Brief Report

blood

LYMPHOID NEOPLASIA

Frequent *NFKBIE* deletions are associated with poor outcome in primary mediastinal B-cell lymphoma

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Key Points

- A recurrent 4-bp deletion in the *NFKBIE* gene is a common event during lymphomagenesis in various lymphoid malignancies.
- The deletion occurs in 22.7% of PMBL cases and is associated with a particularly aggressive clinical disease course.

We recently reported a truncating deletion in the *NFKBIE* gene, which encodes IκBε, a negative feedback regulator of NF-κB, in clinically aggressive chronic lymphocytic leukemia (CLL). Because preliminary data indicate enrichment of *NFKBIE* aberrations in other lymphoid malignancies, we screened a large patient cohort (n = 1460) diagnosed with different lymphoid neoplasms. While *NFKBIE* deletions were infrequent in follicular lymphoma, splenic marginal zone lymphoma, and T-cell acute lymphoblastic leukemia (<2%), slightly higher frequencies were seen in diffuse large B-cell lymphoma, mantle cell lymphoma, and primary central nervous system lymphoma (3% to 4%). In contrast, a remarkably high frequency of *NFKBIE* aberrations (46/203 cases [22.7%]) was observed in primary mediastinal B-cell lymphoma (PMBL) and Hodgkin lymphoma (3/11 cases [27.3%]). *NFKBIE*-deleted PMBL patients were more often therapy refractory (*P* = .022) and displayed inferior outcome compared with wild-type patients (5-year survival, 59% vs 78%; *P* = .034); however, they appeared to benefit from radiotherapy

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(*P* = .022) and rituximab-containing regimens (*P* = .074). *NFKBIE* aberrations remained an independent factor in multivariate analysis (*P* = .003) and when restricting the analysis to immunochemotherapy-treated patients (*P* = .008). Whole-exome sequencing and gene expression profiling verified the importance of NF-κB deregulation in PMBL. In summary, we identify *NFKBIE* aberrations as a common genetic event across B-cell malignancies and highlight *NFKBIE* deletions as a novel poor-prognostic marker in PMBL. (*Blood*. 2016;128(23):2666-2670)

Introduction

Deregulated NF-κB signaling is a hallmark of most lymphoid malignancies, and recurrent mutations in NF-κB transcription factors or upstream signaling components, such as *CD79B*, *CARD11*, *MYD88*, or *TNFAIP3,* are common findings in B-cell neoplasms.[1-3] Genetic aberrations in both the canonical and noncanonical NF-κB pathway are known to lead to NF-κB activation.[4] However, the full compendium of NF-κB pathway genes affected by recurrent mutations in lymphoid malignancies remains to be elucidated.

Recently, we reported a 4-bp truncating mutation in the *NFKBIE* gene, which encodes IκBε, a negative regulator of NF-κB in normal B cells, in chronic lymphocytic leukemia (CLL).[5-8] *NFKBIE* deletions were enriched in clinically aggressive CLL patients (7% to 8%) and associated with shorter time to first treatment. At the functional level, *NFKBIE*-deleted cases showed reduced IκBε levels and decreased p65 inhibition, along with increased phosphorylation and nuclear translocation of p65, compared with wild-type patients. Similarly, loss of IκBε was reported to lead to constitutive NF-κB transcriptional activation in C57B16 mice, which in turn resulted in increased B-cell proliferation and survival.[9]

Preliminary data from limited patient series indicate an increased frequency of *NFKBIE* aberrations in other lymphoid malignancies, such as relapsed/refractory diffuse large B-cell lymphomas (DLBCL) and primary mediastinal large B-cell lymphomas (PMBL).[10,11] By screening a large cohort of 1460 patients with different lymphoid neoplasms, we provide further evidence that *NFKBIE* deletions are a common event during lymphomagenesis and highlight an enrichment in PMBL linked to a particularly poor outcome.

Study design

Patients

In total, 1460 patients diagnosed with different lymphoid malignancies were included from collaborating institutions in Denmark, Germany, Greece, France, Spain, Sweden, and the United Kingdom. The study cohort comprised DLBCL (n = 520), follicular lymphoma (n = 225), PMBL (n = 203),[12,13] mantle cell lymphoma (n = 189), splenic marginal zone lymphoma (n = 175),[14] T-cell acute lymphoblastic leukemia (n = 94), primary central nervous system lymphoma (n = 34), classical Hodgkin lymphoma (cHL; n = 11), and small lymphocytic lymphoma (n = 9). All samples were collected before start of therapy and diagnosed according to the WHO classification.[15] Written consent was obtained in accordance with the Declaration of Helsinki and with ethical approval obtained from the local ethics committees.

Mutational analysis of *NFKBIE*

The entire coding region of *NFKBIE* was investigated by Sanger sequencing in a representative subset (n = 292) of the study cohort, while the 4-bp deletion hotspot located in exon 1 was analyzed either by Sanger sequencing (n = 350) or GeneScan analysis (n = 807) in the remaining cases (detailed in supplemental Methods, available on the *Blood* Web site). In addition, targeted deep sequencing was performed in 44 PMBL and 22 DLBCL samples using the Nextera XT kit (Illumina) for library preparation (supplemental Methods).[6,7,16,17] For the cHL samples, Hodgkin and Reed/Sternberg (HRS) cells were microdissected and sequenced as detailed in supplemental Methods.

Whole-exome sequencing

Whole-exome sequencing (WES) was performed in 7 matched tumor or germline PMBL cases (detailed in supplemental Methods); in addition, previously reported WES data on 7 PMBLs were included.[10,18] WES data have been deposited at the European Nucleotide Archive (http://www.ebi.ac.uk/ena), which is hosted at the European Bioinformatics Institute, under accession number PRJEB15361.

Gene expression profiling

To assess gene expression differences in *NFKBIE*-deleted (n = 8) and *NFKBIE* wild-type (n = 21) PMBL, we applied the NanoString PanCancer Pathways Panel to quantify transcript levels of 770 genes representing 13 canonical cancer pathways plus 30 selected genes reported to be important in PMBL and/or the NF-κB pathway[8,19] (described in supplemental Methods). Gene expression data have been deposited in National Center for Biotechnology Information's Gene Expression Omnibus database and are accessible through Gene Expression Omnibus Series accession number GSE86815.

Statistical analysis

Pairwise comparisons of variables for exploratory purposes were performed using the Mann-Whitney test or the χ^2 test. Overall survival (OS) was calculated from time of diagnosis until date of death or last follow-up. Kaplan-Meier analysis was performed to construct survival curves, and the log-rank test was applied to evaluate differences between subgroups. Cox regression analysis was applied for multivariate analysis. All analyses were carried out using the software package SPSS Version 23.0 (IBM, Armonk, NY).

Results and discussion

Overall, 86 *NFKBIE* mutations were identified in 84 of 1460 patients (5.8%). All but 4 patients (L389P, P440L, and L495V missense mutations and a 4-bp splice-site deletion) exhibited a heterozygous 4-bp deletion (delTTAC), known to result in a truncated protein.[6] Two PMBL cases with the recurrent 4-bp deletion showed additional *NFKBIE* frameshift deletions (a 7-bp and 38-bp deletion). The somatic nature of the 4-bp deletion has previously been confirmed.[5-7,11,18] Using a cutoff of >5% for the mutant allele, GeneScan analysis and deep sequencing revealed a high concordance of allele frequencies between both techniques (n = 10, $r = 0.80$, $P = .005$), with variant allele frequencies ranging from 5% to 62% in *NFKBIE*-deleted cases. This finding indicates that *NFKBIE* mutations may occur at different time points of lymphomagenesis (supplemental Tables 1 and 2; Figure 1).

While *NFKBIE* deletions were relatively rare in patients diagnosed with follicular lymphoma (3/225 [1.3%]), splenic marginal zone lymphoma (3/175 [1.7%]), and T-cell acute lymphoblastic leukemia (1/94 [1.1%]), slightly higher frequencies were detected among DLBCL (18/520 [3.5%]), mantle cell lymphoma (8/189 [4.2%]), primary central nervous system lymphoma (1/34 [2.9%]), and small

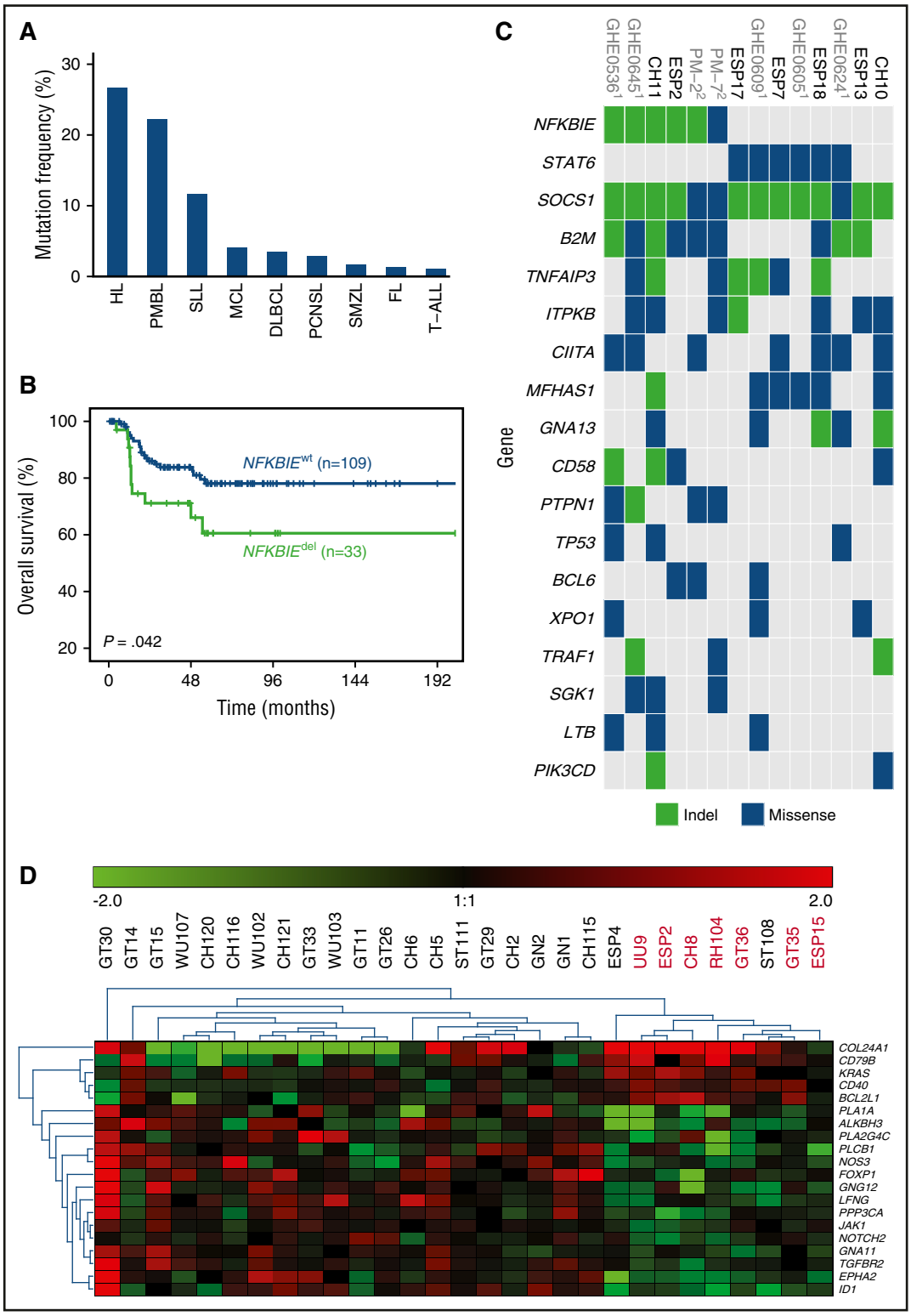

Figure 1. Assessment of *NFKBIE* mutations in 1460 patients diagnosed with different lymphoid malignancies. (A) *NFKBIE* mutation frequency per disease entity. (B) OS in 143 PMBL patients according to *NFKBIE* mutation status (*P* value refers to log-rank test). (C) Recurrently mutated genes in PMBL. Based on WES generated on 7 PMBL cases from this series and available exome data on 7 cases from Mareschal et al.[10] and Gunawardana et al.[18] (D) Differentially expressed genes in *NFKBIE* wild-type (n = 21) vs deleted (n = 8) PMBL cases based on the NanoString PanCancer Pathways Panel plus an additional 30 genes (detailed in supplemental Methods). FL, follicular lymphoma; HL, Hodgkin lymphoma; MCL, mantle cell lymphoma; PCNSL, primary central nervous system lymphoma; SMZL, splenic marginal zone lymphoma; SLL, small lymphocytic lymphoma; T-ALL, T-cell acute lymphoblastic leukemia.

lymphocytic lymphoma (1/9 [11.1%]). In contrast, PMBL patients showed a marked enrichment, with 46 of 203 cases harboring a *NFKBIE* deletion (22.7% vs 2.9% [38/1257 in other entities]; $P < .001$; Figure 1A). Notably, the prevalence of *NFKBIE*-deleted PMBL cases was independent of contributing center (supplemental Table 3).

Table 1. Comparison of pretreatment characteristics of the 142 PMBL patients with available survival data

Characteristic	*NFKBIE* mutated (n = 33)	*NFKBIE* wild-type (n = 109)	*P* value
Age, y			.417
Median	32	35	
Range	19-75	17-79	
Sex			.613
Male	12 (36%)	45 (41%)	
Female	21 (64%)	64 (59%)	
Ann Arbor stage			.581
1	5 (18%)	26 (31%)	
2	15 (54%)	39 (45%)	
3	1 (3%)	4 (5%)	
4	7 (25%)	16 (19%)	
Missing data	5	24	
International Prognostic Index			.885
0	4 (12%)	13 (13%)	
1	15 (46%)	40 (41%)	
2	6 (18%)	23 (24%)	
3	6 (18%)	17 (17%)	
4	2 (6%)	3 (3%)	
5	0 (0%)	2 (2%)	
Missing data	0	11	
Extranodal involvement			.916
Yes	11 (35%)	31 (34%)	
No	20 (65%)	59 (66%)	
Missing data	2	19	
Bone marrow involvement			.231
Yes	1 (4%)	0 (0%)	
No	26 (96%)	90 (100%)	
Missing data	6	19	
Bulky disease			.706
Yes	19 (76%)	65 (72%)	
No	6 (24%)	25 (28%)	
Missing data	8	19	
LDH elevation			.289
Yes	27 (84%)	67 (75%)	
No	5 (16%)	22 (25%)	
Missing data	1	20	
B-symptoms			.375
Yes	8 (25%)	34 (33%)	
No	24 (75%)	68 (67%)	
Missing data	1	7	
CHOP-based treatment			
Yes	33 (100%)	104 (100%)	
No	0 (0%)	0 (0%)	
Missing data	0	5	
Rituximab-containing treatment			.483
Yes	25 (76%)	83 (81%)	
No	8 (24%)	19 (19%)	
Missing data	0	7	
Intensified chemotherapy treatment			
Yes	7 (21%)	26 (25%)	.638
No	26 (79%)	77 (75%)	
Missing data	0	6	
Radiation			.439
Yes	15 (50%)	50 (58%)	
No	15 (50%)	36 (42%)	
Missing data	3	23	
Response to treatment			.022
CR/CRu	20 (62%)	71 (81%)	
Partial remission	4 (13%)	11 (13%)	
Progressive disease	8 (25%)	6 (6%)	
Missing data	1	21	

Values are number and percentage of patients (unless indicated otherwise). *P* values are from 2-sided χ^2 tests for categorical variables and from 2-sided Mann-Whitney *U* tests for comparison.

CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; CR, complete remission; CRu, uncertain complete remission; ECOG, performance status of the Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase.

In an ongoing exome sequencing analysis of microdissected HRS cells in cHL, we obtained indication for *NFKBIE* mutations in 4 out of 11 cases. From these 4 cases, we isolated HRS cells and confirmed somatic *NFKBIE* aberrations (1 deletion and 2 missense mutations) in 3 out of 4 cases by Sanger sequencing (supplemental Table 4). Hence, *NFKBIE* aberrations are also a recurrent event in cHL (Figure 1A) and further explain biologic similarities reported between both lymphoma entities.[19-21]

For 142 out of 203 investigated PMBL cases, clinical follow-up data were available; the median follow-up time for patients alive was 61 months (range, 1-258 months). There were no significant differences between *NFKBIE*-deleted and wild-type PMBL patients with respect to clinical characteristics (Table 1). However, *NFKBIE*-deleted PMBL patients were more likely than wild-type patients to be refractory to primary chemotherapy (25% vs 6%; $P = .022$). Furthermore, these patients had a significantly shorter OS as compared with wild-type patients (5-year survival, 59% vs 78%; $P = .034$; Figure 1B). In multivariate analysis, *NFKBIE* deletion status (n = 111; 95% confidence interval, 1.65-12.04; hazard ratio, 4.46; $P = .003$) remained an independent factor for poor outcome (supplemental Table 5), even when restricting the analysis to patients treated with immunochemotherapy (n = 91; 95% confidence interval, 1.72-39.82; hazard ratio, 8.27; $P = .008$; supplemental Table 6). In contrast, no significant difference in treatment response and OS was seen between 16 *NFKBIE*-deleted and 428 wild-type DLBCL patients (median OS, 65 vs 52 months; $P = .804$; supplemental Table 7) or within immunohistochemistry-based germinal center B-cell–like and non–germinal center B-cell–like subtypes (supplemental Figure 2).

An improved patient outcome was recently demonstrated in PMBL with the addition of rituximab to dose-intense chemotherapy (ie, dose-adjusted etoposide, doxorubicin, cyclophosphamide, vincristine, and prednisone [CHOEP]), resulting in an OS rate of 97%.[22] Patients in the present series received heterogeneous treatment regimens, and data interpretation warrants caution. Nevertheless, all patients received CHOP-based treatment; in ~75% of cases, rituximab was added, and ~25% were treated with dose-intensified schemes. For the latter, the vast majority received CHOEP, while individual cases were treated with mega-CHOEP; or a combination of doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP). While dose escalation did not improve outcome for *NFKBIE*-deleted patients, these patients appeared to benefit from the addition of rituximab (n = 33, $P = .074$) or, particularly, radiotherapy (n = 30, $P = .022$). After restricting this analysis to nonprogressive patients, the benefit of radiation showed borderline significance ($P = .083$; supplemental Figure 3).

Several studies have reported high frequencies of *TNFAIP3* aberrations (ranging from 30% to 60%[10,18,23,24]), another key regulator of NF-κB, in PMBL. Based on exome data on 14 cases,[10,18] most PMBL cases demonstrated a very high number of somatic variants (average 218 mutations). While 3 *NFKBIE*-mutated cases showed concomitant *TNFAIP3* aberrations (Figure 1C), other members of the NF-κB pathway or interrelated pathways, such as the JAK/STAT pathway,[25] were also affected. In addition, we performed gene expression profiling in *NFKBIE*-deleted (n = 8) vs wild-type (n = 21) PMBL cases using NanoString technology and identified 79 differentially expressed genes, including several NF-κB target genes or upstream mediators, such as *BCL2L1*, *NFKBIB*, *EGFR*, *CD79B*, and *CD40* (Figure 1D; supplemental Table 8). Together, these findings support deregulation of NF-κB signaling as a major factor in PMBL pathobiology.

In conclusion, our findings highlight *NFKBIE* deletions as a common, recurrent genetic event among different lymphoid malignancies. *NFKBIE* deletions emerged among the most frequent genetic aberrations in PMBL and were associated with chemorefractoriness

and an inferior clinical outcome. Further validation studies are now warranted to confirm this novel finding.

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Authorship

Contribution: L.M., D.N., R.R., and F.D. designed the research; L.M., D.N., E.Y., E.M., M. Abdulla, M.F., F.A., V.L., M.S., K.Y., A.S., T.P., B.G., A.T., N.W., A.R.-D., M. Angelopoulou, M.Z., C.M.A., L.C., D.L., C.D.B., C.B., J.O., J.F., B.D., H.G.D., D.R.-W., C.A.S., H.D.M.-P., T.Z., M.-L.H., J.C.S., G.E., O.A.B., E.R., M.E., P.K., M. Hultdin, T.P., K.G., A.L.-G., R.K., S.O., K.S., N.S., G.K., A.R., E.C., R.-M.A., G.O., T.P.V., M. Hummel, R.R., and F.D. performed the research and/or contributed patient samples and clinical data; L.M., D.N., E.Y., R.R., and F.D. analyzed the data; and L.M, D.N., R.R., and F.D. wrote the paper. All authors read and agreed to the final version of the manuscript.

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