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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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Authors' contribution

DS collected and reviewed clinical data, analyzed data, designed the research, and wrote the manuscript. BB performed morphological diagnoses, performed FISH studies, analyzed data and wrote the manuscript; ChrB, EB, DC, FL, KB, NK, GB, AC, GD, OT reviewed and interpreted clinical data. EP performed morphological diagnoses and FISH analyses. VF supported material and data acquisition and collected data. ChlB and AD performed FISH analyses. FD, JB, CélB, MP performed morphological diagnoses. JPJ analyzed data and

supervised the statistical analyses; PG and LdL performed morphological diagnoses, designed and sustained the research, collected and analyzed data, and wrote the manuscript.

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Abstract

ALK-negative anaplastic large cell lymphoma (ALCL) comprises subgroups harboring rearrangements of *DUSP22* (*DUSP22*-R) or *TP63* (*TP63*-R). Two studies respectively reported 90% and 40% 5-year overall survival (OS) in 21 and 12 *DUSP22*-R/*TP63*-not rearranged (NR) patients, making the prognostic impact of *DUSP22*-R unclear. Here, 104 newly diagnosed ALK-negative ALCL patients (including 37 from first-line clinical trials) from the LYSA TENOMIC database were analyzed by break-apart FISH assays for *DUSP22*-R and *TP63*-R. There were 47/104 (45%) *DUSP22*-R and 2/93 (2%) *TP63*-R cases, including one *DUSP22*-R/*TP63*-R. *DUSP22*-R tumors showed more frequent CD3 expression (62% *versus* 35%, *P*=0.01), and less commonly a cytotoxic phenotype (27% *versus* 82%; *P*<0.001). At diagnosis, *DUSP22*-R ALCL patients had more frequent bone involvement (32% *versus* 13%, *P*=0.03). The patient with *DUSP22*-R/*TP63*-R ALCL had a rapidly fatal outcome. After a median follow-up of 4.9 years, 5-year progression-free survival (PFS) and OS of 84 patients without *TP63*-R treated with curative intent anthracycline-based chemotherapy were 41% and 53%, respectively. According to *DUSP22* status, 5-year PFS was 57% for 39 *DUSP22*-R *versus* 26% for 45 triple-negative *(DUSP22*-NR/*TP63*-NR/ALK-negative) patients (*P*=0.001). The corresponding 5-year OS rates were 65% and 41%, respectively (*P*=0.07). In multivariate analysis, performance status and *DUSP22* status significantly affected PFS, and distinguished four risk groups, with 4-year PFS and OS ranging from 17% to 73% and 21% to 77%, respectively. Performance status but not *DUSP22* status impacted OS. The use of Brentuximab vedotin (BV) in relapsed/refractory patients improved OS2 independently of *DUSP22* status. Our findings support the biological and clinical distinctiveness of *DUSP22*-R ALK-negative ALCL. Its relevance to outcome in patients receiving frontline BV remains to be determined.

Introduction

Anaplastic lymphoma kinase (ALK)-negative anaplastic large cell lymphoma (ALCL) is one of the four ALCL entities recognized in the current WHO classification of lymphoid neoplasms. It is a systemic disease entity defined as a CD30-positive T-cell neoplasm that is not reproducibly distinguishable on morphological grounds from ALK-positive ALCL but lacks ALK protein expression.[1] Before 2017, ALK-negative ALCL was listed as a provisional entity, because of overlapping features with CD30-positive peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), and the lack of established diagnostic criteria. Improved criteria for routine diagnostic practice plus results from several studies suggesting distinguishing molecular features, led to validate ALK-negative ALCL as a definitive entity.[1,2]

Multiple studies over the past years have highlighted heterogeneity of ALK-negative ALCL, and emphasized that this entity is not merely defined by the lack of *ALK* gene fusions, but comprises a heterogeneous genomic landscape including subgroups harboring *DUSP22* or *TP63* rearrangements (*DUSP22*-R or *TP63*-R) or lacking both (*DUSP22*-NR/*TP63*-NR/ALK-negative, referred to as triple-negative ALCL). Other recurrent alterations consist of somatic mutations of *JAK1*, *STAT3* or *MSC*, the expression of ERBB4-aberrant transcripts, or a deregulated BATF3/IL-2R–module.[3–7] Especially, it has been shown that ALK-negative ALCL with *DUSP22*-R is characterized by a distinct gene expression signature, recurrent *MSC* mutations, lack of STAT3 activation and DNA hypomethylation.[6,8] For these reasons, the recently released International Consensus Classification of lymphoid neoplasms, but not as yet the 5th Edition of the WHO-HAEM classification, considers *DUSP22*-R ALCL as a distinct genomic subtype.[9,10]

With conventional therapy, 5-year overall survival (OS) of ALK-negative ALCL patients is approximately 50%.[11–15] It has been suggested that *DUSP22*-R could impact this survival. In the first clinical report from a multi-institution US study, the 5-year OS of 21 patients with *DUSP22*-R/*TP63*-NR ALK-negative ALCL was 90%. Later on, a similar favorable outcome was reported in 5 patients in a Danish study (5-year OS, 80%) and in 4 patients from Spain (5-year OS, 100%).[16,17] However, in another recent work from the British Columbia Cancer database, the 5-year OS of 12 patients with *DUSP22*-R/*TP63*-NR ALK-negative ALCL was 40%.[18] Thus,

the prognostic impact of *DUSP22*-R in ALK-negative ALCL is currently unclear. The NCCN guidelines suggest that treatment of the *DUSP22*-R subgroup according to the ALK-positive ALCL algorithm may be considered.[19] However, this could lead to undertreating patients if the prognosis of *DUSP22*-R is not as favorable as expected.

In this retrospective study of 104 patients with ALK-negative ALCL from the TENOMIC database of the Lymphoma Study Association (LYSA), we analysed the pathological characteristics, clinical features, and outcomes of patients according to *DUSP22* and *TP63* status.

Methods

Patients and samples

Patients with ALK-negative ALCL diagnosed between January 2001 and January 2020 were retrieved from the TENOMIC database, the translational T-cell lymphoma research consortium of the LYSA. Thirty-seven patients had been enrolled in first-line clinical trials (26 Ro-CHOP, 8 AATT, 3 ECHELON-2 studies), and 6 in the TOTAL study for relapsed/refractory (R-R) patients, the results of which have been reported[20–23], and 9 patients were from a previous study.[24] Other patients had been treated in routine care. Inclusion criteria required availability of diagnostic tissue (or existing documentation of *DUSP22* rearrangement), and of clinical data including treatment and follow-up. Among the cases for which *DUSP22* FISH has been performed secondarily, we recorded a failure in 5 cases. These cases have not been included in the series. Special attention was paid in order to exclude patients with primary cutaneous ALCL. Diagnostic histological slides were reviewed by at least two expert pathologists and clinical data were collected (details are provided in the *Online Supplementary Appendix*). The study was approved by the ethic committee of the TENOMIC program (Comité de Protection des Personnes Ile-de-France IX 08-009).

Fluorescence in situ hybridization (FISH)

Break-apart FISH assays to explore rearrangements of *DUSP22/IRF4* and *TP63* were performed on formalin-fixed paraffin-embedded (FFPE) tissue sections, using laboratory-developed probes,[25] or commercial probes (Zyto*Light* SPEC *IRF4*, *DUSP22* Dual Color Break

Apart Probe (ZytoVision GmbH, Bremerharven, Germany) and *TP63* Split FISH Probe (Abnova, Taipei, Taiwan), as previously described.[26] At least 50 tumor nuclei were evaluated. The cut-off to consider a rearrangement was ≥10% of rearranged nuclei. Copy gains or losses of the explored loci were recorded qualitatively for rearranged and non-rearranged alleles.

Statistical analyses

This part is provided in the *Online Supplementary Appendix.*

Results

Patient and disease characteristics

In total, 104 ALK-negative ALCL patients newly diagnosed between January 2001 and January 2020 were analyzed, including 37 patients from first-line clinical trials and 67 patients treated in routine care. Baseline patient and disease characteristics did not differ significantly between patients included in first-line clinical trials and the others (*Online Supplementary Table S1*). At diagnosis, the median age of the 104 patients was 60 years (range 39-86), 74% were male, 36% had performance status (PS) ≥2, 72% were stage 3-4, bone was the most frequently involved extranodal site, and IPI score was equally distributed across the 4 risk groups (Table 1). Ten patients who had skin involvement had advanced stage disease and not just involvement drained lymph node. Most patients (97/104, 93%) were treated frontline with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)/CHOP-like regimens, and 7 patients received non-curative intent care.

The diagnostic samples were mostly lymph nodes (91/104 cases, 88%), and the majority were surgical biopsies. The other tissues examined were from the nasopharynx and tonsil (3/104); liver (3/104); mediastinum (1/104); and other extranodal organs (parotid, lung, intestine, maxillary sinus) (6/104). In all cases the tumor consisted of large cells strongly positive for CD30 and negative for ALK protein expression. Other immunophenotypic features are summarized in Table 2. Expression of pan-T-cell antigens was variably detected; most commonly expressed was CD2 (66/87, 76%) followed by CD3 (49/104, 47%), CD5 (36/97, 37%) and CD7 (11/75, 15%). Expression of at least one cytotoxic molecule was demonstrated in 45/101 (45%) cases. Coexpression of EMA was common

(41/87 cases, 47%). CD4 and CD8 were expressed in 72/97 (74%) and 11/89 (12%) cases, respectively. Phospho-STAT3 (pSTAT3) was positive in 21/44 (48%) samples.

FISH results

DUSP22 locus was rearranged in 47/104 cases (45%), with several distinct hybridization patterns observed (Figure 1). Among *DUSP22*-R cases, 38/47 (81%) showed a classical break-apart pattern, i.e. one normal fusion signal and one red and one green separated (split) signals representing the rearranged allele (Figure 1 C); or variant classical patterns, comprising several pairs of separated red and green signals. This group included 3 cases in which two rearranged alleles were present in the absence of any non-rearranged allele, reflecting biallelic rearrangements (Figure 1 D). The remaining 9/47 (19%) *DUSP22*-R cases featured "atypical" hybridization patterns, consisting of at least one isolated green (3') signal, in the absence of isolated red (5') signals (Figure 1 E); in one of these cases, tight clusters of >10 green signals were detected, in addition to fusion signals (Figure 1 F); in another case, only one or two isolated green signals could be seen, without any detectable fusion signal.

FISH assay for *TP63* was contributive in 93/99 cases, indicating a failure rate of 6%, and could not be performed in 5 cases (no material available). *TP63* locus was rearranged in 2/93 cases (2%), including one case with dual *DUSP22*-R and *TP63*-R. Both *TP63*-R cases showed a "classical" break-apart pattern, with a relatively small distance between the separated red and green signals of the rearranged allele (Figure 2), consistent with an inv(3)(q26q28) resulting in the *TBL1XR1::TP63* fusion, although dual fusion FISH probes were not tested to prove it. Amongst the samples lacking structural alterations of the explored loci, low-level (3 to 4) (Figure 1 A) or high-level (≥5) copy gains of *DUSP22* were observed in the majority of the cases (23/57 (40%) and 15/57 (26%), respectively), including 3 samples with tight clusters of up to 20 fusion signals, consistent with *DUSP22* locus amplification (Figure 1 B). Copy gains of *TP63* were mostly of low level (47/91, 52%), with 4/91 samples (4%) showing up to 5 copies per nucleus.

Distinctive pathological and clinical features according to *DUSP22* status

A morphologic spectrum was observed irrespective of *DUSP22* rearrangement, with marked overlap between the two genomic groups (*Online Supplementary Figure S1*).

Although doughnut-type cells were essentially seen in the *DUSP22*-R subgroup, hallmark-type cells were otherwise seen as a prominent or more discrete component of the tumor cell population irrespective of the genomic status in most cases. Marked pleomorphism was seen in some cases, either *DUSP22*-R or -NR.

Considering the immunophenotype of the neoplastic cells (Table 2), CD3 and CD2 were more often positive among *DUSP22*-R cases than in *DUSP22*-NR tumors in 62% *versus* 35%, (*P*=0.01); and 87% *versus* 67% (*P*=0.044) of the cases, respectively. The expression of other T-cell markers (CD4, CD5, CD7, CD8) was otherwise not significantly different between the two groups. Remarkably, the distribution of the tumors according to the CD4 and CD8 expression was almost identical in the two subgroups, the usual profile being CD4+ CD8- (71% and 67% of the cases in *DUSP22*-R and -NR, respectively), followed by CD4- CD8- (19% of the cases in both subgroups) and CD4- CD8+ (9% and 10% of the cases in *DUSP22*-R and -NR, respectively). There were overall only three CD4+ CD8+ cases. Conversely, both genetic subgroups markedly differed in the frequency of expression of cytotoxic protein, EMA and pSTAT3. Expression of TIA1, granzyme B or perforin were seen in 11-13% in *DUSP22*-R group *versus* 40-63% in *DUSP22*-NR cases. Overall, considering the cases tested for all three cytotoxic markers, 8/30 (27%) of *DUSP22*-R cases *versus* 37/45 (82%) of *DUSP22*-NR cases (*P*<0.001) exhibited a cytotoxic profile, i.e. expressed at least one cytotoxic marker. Similarly, EMA was significantly less expressed in *DUSP22*-R cases, being positive in 13% *versus* 73% of *DUSP22*-R *versus* -NR cases (*P*<0.001). Phospho-STAT3 was positive in only 2/20 (10%) *DUSP22*-R samples *versus* 19/24 (79%) in *DUSP22*-NR cases (*P*<0.001).

Comparing *DUSP22*-R and -NR patient characteristics (Table 1), there was no significant difference in median age or sex, and IPI score was equally distributed. The only statistically significant difference was bone involvement, more frequent in *DUSP22*-R cases (32% *versus* 13%, *P*=0.031). The two groups of patients did not otherwise differ regarding involvement of other extranodal sites. Of note, the frequency of *DUSP22*-R was 35% (13/37) for patients included in clinical trials and 51% (34/67) for patients routinely treated (*P*=0.185) (*Online Supplementary Table S1*).

After a median follow-up of 5 years, 5-year PFS et OS of the 104 patients were 36% and 50%, respectively (Figure 3 A-B). According to *DUSP22* status, 5-year PFS was 48% *versus* 25% for 47 *DUSP22*-R and 57 *DUSP22*-NR patients, respectively (*P*=0.025, Figure 3C), and 5-

year OS was 58% *versus* 44% for *DUSP22*-R and *DUSP22*-NR patients, respectively (*P*=0.2, Figure 3D).

Treatment response, survival, and prognostic factors

Analyses of treatment response, survival, and prognostic factors were restricted to patients who had complete FISH information with a confirmed *TP63*-NR status, and who were treated with curative intent front-line anthracycline-based chemotherapy. These comprised 84 patients (39 *DUSP22*-R/*TP63*-NR and 45 triple-negative ALCL). Patient and disease characteristics are shown in *Online Supplementary Table S2*, and immunophenotypic characteristics are described in *Online Supplementary Table S3*.

Four patients (1 *DUSP22*-R and 3 *DUSP22*-NR) were not evaluable for response due to early death (mainly due to infections). In all, the ORR/CR rates were 75%/67%, without significant difference between triple-negative and *DUSP22*-R/*TP63*-NR patients (*Online Supplementary Table S4*).

The median follow-up of the 84 patients was 4.9 years (range, 0.9 to 10 years). Their 2- and 5-year PFS rates were 45% (95% CI, 36% to 57%) and 41% (95% CI, 31% to 53%), respectively, and the 2- and 5-year OS rates were 67% (95% CI, 57% to 78%) and 53% (95% CI, 42% to 66%), respectively. PFS rates were significantly higher in *DUSP22*-R/*TP63*-NR patients than in triple-negative patients (2-year PFS, 67% *versus* 26%; 5-year PFS, 57% *versus* 26%, *P*=0.001; Figure 4A). However, the OS rates were not significantly different in *DUSP22*-R/*TP63*-NR *versus* triple-negative patients (2-year OS, 74% *versus* 60%; 5-year OS, 65% *versus* 41%, *P*=0.07; Figure 4B). Importantly, PFS and OS were similar for patients included or not in first-line clinical trials (*Online Supplementary Figure S2*).

Clinical and laboratory features were subjected to univariate analyses to evaluate their impact on PFS and OS (*Online Supplementary Table S5*). PS (Figure 4 C-D), Beta-2-microglobulin level, granzyme B and perforin expression significantly impacted PFS and OS, whereas *DUSP22* status and cytotoxic profile affected only PFS. Only PS (0-1 *versus* ≥ 2) and *DUSP22*-R/NR status were retained for multivariate analysis because of missing data for the other factors. Both PS and *DUSP22* status significantly affected PFS, but only PS remained significant for OS (Table 3). These two variables delineated four risk groups (Figure 4 E-F): *DUSP22*-R/*TP63*-NR and PS-0-1, with 4-year PFS and OS rates of 73% and 77%, respectively; *DUSP22*-R/*TP63*-NR and PS ≥ 2, with 4-year PFS and OS rates of 27% and 29%, respectively;

triple-negative and PS 0-1, with 4-year PFS and OS rates of 33% and 62%, respectively; and triple-negative and PS ≥2, with 4-year PFS and OS rates of 17% and 21%, respectively (*P*<0.001 for PFS and *P*=0.001 for OS).

Post-progression survival

Of the 84 patients, 43 (14 *DUSP22*-R and 29 triple-negative) progressed or relapsed after frontline treatment. From this event, the 4-year OS (OS2) was 29% (21% in *DUSP22*-R/*TP63*-NR *versus* 34% in triple-negative patients, *P*=0.62; Figure 5A). Information on salvage treatment was retrieved for 40/43 patients. The 4-year OS2 was 44% for the 27 patients having received BV at relapse (only one patient had previously received frontline BV) *versus* 0% for the 13 patients having received standard treatment, mainly cytarabine-based regimens or bendamustine (*P*<0.001, Figure 5B). Figure 5C illustrates OS2 according to *DUSP22* status and BV as salvage treatment. In multivariate analysis of these 2 parameters, only BV impacted OS2 (*P*<0.001; HR 0.119 (95% CI 0.041 to 0.343)). Indeed, when restricting the OS2 analysis to the patients who received BV as salvage treatment, there was no significant difference according to *DUSP22* status (Figure 5D).

Characteristics of the two patients with *TP63*-R ALK- ALCL

The patient with the dual *TP63* and *DUSP22* rearrangement was a 43-year-old man presenting with cervical lymphadenopathy and an IPI score at 0. The tumor consisted of diffuse sheets of medium to large atypical lymphoid cells with frequently reniform or horseshoe-shaped nuclei (Figure 2). In addition to CD30, the tumor cells were CD3+, CD4+, CD5+, CD7-, CD8-, EMA-, TIA-1-, granzyme B-, perforin-, pSTAT3- and p63+. Rebiopsy at relapse one year later showed identical features.

The patient with an isolated *TP63* gene rearrangement was a 52-year-old woman with an IPI score at 2 (Ann Arbor stage 3 and elevated LDH). A lymph node biopsy showed cohesive sheets of large cells with oval nuclei and prominent nucleoli, associated with diffuse interstitial fibrosis (*Online Supplementary Figure S3*). The neoplastic cells were strongly positive for CD30, CD2+, CD3-, CD4+, CD5-, CD8-, TIA1+, granzyme B+, perforin+ with nuclear p63 protein expression.

Both patients reached CR after CHOP (*DUSP22-R*/*TP63*-R case) or CHOEP (*TP63*-R case) regimens and underwent consolidative autologous stem-cell transplantation. They both relapsed after transplantation, the patient with a dual rearrangement died from

lymphoma 5 months after relapse, and the other is remaining in CR more than 2 years after salvage treatment with BV + gemcitabine and allogeneic stem-cell transplantation.

Discussion

We report here the clinical and pathological findings of 104 patients with ALK-negative ALCL according to *DUSP22-R* status (47 *DUSP22*-R and *57 DUSP22*-NR) and *TP63*-R status (2 *TP63*-R and 91 *TP63*-NR), including 39 *DUSP22*-R/*TP63*-NR and 45 triple-negative cases. This represents the largest such series published so far. The main conclusions of our study are 1) *DUSP22*-R ALCL encompasses a spectrum of FISH patterns, has distinctive immunophenotypic features and more frequently involves bone; 2) the 65% 5-year OS of *DUSP22*-R patients is intermediate between those previously reported in the US study (90%) and by the BCCA investigators (40%); 3) both *DUSP22* status and PS have an independent impact on PFS; 4) OS was mainly affected by PS; and 5) OS2 was markedly improved by the use of BV as salvage treatment, without a significant influence of *DUSP22* status.

With the comparison group (*DUSP22*-NR ALK-negative ALCL) comprised of 57 individuals, the *DUSP22*-R cases constitute 45% of our study population. Strikingly, this proportion is higher than in other studies from North America and Europe, where a frequency of 18% to 30% *DUSP22* rearrangements has been reported.[3,16–18] However, the mode of recruitment of samples and patients precludes drawing conclusions regarding the relative prevalence of ALK-negative ALCL genomic subgroups. In particular, the distribution of *DUSP22*-R/-NR cases was different among the 37 patients enrolled in first-line clinical trials (13/37 (35%) *DUSP22*-R, including 6/26 (23%) in Ro-CHOP study) *versus* the others collected through the TENOMIC network (34/67, 51%). Since all cases of ALK-negative ALCL patients from the clinical trials were included in this study when possible, they represent an « unbiased » group of cases and their characteristics in terms of *DUSP22* status are much consistent with the existing literature, confirming the 30% prevalence of *DUSP22*-R in the multi-institution US study.[3]

Several reasons explain the relatively numerous *DUSP22*-R cases among the non-clinical trial patients in our study. The collection of patients' data and samples through TENOMIC primarily aims at collecting high-quality data and cases of medical and scientific

interest, which may be influenced by specific topics of interest like the current project on ALCL with *DUSP22*-R.[27] Moreover, the most active participants are referral centers with expert pathologists being consulted for unusual or difficult cases, or for ancillary techniques like FISH. In addition, it is also worth mentioning the use of cases from a former publication, among which a majority (7/9) harboring a *DUSP22* rearrangement.[24] In fact, five of these cases, all *DUSP22*-R, that had been coded as CD30-positive PTCL-NOS in that study, because they did not fulfill the stringent immunophenotypic criteria originally used for the diagnosis of ALK-negative ALCL (i.e., requiring the expression of at least one cytotoxic molecule or EMA), became consistent with ALK-negative ALCL in the light of updated criteria developed later.

We found only 2/93 (2%) *TP63*-R cases in our series, which is at the lower end of previously reported frequencies (2- 8%) in ALK-negative ALCL. [3,16,18] It might be argued that the exclusive use of a break-apart FISH probe to explore the *TP63* locus may have missed cases harboring a *TBL1XR1::TP63* intrachromosomal inversion, due to the small distance between the split signals in this context. Nonetheless, being aware of the risk of false negative results, the slides were examined very carefully, and we believe that the low prevalence of *TP63*-R truly reflects the biology of our cohort. On the other hand, cryptic *TP63* rearrangements cannot formally be excluded, as recently described.[28] These latter would however not have been detected in previously published series based on FISH assays.

A spectrum of *DUSP22* FISH patterns were observed (Figure 1). In addition to extra-copies of the intact (non-rearranged) *DUSP22* locus, which could represent either specific gains or polysomy of chromosome 6, three *DUSP22*-NR cases featured a FISH pattern consistent with *DUSP22* locus amplification. This observation has not previously been reported, and its biological consequence is unclear. The *DUSP22* gene encodes a dual specificity phosphatase that functions as a tumor-suppressor gene by exerting an inhibitory effect on various signaling pathways.[29,30] While it has been shown that *DUSP22* gene rearrangements lead to the downregulation of the enzyme, it is questionable how an amplification could result in its silencing, unless the amplified allele encodes an altered, non-functional isoform. Alternatively, the pathogenic effect in such cases could be mediated by the amplification of another neighbouring gene with an oncogenic function (e.g., *IRF4*).

Among *DUSP22*-R cases, we observed both the most classical break-apart FISH pattern and variants of it, including cases with biallelic rearrangements or extra copies of

both the rearranged and non-rearranged alleles. Although details regarding the encountered FISH patterns are frequently missing in the literature (the result being commonly limited to a binary information: rearranged or not), the classical break-apart pattern is the most frequently described one in the series and case reports published so far on *DUSP22*. In our cohort however, approximately 20% of *DUSP22*-R cases were characterized by atypical hybridization patterns, featuring one or several extra copies of isolated green signals, suggesting a rearrangement with subsequent deletion of the 5' side of the locus (telomeric red probe) and preservation of its 3' side (centromeric green probe). This configuration, which reflects an unbalanced translocation, has recurrently been described in earlier series of cutaneous CD30+ T-cell lymphoproliferations, when the gene believed to be involved in 6p25.3 locus rearrangements was *IRF4*, but it has been reported once in systemic ALK-negative ALCL.[31,32] Nonetheless, in a case of lymphomatoid papulosis characterized by a similar atypical *DUSP22* FISH pattern, Karai and colleagues could demonstrate by FISH that the partner locus of the translocation was at 7q32.3, similar to what has been described for the classical break-apart pattern.[29,33]

The immunohistochemistry results on our series are overall consistent with the range reported in previous reports.[3,18,34] In addition, we document CD4 and CD8 expression profiles which were evaluated in the majority of cases (87/104) and were remarkably similar irrespective of *DUSP22* status, most commonly CD4+ CD8- (67% of the cases) or double negative for CD4 and CD8 (21% of the cases). In addition, our findings confirm significant differences between *DUSP22*-R and -NR cases in terms of cytotoxic profile. Of note, while confirming the lack of cytotoxic phenotype as a characteristic feature of *DUSP22*-R cases, we also found that a significant minority of these (8/30, 27%) expressed one or several cytotoxic marker(s), which is a higher proportion than the +/- 10% in previously reported series.[3,18] EMA and pSTAT3 expression were also much less common in *DUSP22*-R cases, and there was less frequent CD3 positivity in *DUSP22*-NR ALCL.[3,8,18] The case with dual *DUSP22* and *TP63* rearrangements (Figure 2) was CD3+ CD4+ CD8- EMA- pSTAT3- and non-cytotoxic. Similar findings have been reported in the other ALK-negative ALCL cases with that rare genomic configuration, suggesting that the immunophenotype is likely driven by the *DUSP22* rearrangement in those tumors.[35,36]

We found that among ALCL patients treated with curative intent chemotherapy, *DUSP22*-R was a significant determinant of improved PFS, in uni- and multivariate analyses,

with 57% 5-year PFS in *DUSP22*-R/*TP63*-NR *versus* 26% in triple-negative patients. In comparison, in the BCCA study, the 5-year PFS of 11 *DUSP22*-R/*TP63*-NR patients treated with curative intent chemotherapy was 44%.[18] In the US study, PFS was not reported.[3] Unlike previous reports the advantage in OS for our *DUSP22*-R/*TP63*-NR–patients (65% 5-year OS) compared to triple-negative patients (41%) did not reach statistical significance. We also found that PS affected PFS and was the prominent factor affecting OS in multivariate analysis in our series. Further, we identified a low-risk group characterized by *DUSP22*-R and PS 0-1, with 4-year PFS of 73% and 4-year OS of 77%. Conversely, patients with *DUSP22*-R and PS ≥ 2 had 4-year PFS and OS rates of 27% and 29%, respectively, demonstrating the major impact of PS on outcome. In a recent report from the International T-Cell Project, PS ≥2 was the factor with the highest impact on PFS and OS in multivariate analysis with respective HR of 3.69 and 4.04, but genomic subtyping of these ALK-negative ALCL was not studied.[15]

BV has previously been shown to improve OS2 after progression/relapse of ALK-negative ALCL patients compared to historical controls.[37,38] Here, we also confirm that OS2 was markedly improved by salvage treatment with BV, which was the main prognostic factor in multivariate analysis. Interestingly, we found no significant difference in OS2 according to *DUSP22* status and an overall similarly good outcome in patients who received BV at relapse/progression in *DUSP22*-R/*TP63*-NR and triple-negative patients, suggesting that response to BV in R-R patients is not influenced by the *DUSP22* status.

PFS rather than OS may better capture the prognostic impact of *DUSP22*-R since it is not influenced by salvage treatment, while in turn OS analysis is more complex to interpret and should take into account potential differences in salvage treatment. It turned out that, at relapse/progression, 21/26 (81%) triple-negative patients but only 6/14 (43%) *DUSP22*-R patients received BV. Therefore, this imbalance could contribute to the absence of a significant difference in OS between *DUSP22*-R and -NR patients.

Despite limitations inherent to a retrospective study with unbalanced distribution of *DUSP22*-R/NR patients, incomplete *TP63* FISH data, heterogeneity in first-line treatments, our findings support the biological and clinical distinctiveness of *DUSP22*-R ALK-negative ALCL. Moreover, our results confirm a better PFS of *DUSP22*-R/*TP63*-NR cases compared to triple-negative ALCLs, but clearly inferior to historical series of ALK-positive ALCL patients.[39] Of note, with the limitation of underpower of small groups, outcome did not differ according to first-line treatment (CHOP, CHOEP or BV-CH(E)P; data not shown), but only a small

fraction of our patients received frontline BV. Given the benefit of BV-CHP over CHOP in ALK-negative ALCL in the ECHELON-2 trial with an improved 5-year PFS (but not OS), BV-CHP has become the standard of care for first-line treatment of ALK-negative ALCL.[22] However, since genomic subtyping was not reported, its potential impact on the PFS difference observed between BV-CHP and CHOP arms is unknown. Future studies will be necessary to clarify this point and the impact of *DUSP22* status in newly diagnosed patients with ALK-negative ALCL treated with frontline BV.

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Table 1. Patient and disease characteristics.

Clinical features at diagnosis	All patients	*DUSP22*-non rearranged ALK-negative ALCL	*DUSP22*-rearranged ALK-negative ALCL	*P*
n	104	57	47	
Diagnosis era	2001-2020	2001-2020	2004-2019	
Age (years)				
Median (range)	60 (39-86)	61 (39-85)	60 (40-86)	
>60	53/104 (51%)	29/57 (51%)	24/47 (51%)	1
Male	77/104 (74%)	39/57 (68%)	38/47 (81%)	0.225
Performance status ≥ 2	37/103 (36%)	23/57 (40%)	14/46 (30%)	0.403
Staging at diagnosis				0.701
PET	84/100 (84%)	45/55 (82%)	39/45 (87%)	
CT	16/100 (16%)	10/55 (18%)	6/45 (13%)	
Ann Arbor stage (1-2 *vs* 3-4)				0.862
1	8/104 (8%)	3/57 (5%)	5/47 (11%)	
2	21/104 (20%)	12/57 (21%)	9/47 (19%)	
3	20/104 (19%)	16/57 (28%)	4/47 (8%)	
4	55/104 (53%)	26/57 (46%)	29/47 (62%)	
Involved site (any)				
Bone	22/103 (21%)	7/56 (13%)	15/47 (32%)	**0.031**
Liver	17/103 (17%)	8/56 (14%)	9/47 (19%)	0.692
Bone marrow	13/103 (13%)	7/56 (13%)	6/47 (13%)	1
Lung	13/103 (13%)	5/56 (9%)	8/47 (17%)	0.350
Spleen	12/103 (12%)	5/56 (9%)	7/47 (15%)	0.528
Soft tissue	12/103 (12%)	10/56 (18%)	2/47 (4%)	0.067
Skin	10/103 (10%)	3/56 (5%)	7/47 (15%)	0.196
Gastrointestinal tract	7/103 (7%)	4/56 (7%)	3/47 (6%)	1
Parotid	4/103 (4%)	1/56 (2%)	3/47 (6%)	0.490
Nasopharynx	3/103 (3%)	1/56 (2%)	2/47 (4%)	0.877
Tonsil	2/103 (2%)	1/56 (2%)	1/47 (2%)	1
Sinus	2/103 (2%)	1/56 (2%)	1/47 (2%)	1
Thyroid	1/103 (1%)	0/56 (0%)	1/47 (2%)	0.930
Adrenal	1/103 (1%)	0/56 (0%)	1/47 (2%)	0.930
Blood	1/103 (1%)	1/56 (2%)	0/47 (0%)	1
Ascites	1/103 (1%)	0/56 (0%)	1/47 (2%)	0.930
Pleura	0/103 (0%)	0/56 (0%)	0/47 (0%)	---
Extranodal site >1	29/104 (28%)	15/57 (26%)	14/46 (30%)	0.862
Elevated lactate dehydrogenase	58/103 (56%)	30/57 (53%)	28/46 (61%)	0.523
Beta-2-microglobulin ≥ 3 mg/L	24/55 (44%)	17/34 (50%)	7/21 (33%)	0.352
IPI score				0.358
0-1	29/103 (28%)	13/57 (23%)	16/46 (35%)	
2	24/103 (23%)	16/57 (28%)	8/46 (17%)	
3	26/103 (25%)	16/57 (28%)	10/46 (22%)	
4-5	24/103 (23%)	12/57 (21%)	12/46 (26%)	
Patients in first-line clinical trials	37/104 (36%)	24/57 (42%)	13/47 (28%)	0.185
Primary therapy				0.292
CHOP	45/104 (43%)	23/57 (40%)	22/47 (47%)	
CHOEP	24/104 (23%)	13/57 (23%)	11/47 (23%)	
Romidepsin-CHOP	10/104 (10%)	9/57 (16%)	1/47 (2%)	
BV-CH(E)P	6/104 (6%)	3/57 (5%)	3/47 (6%)	
Mini-CHOP	7/104 (7%)	2/57 (4%)	5/47 (11%)	
ACVBP	5/104 (5%)	3/57 (5%)	2/47 (4%)	
Non-curative care	7/104 (7%)	4/57 (7%)	3/47 (6%)	
Consolidative transplantation				0.218
AutoSCT	14/104 (13%)	5/57 (9%)	9/47 (19%)	

AlloSCT	5/104 (5%)	2/57 (4%)	3/47 (6%)	
Auto-minialloSCT tandem	1/104 (1%)	1/57 (2%)	0/47 (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 2. Immunophenotypic characteristics of the 104 tumors.

	All patients (n=104)	***DUSP22*-NR ALK-negative ALCL (n=57)**	***DUSP22*-R ALK-negative ALCL (n=47)**	***P***
CD30	104/104	57/57	47/47	1
ALK	0/104	0/57	0/47	1
T-cell antigens				
CD3	49/104 (47%)	20/57 (35%)	29/47 (62%)	**0.01**
CD5	35/97 (36%)	17/53 (32%)	19/44 (43%)	0.296
CD2	66/87 (76%)	33/49 (67%)	33/38 (87%)	**0.044**
CD7	11/75 (15%)	7/40 (18%)	4/35 (11%)	0.528
CD4	72/97 (72%)	38/50 (76%)	34/47 (72%)	0.817
CD8	11/89 (12%)	5/45 (11%)	6/44 (11%)	0.758
CD4+ CD8-	60/87 (69%)	28/42 (64%)	32/45 (69%)	0.817
CD4- CD8-	16/87 (18%)	8/42 (19%)	8/45 (18%)	1
CD4- CD8+	8/87 (9%)	4/42 (10%)	4/45 (9%)	1
CD4+ CD8+	3/87 (3%)	2/42 (5%)	1/45 (2%)	0.608
EMA	41/87 (47%)	36/49 (73%)	5/38 (13%)	**<0.0001**
Cytotoxic markers				
TIA1	21/78 (27%)	16/40 (40%)	5/38 (13%)	**0.01**
Granzyme B	26/92 (28%)	21/48 (44%)	5/44 (11%)	**0.001**
Perforin	31/76 (41%)	27/43 (63%)	4/33 (12%)	**<0.0001**
Cytotoxic profile*	45/75 (60%)	37/45 (82%)	8/30 (27%)	**<0.0001**
pSTAT3	21/44 (48%)	19/24 (79%)	2/20 (10%)	**<0.001**

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

Table 3. Parameters influencing PFS and OS in multivariate analyses.

Parameter	PFS				OS			
Patients (no. used., n=83)	*P*	HR	95% CI low	95% CI high	*P*	HR	95% CI low	95% CI high
PS ≥2	**0.005**	2.259	1.271	4.013	**<0.001**	3.024	1.593	5.741
DUSP22-NR	**0.008**	2.256	1.233	4.127	0.194	1.556	0.799	3.031

Figures Legends

Figure 1. *DUSP22* FISH patterns.

The range of FISH patterns observed for *DUSP22* locus (right column: Zyto*Light* SPEC *IRF4*, *DUSP22* Dual Color Break Apart Probe, ZytoVision) is illustrated, with the corresponding HE images (left column). *DUSP22* non-rearranged cases (A-B) included a majority of samples showing copy gains (A: 3 to 4 fusion signals per nucleus), and a few characterized by an amplification of *DUSP22* locus (B: tight clusters of fusion signals). Among *DUSP22*-rearranged cases (C-F), approximately 80% showed a classical break-apart pattern of *DUSP22* locus or variants thereof (C: separated red and green signals for the rearranged allele, with an additional fusion signal representing the non-rearranged allele; D: biallelic rearrangements), while 20% featured various atypical break-apart patterns (E: rearrangement with deletion of the red (5') portion of the probe, resulting in an isolated green (3') signal, in addition to the non-rearranged allele; F: variant of pattern shown in E, presenting tight clusters of green (3') signals, in addition to fusion signals representing the non-rearranged allele). All HE images are taken at original x400 magnification and the FISH images are taken at x630.

Figure 2. ALK-negative ALCL with dual *TP63* and *DUSP22* rearrangement.

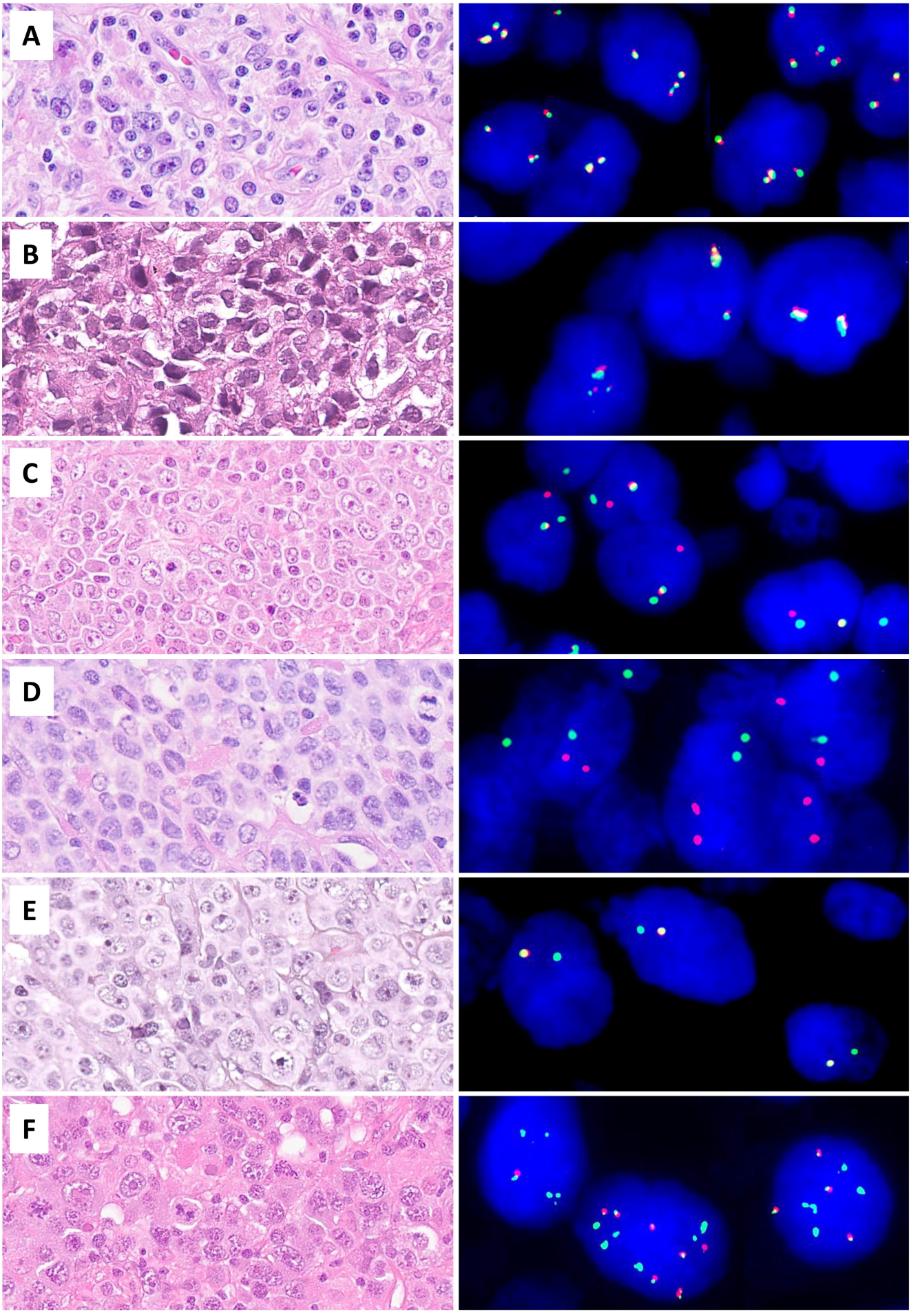
(A-B) The tumor comprises cohesive sheets of atypical lymphoid cells including anaplastic-type "hallmark" cells (hematoxylin and eosin, original magnifications x400 and x800); (C-J) on immunohistochemical stains the neoplastic cells are strongly CD30+ (C), CD3+ (D), CD5+ (E), CD7- (F), CD4+ (G), CD8- (H), with a high Ki67 proliferation index (I) and negative for TIA-1 (J) (all immunoperoxidase; original magnification x400); (K-L) representative nuclei from the

FISH assays for *DUSP22* (K) and *TP63* rearrangement (L) showing a pattern indicative of a break for the two tested loci (original magnification x630).

Figure 3. Survival of the 104 patients. (A) Progression-free survival (PFS) and (B) overall survival (OS) of the whole cohort. (C) PFS and (D) OS according to *DUSP22* status.

Figure 4. Survival of the 84 *TP63*-NR patients treated with curative intent anthracycline-based chemotherapy. (A) Progression-free survival (PFS) and (B) overall survival (OS) according to *DUSP22* status. (C) PFS and (D) OS according to performance status. (E) PFS and (F) OS according to both factors.

Figure 5. Post-progression overall survival (OS2). (A) According to *DUSP22* status, (B) according to Brentuximab vedotin (BV) use at relapse/progression, (C) according to both parameters, and (D) when restricting the analysis to the patients who received BV as salvage treatment.


A
B
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E
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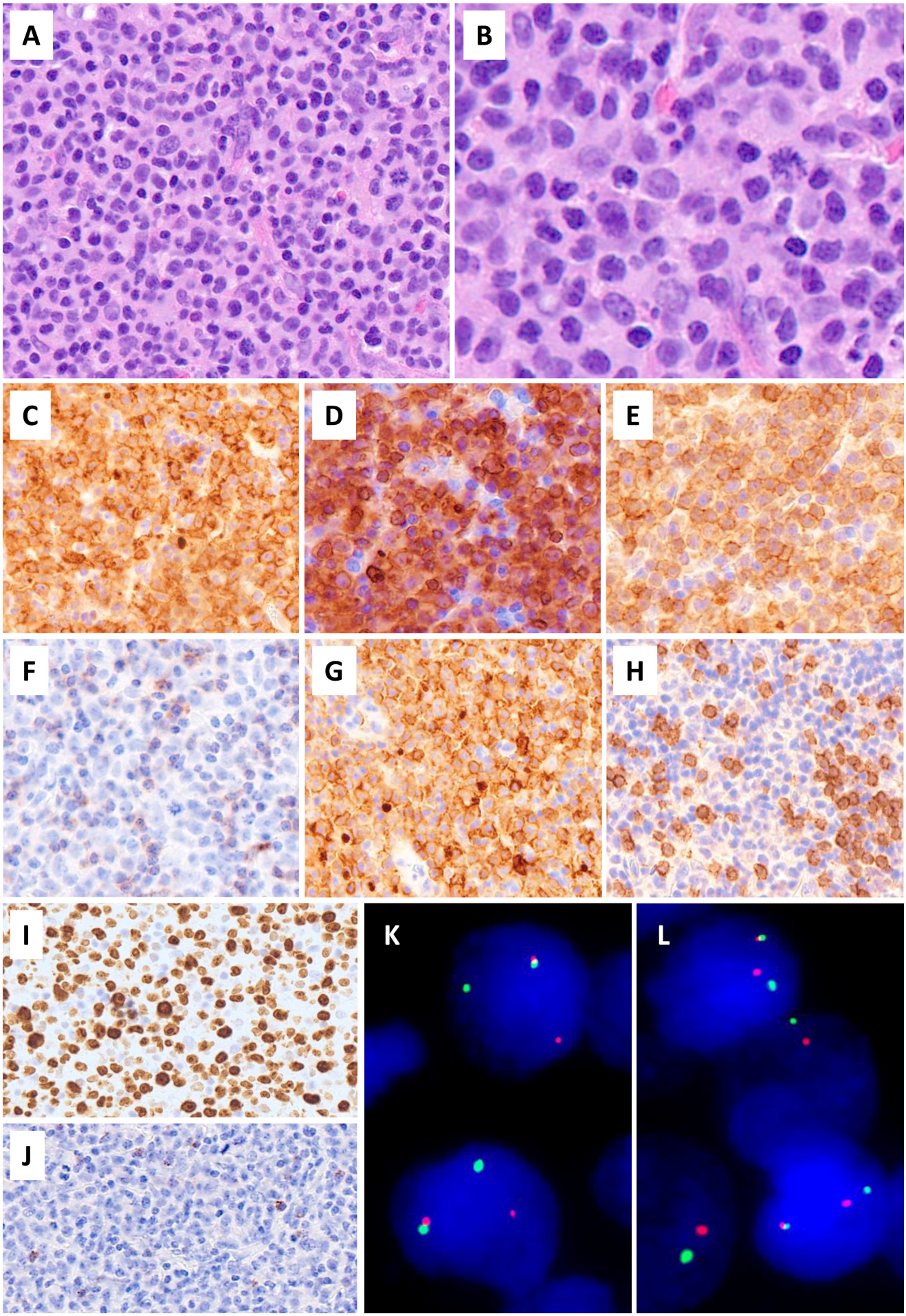
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Figure 3

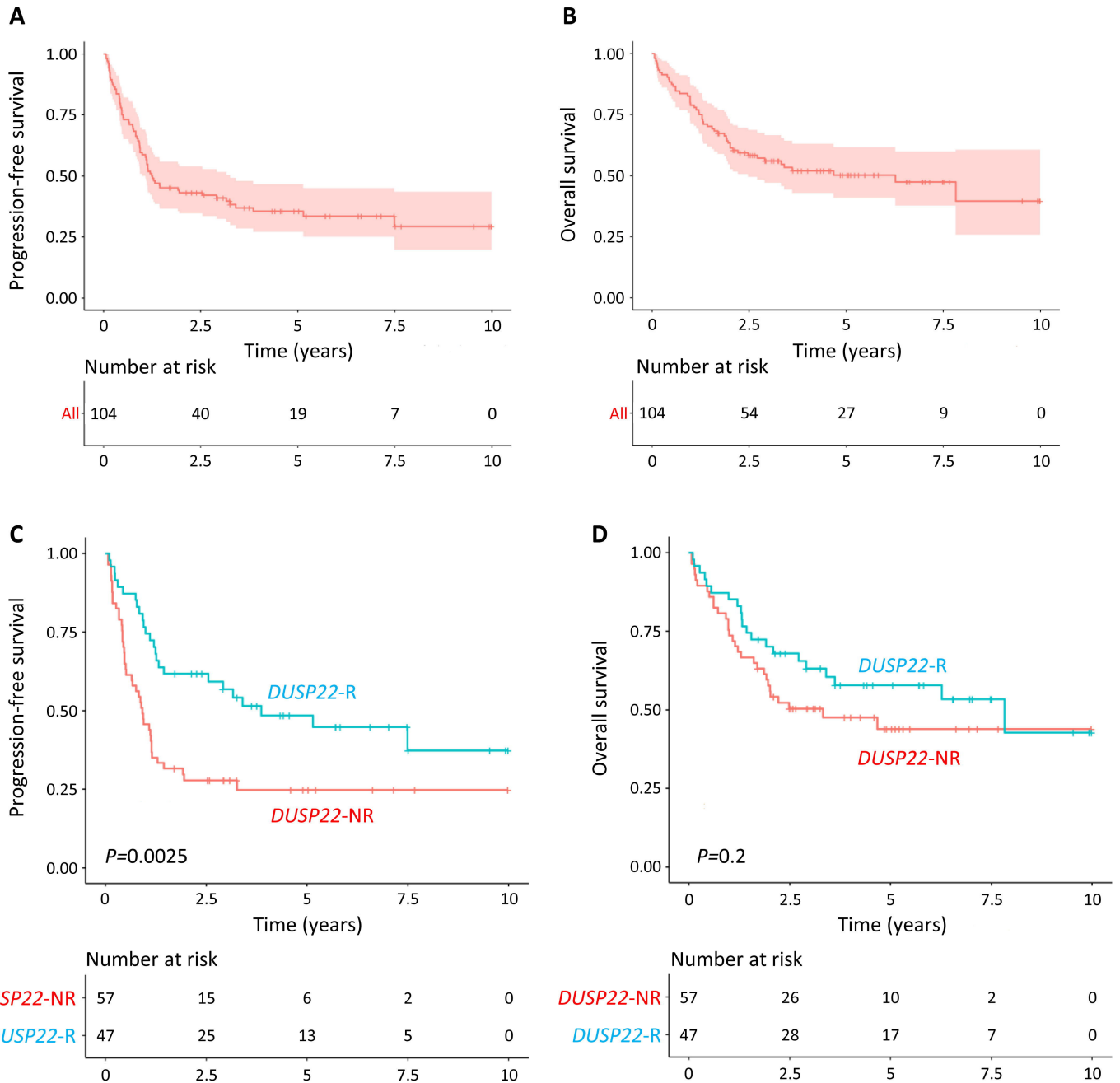

A
Progression-free survival
1.00
0.75
0.50
0.25
0.00
0
2.5
5
7.5
10
Time (years)
Number at risk
All 104 40 19 7 0
B
Overall survival
Time (years)
Number at risk
All 104 54 27 9 0
C
Progression-free survival
DUSP22-R
DUSP22-NR
P=0.0025
Time (years)
Number at risk
DUSP22-NR 57 15 6 2 0
DUSP22-R 47 25 13 5 0
D
Overall survival
DUSP22-R
DUSP22-NR
P=0.2
Time (years)
Number at risk
DUSP22-NR 57 26 10 2 0
DUSP22-R 47 28 17 7 0

Figure 4

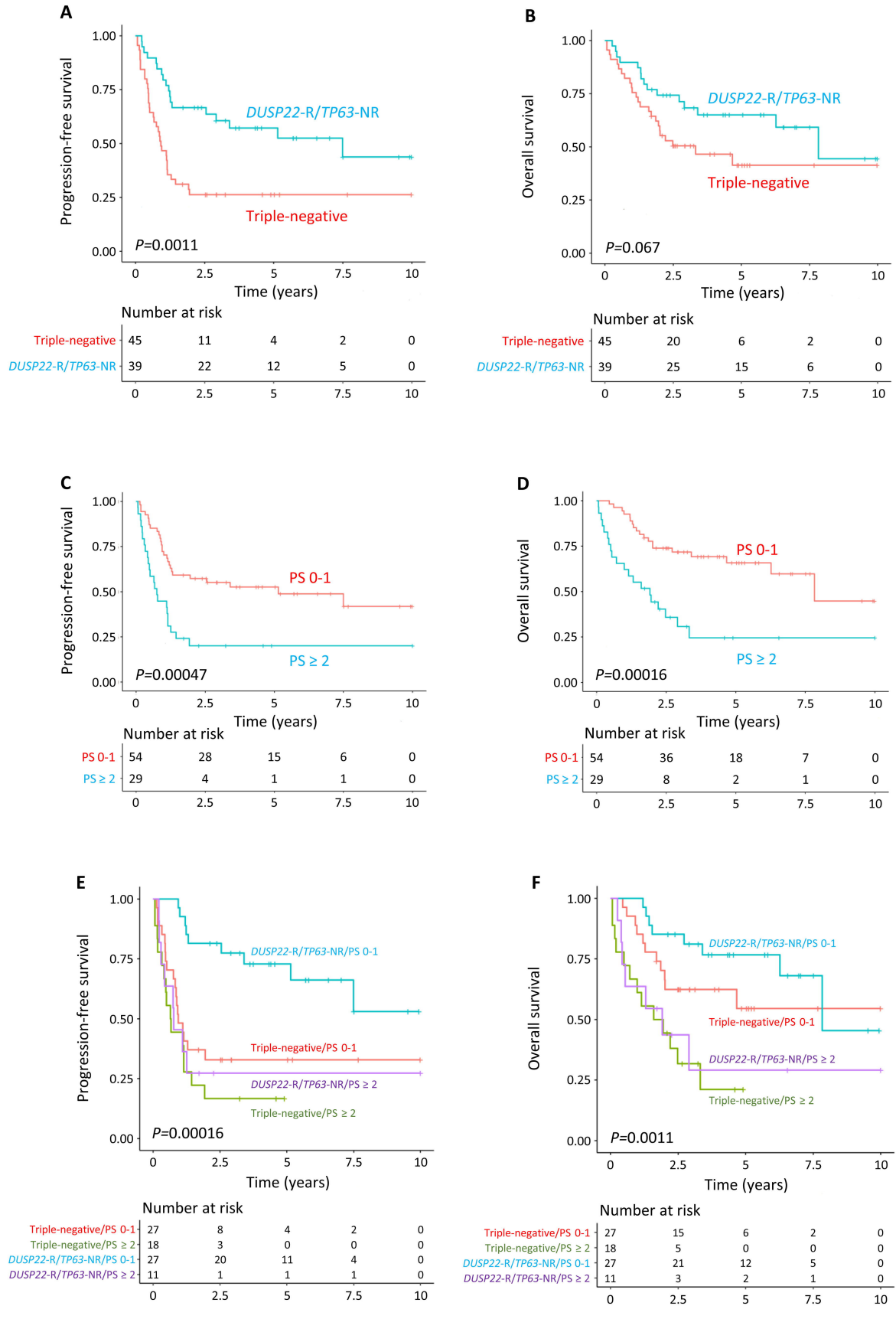
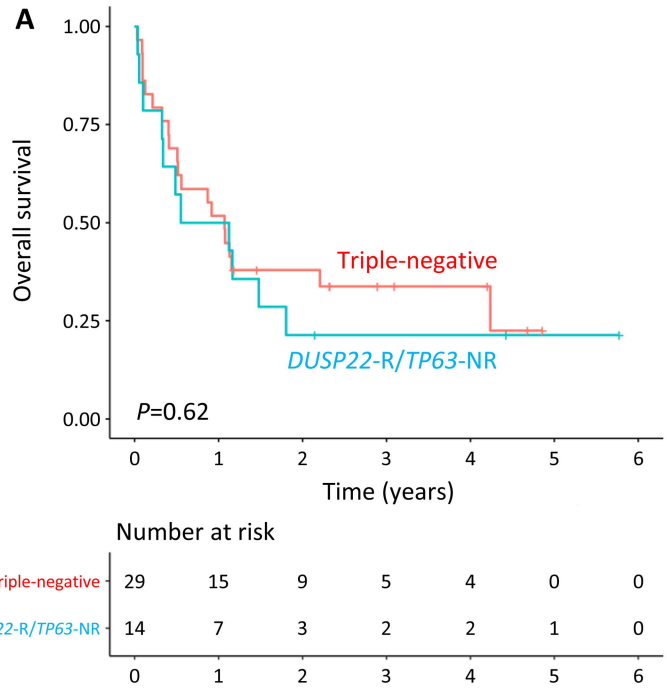
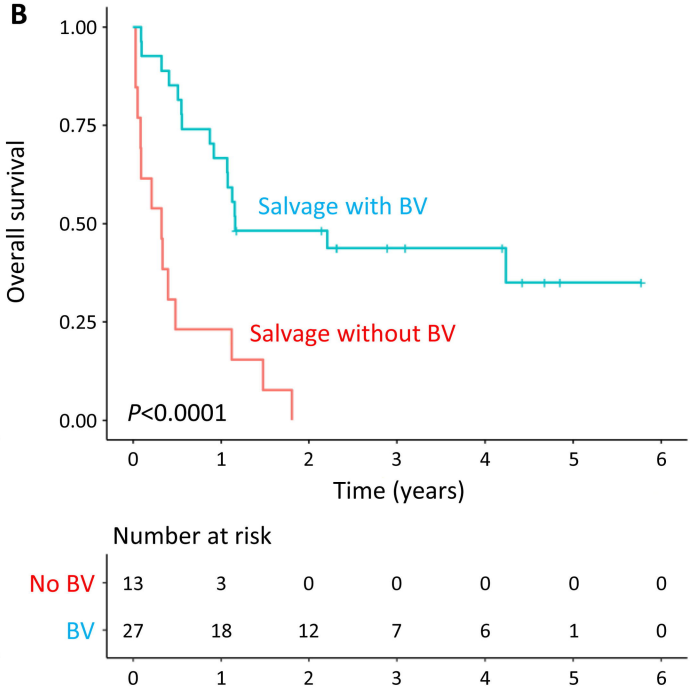
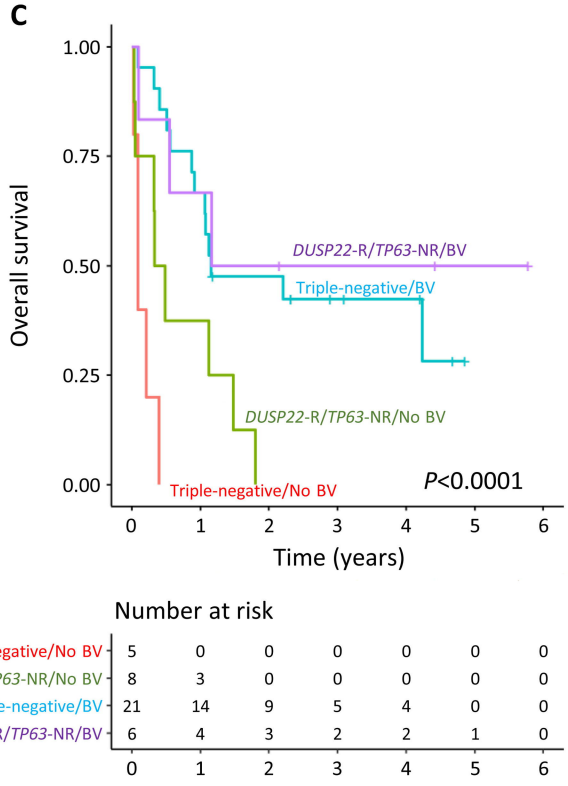
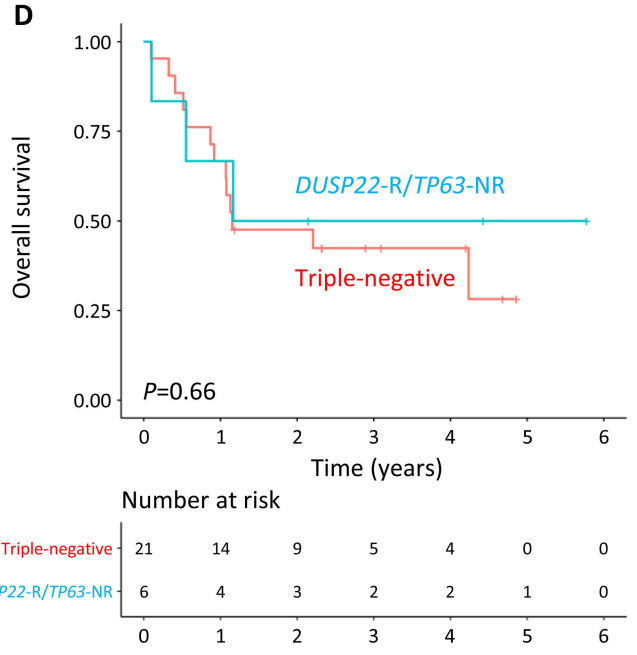

A
Progression-free survival
DUSP22-R/TP63-NR
Triple-negative
P=0.0011
Time (years)
Number at risk
Triple-negative 45 11 4 2 0
DUSP22-R/TP63-NR 39 22 12 5 0
B
Overall survival
DUSP22-R/TP63-NR
Triple-negative
P=0.067
Time (years)
Number at risk
Triple-negative 45 20 6 2 0
DUSP22-R/TP63-NR 39 25 15 6 0
C
Progression-free survival
PS 0-1
PS ≥ 2
P=0.00047
Time (years)
Number at risk
PS 0-1 54 28 15 6 0
PS ≥ 2 29 4 1 1 0
D
Overall survival
PS 0-1
PS ≥ 2
P=0.00016
Time (years)
Number at risk
PS 0-1 54 36 18 7 0
PS ≥ 2 29 8 2 1 0
E
Progression-free survival
DUSP22-R/TP63-NR/PS 0-1
Triple-negative/PS 0-1
DUSP22-R/TP63-NR/PS ≥ 2
Triple-negative/PS ≥ 2
P=0.00016
Time (years)
Number at risk
Triple-negative/PS 0-1 27 8 4 2 0
Triple-negative/PS ≥ 2 18 3 0 0 0
DUSP22-R/TP63-NR/PS 0-1 27 20 11 4 0
DUSP22-R/TP63-NR/PS ≥ 2 11 1 1 1 0
F
Overall survival
DUSP22-R/TP63-NR/PS 0-1
Triple-negative/PS 0-1
DUSP22-R/TP63-NR/PS ≥ 2
Triple-negative/PS ≥ 2
P=0.0011
Time (years)
Number at risk
Triple-negative/PS 0-1 27 15 6 2 0
Triple-negative/PS ≥ 2 18 5 0 0 0
DUSP22-R/TP63-NR/PS 0-1 27 21 12 5 0
DUSP22-R/TP63-NR/PS ≥ 2 11 3 2 1 0

Figure 5


A
Overall survival
1.00
0.75
0.50
0.25
0.00
Triple-negative
DUSP22-R/TP63-NR
P=0.62
0
1
2
3
4
5
6
Time (years)
Number at risk
Triple-negative 29 15 9 5 4 0 0
DUSP22-R/TP63-NR 14 7 3 2 2 1 0
0 1 2 3 4 5 6


B
Overall survival
1.00
0.75
0.50
0.25
0.00
Salvage with BV
Salvage without BV
P<0.0001
0
1
2
3
4
5
6
Time (years)
Number at risk
No BV 13 3 0 0 0 0 0
BV 27 18 12 7 6 1 0
0 1 2 3 4 5 6


C
Overall survival
1.00
0.75
0.50
0.25
0.00
DUSP22-R/TP63-NR/BV
Triple-negative/BV
DUSP22-R/TP63-NR/No BV
Triple-negative/No BV
P<0.0001
0
1
2
3
4
5
6
Time (years)
Number at risk
Triple-negative/No BV 5 0 0 0 0 0 0
DUSP22-R/TP63-NR/No BV 8 3 0 0 0 0 0
Triple-negative/BV 21 14 9 5 4 0 0
DUSP22-R/TP63-NR/BV 6 4 3 2 2 1 0
0 1 2 3 4 5 6


D
Overall survival
1.00
0.75
0.50
0.25
0.00
DUSP22-R/TP63-NR
Triple-negative
P=0.66
0
1
2
3
4
5
6
Time (years)
Number at risk
Triple-negative 21 14 9 5 4 0 0
DUSP22-R/TP63-NR 6 4 3 2 2 1 0
0 1 2 3 4 5 6

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

SUPPLEMENTARY APPENDIX

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),

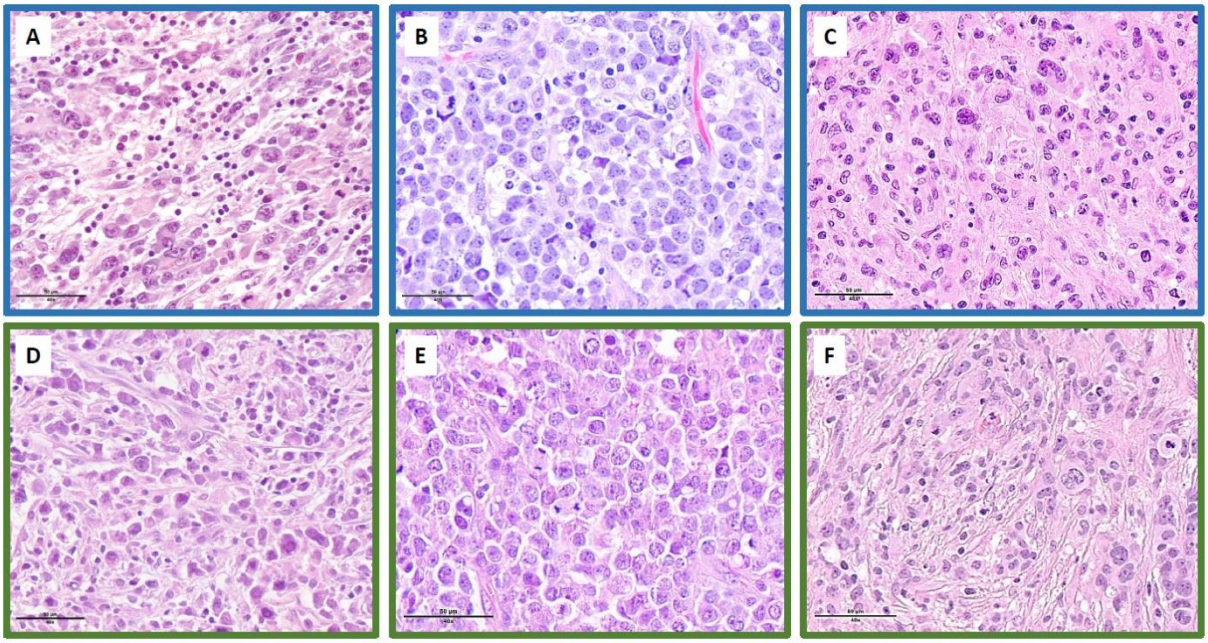
stable disease (SD), or progressive disease (PD), was assessed for evaluable patients. Objective response rate (ORR) was defined as the proportion of patients with a CR or PR to treatment. Response assessment was based on international response criteria, depending on the era (Cheson 1999, Cheson 2007 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.

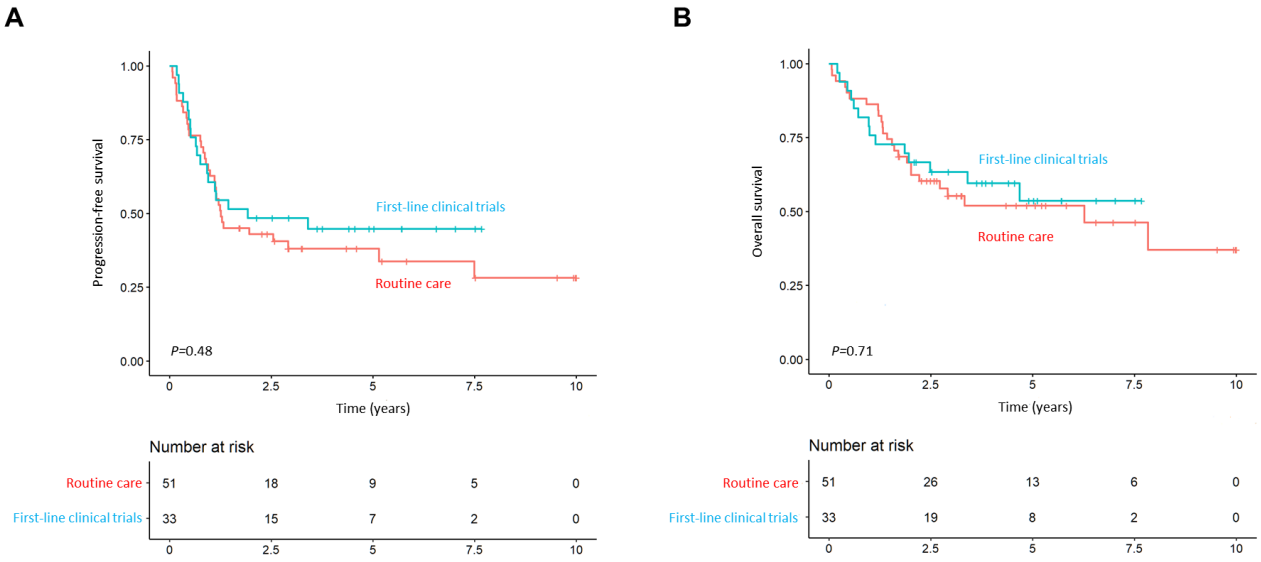
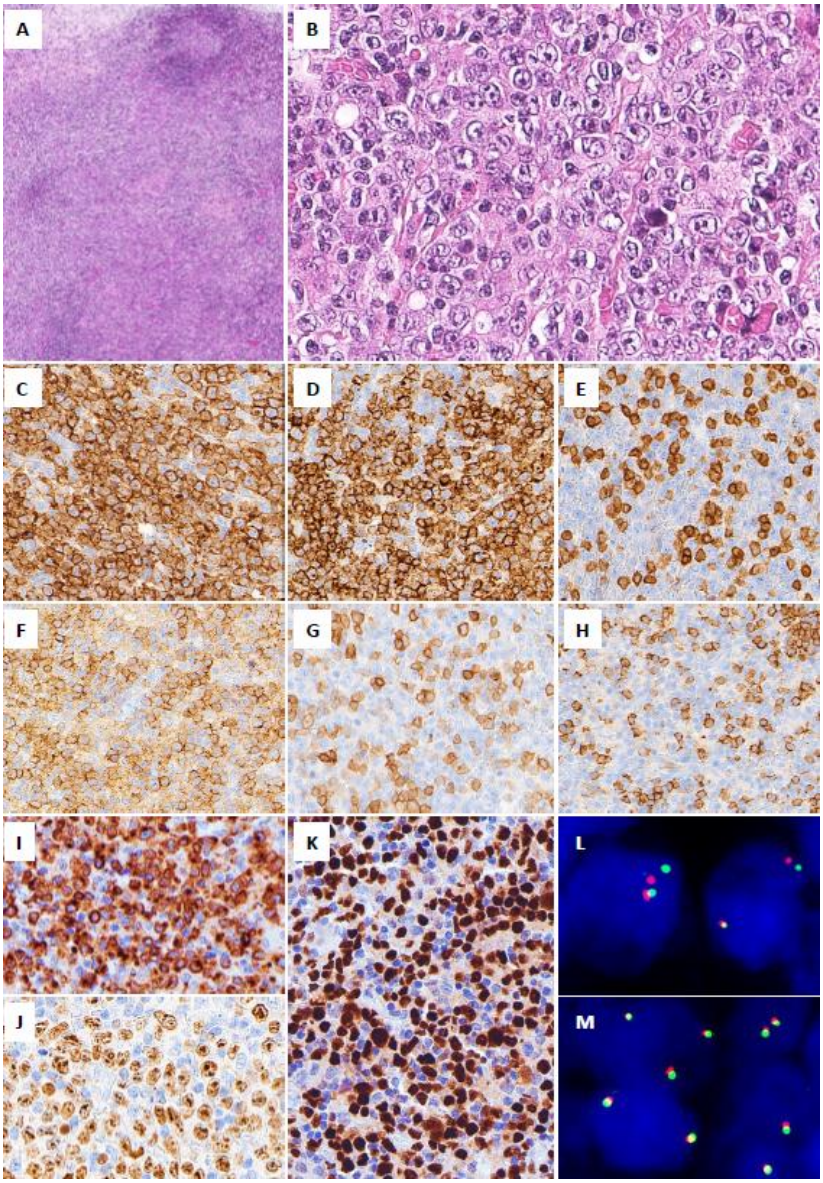


Figure S3. ALK-negative ALCL with *TP63* rearrangement.



(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.

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