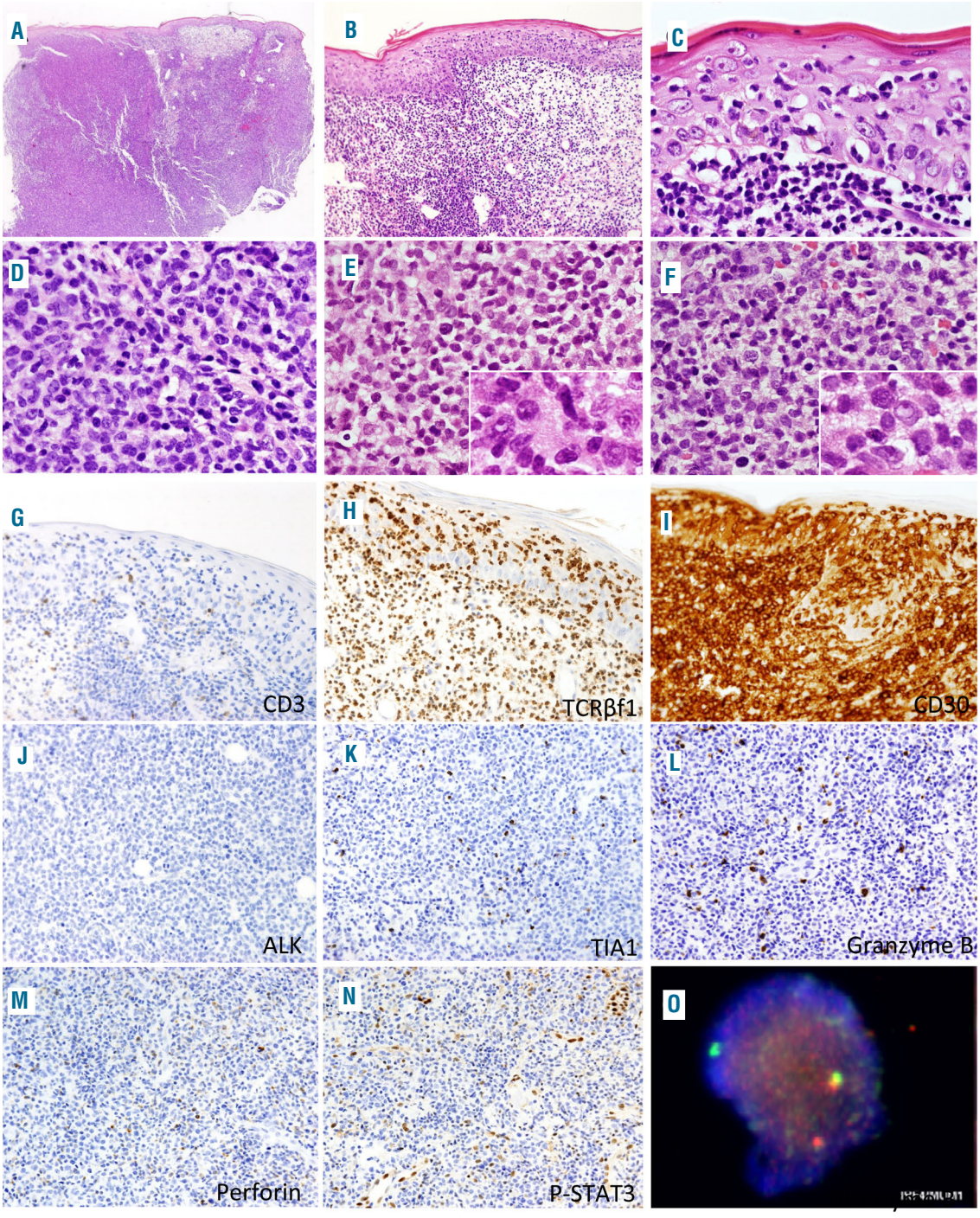

Figure 3. Histological and immunophenotypic features of a primary cutaneous anaplastic large cell lymphoma with *DUSP22* rearrangement (case 5). (A) Low-power microscopic image of the skin biopsy showing diffuse dermal infiltration, characterized histologically by a dense dermal infiltrate with epidermal involvement by small lymphocytes (B and C). (D) Dermal infiltrate of medium-sized and atypical lymphocytes, with a monomorphic appearance, including hallmark and occasional doughnut cells (E, inset; F, inset). Neoplastic cells were CD3-negative (G), TCRβF1-positive (H), and CD30-positive (I). ALK (J), TIA-1 (K), granzyme B (L), and perforin (M), and P-STAT3 (N) were negative. (O) Fluorescence *in situ* hybridization using a break-apart probe at the *DUSP22* locus shows a rearrangement, with one normal fusion signal and an abnormal split signal.

lished by other groups,[9] identifying lack of activation of the JAK/STAT pathway in *DUSP22*-rearranged cases, despite the fact that this had initially been proposed as a universal finding in ALK-positive and ALK-negative ALCLs.[10]

We describe histological and immunophenotypic features that may help recognize *DUSP22*-rearranged cases. The presence of sheets of intermediate-to-large cells, with relatively monomorphous large-cell cytology, including hallmark and doughnut cytology, with no expression of cytotoxic markers, is useful for further FISH testing in systemic cases. In the pcALCL cases, the presence of the previously described biphasic pattern is a useful indicator of *DUSP22*-rearrangement. The same translocation involving locus 6p25 was also described in lymphomatoid papulosis (LyP),[8,11] suggesting that this molecular alteration could determine a better outcome, both in cutaneous and systemic ALK-negative ALCL.

Constant expression of T-cell markers and a lack of cytotoxic markers and markers of activation of the STAT pathways seem to be linked to *DUSP22* translocation in this series.

It would be of interest to explore whether this combination of markers in other ALK-negative ALCLs identifies cases with specific morphology, immunophenotype or clinical features.

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