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LETTER

Lymphoma

**STAT3 and TP53 mutations associate with poor prognosis in anaplastic large cell lymphoma**

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To the Editor:

Systemic anaplastic large cell lymphoma (sALCL) encompasses two distinct clinical entities of T-cell non-Hodgkin lymphoma: anaplastic lymphoma kinase-positive (ALK+) ALCL and ALK-negative (ALK−) ALCL. These entities are characterized by either the presence or absence of an ALK translocation. It has been reported that ALK+ ALCL has a better prognosis compared to ALK−, with a 5-year overall survival (OS) of 70–80% versus 40–60%, respectively, [1–3]. Furthermore, more than 30% of ALK+ ALCL patients relapse [4, 5]. Despite the distinction between the two sALCL subtypes, frontline treatment for adults is similar and is based on CHOP or CHOEP, instead pediatric ALCL patients are mainly treated following the ALCL99 protocol [6–8]. Whilst high-throughput genomic studies in sALCL have shown recurrent genetic alterations, their association with outcome has not been fully investigated [9–13].

In this study, the mutational landscape of sALCL patient tumors was investigated to discover potential biomarkers that may improve risk stratification and patient management.

A cohort of 82 sALCL patient tumors (47 ALK+ and 35 ALK−) and 6 ALCL cell lines (4 ALK+, 2 ALK−) (Table S1) were subjected to deep targeted next-generation sequencing analyzing the whole coding regions of 275 cancer related genes (Table S2). The average depth achieved across all the samples sequenced was ~2000×. Sequencing data are available at Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/, SRA identifier PRJNA602225).

Male subjects were predominant in both subgroups of our cohort, 57.4% in ALK+ versus 67.6% in ALK−. ALK+ patients were significantly younger than ALK− patients with an average age of 22.7 (3–61) and 55.2 (27–81) years, respectively. ALK+ ALCL patients had a longer survival than ALK− ALCL with a 7-year OS of 77.6% and 46.7%, respectively, and with 7-year progression free survival (PFS) being comparable at 58.7% for ALK+ and 44.1% for ALK− patients (Fig. S1). The first line of treatment for all the adult patients was systemic chemotherapy, and most of the childhood ALK+ ALCL patients (80%) were treated following the ALCL99 or ALCL98 protocols. Although ALK+ patients have a longer

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OS, more than 30% relapsed after first-line treatment. Among the 275 genes analyzed, we identified 148 (54%) genes harboring at least one mutation throughout the entire cohort; 132 genes among the patients and 43 among the cell lines, with 27 genes in common (Fig. S2, Table S3). Overall, 72 out of 82 (88%) patients carried at least one STAT3 and TP53 mutations associate with poor prognosis in anaplastic large cell lymphoma.
mutation within the genes analyzed. We detected an average of 4.2 mutations per patient in ALK− ALCL and an average of 2.7 in ALK+ ALCL. The most recurrently mutated gene in the entire cohort was TP53 found in 16% of sALCL patients (11% ALK+, 23% ALK− and in all ALK+ cell lines). Interestingly, for the ALK+ group, mutated TP53 was more frequent in young patients (p < 0.04). LRP1B was prevalently mutated in ALK+ patients (19%) and in three cell lines. STAT3 and JAK1 were mutated solely in ALK− ALCL, both with a prevalence of 26%, and were the most mutated genes in this group (Fig 1a). Recurrent mutations were detected in epigenetic modifier genes also recently reported to be frequently mutated in BIA-ALCL [14]. KMT2D and TET2 were found mutated in ALCL patients regardless of ALK status and EP300 and KMT2C only in ALK+ patients. Pathway enrichment analysis showed a significant enrichment in mutated genes involved in JAK/STAT (p < 0.003) and PI3K/AKT signaling pathways (p < 0.02) for ALK− ALCL compared with ALK+ ALCL (Fig 1b). We investigated possible correlations between the existence of mutations in the most mutated genes and the clinical characteristics of our cohort. Poor prognostic outcome was defined as patients meeting at least one of the following criteria: deceased, unresponsive to treatment and/or disease relapse. The most recurrently mutated genes in the poor prognostic sub-cohort independent of ALK status were TP53 (27%), STAT3 (24%), EPHA5 (16%), JAK1 (16%), PRDM1 (13.5%), LRP1B (11%) and KMT2D (11%). Considering only refractory/relapsed ALCL patients, mutations within TP53 (28%) and EPHA5 (19%) were the most common (Table S4). In relation to the prognosis, ALK+ patients did not show any significant difference in the signaling pathways affected by mutations. On the contrary, the JAK/STAT (p < 0.005) and PI3K/AKT pathways (p < 0.036) were enriched in ALK− ALCL patients with an inferior outcome (Fig 1b). Pathogenetic variants of STAT3 were detected in 9/35 (26%) of ALK− ALCL patients. Mutations were located mainly within the SH2 domain (S614R, E616G, Y640F, N647I, K658delinsNM and D661V) and in one case within the DNA binding domain (C426R). Mutated JAK1 was detected in 9/35 (26%) of ALK− ALCL patients and of those, 6/9 were at the hotspot codon 1097 (G1097D/F/N/S) (Fig 1d). For four patients, JAK1 was mutated together with STAT3, thereby emphasizing the importance of the JAK/STAT signaling axis. To evaluate the prognostic value of mutations in the JAK/STAT pathway, we performed Cox regression analysis and showed that ALK− ALCL patients harboring STAT3 and/or JAK1 mutation have a shorter OS (hazard ratio [HR] = 2.8; 95% confidence interval [CI], 1.1–7.1, p < 0.03) (Fig S3A). Furthermore, the prognostic value of the most mutated genes in ALK− ALCL: STAT3 (9/35), JAK1 (9/35), TP53 (8/35) and KMT2D (7/35) were investigated. Cox regression analysis showed that patients with STAT3 mutations have a significantly shorter OS compared to those with wild-type STAT3 (HR = 4.1; 95% CI, 1.56–10.71, p < 0.002) (Fig 1c). In addition, while JAK1 and KMT2D mutations did not significantly correlate with OS (p < 0.2 and p < 0.3, respectively), TP53 mutations clearly displayed the correlation (p < 0.01) (Fig S3B–D). To further confirm that mutations in STAT3 are associated with shorter OS, we applied Akaike’s informative criteria model to the four aforementioned genes. STAT3 mutations were found to be the best predictor of OS in ALK− ALCL (Table S5). Moreover, no significant differences were found between mutation status of these genes with age, gender, disease stage, eastern cooperative oncology group performance status or age-adjusted international prognostic index (AA-IPI). As expected [9, 13, 15], expression of p-STAT3 (Y705) was detected at a high level in all ALK− ALCL patients harboring STAT3 mutations, although low/medium expression of p-STAT3 was also detected in STAT3 wild-type patient tumors (Fig S4, Table S6). Mutations in the LRP1B gene were detected in 12/82 (15%) of sALCL patients (19% ALK+ and 9% ALK−) and three cell lines. Since LRP1B was the most recurrently altered gene in ALK+ ALCL, we assessed its possible association with outcome, but no differences were found between mutated and nonmutated patients. To investigate somatic mutations with a possible role in disease relapse, we sequenced paired diagnostic and relapse samples available for four patients (1 ALK+ and 3 ALK−) (Fig 2a). Two different acquired mutations in EPHA5 were detected in each of the two relapse samples (patient tumors...
ALK+1R and HK-10R): a stop codon at S566 and a glycine–valine change at residue 723. In the latter patient (HK-10), identification of mutated *EPHA5* appears to be the result of the emergence of a new malignant clone, harboring novel mutations in several other genes consistently with a similar variant allele frequency (Fig. 2b). Interestingly,
lesions in sALCL with a clinical implication [12]. PRDM1 genes have been shown to be the most common TP53 co-occurring with an inferior outcome, in the former case in ALK reporting an association between mutated gene mutations, thereby demonstrating either copy number loss or concomitant mutations are mechanisms which have the potential to alter p53 and PRDM1 pathways activity.

In summary, within one of the largest cohort of 82 sALCL patients, we provide robust information on the genetic spectrum of genes either solely mutated in ALK–ALCL (STAT3, JAK1) or across the whole spectrum of ALCL (TP53, LRP1B, EPHA5, KMT2D). In addition, we describe novel biomarkers for predicting treatment outcome reporting an association between mutated STAT3 and TP53 with an inferior outcome, in the former case in ALK– disease and in the latter case all sALCL independent of ALK status. Finally, this mutational landscape provides further candidate genes that deserve consideration for their possible role in the patient outcome, such as EPHA5, KMT2D, PRDM1 and SOCS1.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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