The presence of TP53 mutation at diagnosis of follicular lymphoma identifies a high-risk group of patients with shortened time to disease progression and poorer overall survival

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The International Prognostic Index and the Follicular Lymphoma International Prognostic Index are widely used for the risk assessment of follicular lymphoma (FL). Although molecular studies have provided insight into the biology of FL, no molecular marker has impacted on treatment stratification. Because TP53 mutations are associated with poor prognosis in hematologic malignancies, we investigated the prognostic value of TP53 mutation at diagnosis in FL. Heterozygous TP53 mutation was detected in 12 of 185 (6%) analyzed cases. Mutation was associated with older age (P = .02) and higher International Prognostic Index score (P = .04). On multivariate analysis, TP53 mutation correlated with shorter progression-free survival (P < .001) and overall survival (P = .009). TP53 mutation was associated with low expression of the immune-response 1 gene expression signature (P = .016) and with an unfavorable gene expression-based survival predictor score (P < .001), demonstrating for the first time that molecular features of the malignant cell may correlate with the nature of the immune response in FL. (Blood. 2008;112:3126-3129)

Introduction

Follicular lymphoma (FL) is characterized by episodes of progression alternating with periods of remission and is associated with a median survival of 8 to 10 years.1,2 However, a proportion of patients die within the first 2 years; furthermore, histologic transformation may occur, dramatically reducing overall survival (OS).3,4 Despite the introduction of immunotherapy, which has improved outcome,5-7 the management of high-risk patients remains challenging. The International Prognostic Index (IPI) and the Follicular Lymphoma International Prognostic Index are widely used for risk assessment in FL,8 the latter retaining its predictive capacity with the current use of upfront immunotherapy.9 Newer molecular studies have provided insight into the biology of FL, and as yet no molecular markers have impacted on treatment stratification. Gene expression profiling studies have identified 2 prognostic signatures, immune-response 1 (IR1) and immune-response 2 (IR2), both based on nonmalignant tumor-infiltrating cells.10 The IR1 signature is a molecular correlate of a T cell–rich tumor microenvironment, whereas the IR2 signature reflects a microenvironment enriched in myeloid-lineage cells. A survival predictor score was formed from these expression signatures, high values of which indicated enrichment for the IR2 signature and unfavorable OS, which has been confirmed recently.11

Mutations in TP53 are frequent in cancer12 and hematologic malignancies where they correlate with unfavorable prognosis and chemotherapy resistance.13-15 TP53 mutation has been reported in 10% to 20% of various histologic subtypes of non-Hodgkin lymphoma.16 In FL, TP53 mutation occurs infrequently at diagnosis and usually in association with transformation.17-20 We therefore set out to clarify the role of TP53 mutation in a large series of previously untreated FL patients and assess its impact on patient prognosis and clinical outcome.

Methods

Patient information

DNA from 191 untreated patients with FL presenting between 1974 and 2001 was obtained through the Lymphoma/Leukemia Molecular Profiling Project; 185 cases were analyzed for TP53 mutation. These samples were chosen because they were fully characterized molecularly.10 Clinical data


An Inside Blood analysis of this article appears at the front of this issue.
were available in 172 cases. Approval to use clinical material for mutation analysis was obtained from the London Research Ethics Committee and East London and the City London Research Ethics Committee, and their stipulations regarding patient consent, confidentiality, and data protection were followed. The ethics submission covering this project is 06/Q0605/69. This study was conducted in accordance with the Declaration of Helsinki.

**TP53 mutation detection**

Because our previous data\(^26\) showed poor correlation between TP53 protein status by immunohistochemistry and mutation status, and in keeping with the recommendation from the International Agency for Research on Cancer (IARC) TP53 database,\(^21\) we screened genomic DNA samples for DNA sequence variants using high-resolution melting curve analysis followed by bidirectional sequencing. Primers were designed to amplify the coding sequence and flanking 3′ and 5′ splice sites of exons 5 to 8 of TP53 using Primer3\(^22\) (primers and conditions are available on request). Analysis was restricted to those exons as they harbor 94.2% of all somatic mutations in TP53.\(^8\) Bidirectional sequencing. Primers were designed to amplify the coding sequence and flanking 3′ and 5′ splice sites of exons 5 to 8 of TP53 using Primer3\(^22\) (primers and conditions are available on request). Analysis was restricted to those exons as they harbor 94.2% of all somatic mutations in TP53.\(^8\) Primers were designed to amplify the coding sequence and flanking 3′ and 5′ splice sites of exons 5 to 8 of TP53 using Primer3\(^22\) (primers and conditions are available on request). Analysis was restricted to those exons as they harbor 94.2% of all somatic mutations in TP53.\(^8\) Primers were designed to amplify the coding sequence and flanking 3′ and 5′ splice sites of exons 5 to 8 of TP53 using Primer3\(^22\) (primers and conditions are available on request). Analysis was restricted to those exons as they harbor 94.2% of all somatic mutations in TP53.\(^8\) Primers were designed to amplify the coding sequence and flanking 3′ and 5′ splice sites of exons 5 to 8 of TP53 using Primer3\(^22\) (primers and conditions are available on request). Analysis was restricted to those exons as they harbor 94.2% of all somatic mutations in TP53.\(^8\)

Statistical analysis

Association with clinical characteristics was investigated by Fisher exact test for (not ordered) categorical data, \(t\) test for normally distributed continuous data, and Mann-Whitney \(U\) test for ordered data. OS was defined as the time from diagnosis to death, or for patients remaining alive, the time from diagnosis to last contact. Progression-free survival (PFS) was defined as the time from diagnosis to first progression, transformation or death from any cause, or for patients remaining alive and disease free, the time from diagnosis to last contact. Transformation was defined histologically (\(n=27\)) or clinically (\(n=12\)) with signs including: rapid nodal or extranodal growth, unusual extranodal sites, or sudden rise in lactate dehydrogenase. Kaplan-Meier survival estimates were obtained and the log-rank test used to compare differences between the mutated and wild-type subgroups. Multivariate Cox regression was used to determine whether mutation remained independently predictive of PFS and OS after adjusting for clinical variables. Significance was set at \(P\) less than .05. Gene expression correlation was performed as previously described.\(^10\)

**Results and discussion**

TP53 mutation was detected in 12 of 185 (6%) cases of diagnostic FL. All were heterozygous. Eleven mutations were missense (exon 5 (1), exon 7 (5), and exon 8 (5)), and a single mutation arose in the splice site of intron 7 (Table 1). All mutations were previously reported in the IARC TP53 mutation database.\(^12\) Single nucleotide polymorphism and comparative genomic hybridization analysis showed 5 cases with loss of heterozygosity involving the TP53 locus (data not shown), suggesting that del 17p is a rare event at diagnosis.

The major clinical characteristics of the 172 patients are summarized in Table S1 (available on the Blood website; see the Supplemental Materials link at the top of the online article). TP53 mutation occurred more frequently in older patients (\(P=0.02\)) and those with higher IPI (\(P=0.04\)). Mutated patients were treated heterogeneously (Table 1); and although most responded well, responses were frequently of short duration (median, 15 months; range, 4-53 months). This resulted in inferior PFS (log-rank, \(P=0.001\)) and OS (log-rank, \(P<0.001\)) for patients with mutation (Figure 1A and 1B, respectively).

Multivariate analyses confirmed that TP53 mutation was predictive for shorter PFS when adjusted for IPI (hazard ratio [HR] = 3.6; 95% confidence interval [CI], 1.8-7.2; \(P<0.001\)), or for age and stage (HR = 3.4; 95% CI, 1.7-6.7; \(P<0.001\)). However, the effect of TP53 mutation on OS was significant only after adjustment for IPI (HR = 2.7; 95% CI, 1.3-5.6; \(P=0.009\)) but not when adjusted for age and stage. Transformation data were available on 142 cases. There was no association seen between TP53 mutation and transformation in this group; 3 of 11 patients with TP53 mutation transformed in contrast to 36 of 131 with normal TP53 status (Table S1; \(P=1.0\)).

### Table 1. Analysis of TP53 mutations in FL and correlation with tumor stage and clinical outcome

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at diagnosis, y</th>
<th>Genomic no. †</th>
<th>Predicted amino acid no. †</th>
<th>Location</th>
<th>Predicted effect ‡</th>
<th>Predicted TP53 function ‡</th>
<th>Disease stage at presentation</th>
<th>Initial treatment</th>
<th>PFS, mo</th>
<th>OS, y</th>
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<tr>
<td>1</td>
<td>45</td>
<td>g.13216T&gt;A</td>
<td>p.H179Q</td>
<td>Exon5</td>
<td>Missense</td>
<td>Nonfunctional</td>
<td>Ile Single-agent chemotherapy</td>
<td>10</td>
<td>7.7</td>
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<td>p.Y234C</td>
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<td>Missense</td>
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<td>p.G244S</td>
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<td>Missense</td>
<td>Nonfunctional</td>
<td>III Multiagent chemotherapy</td>
<td>11</td>
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<td>p.G245S</td>
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<td>II Radiotherapy</td>
<td>21</td>
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<td>Splice</td>
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</table>

PFS indicates progression-free survival; OS, overall survival; and NA, not applicable.

*Genomic numbers refer to reference sequence U94788.

†Predicted amino acid numbers refer to a translation of NM000546.

‡Predicted structural and functional characteristics of the mutations were obtained from the mutation validation tool of the IARC TP53 Mutation Database.
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References

13. Prokocimer M, Rotter V. Structure and function of complex interaction between the tumor and its microenvironment in FL. 10
14. This group of patients was treated heterogeneously; because TP53 mutation is associated with high-risk disease, this raises the issue of whether these patients should receive more aggressive therapy. Because none of the mutated cases received upfront chemo-immunotherapy, it remains to be determined whether rituximab will improve survival in this group. Patients with chronic lymphocytic leukemia and TP53 abnormalities have not seen improvements in survival with rituximab. 24 Therapeutic strategies could include alemtuzumab or lenalidomide, which have demonstrated activity in chronic lymphocytic leukemia patients with TP53 dysfunction. Although TP53 mutation is more frequent in older patients, 4 of 12 mutated cases here were younger than 60 years, and nonmyeloablative allogeneic stem cell transplantation may be an option. Radiotherapy would not be predicted to prolong responses, 25 as the majority of mutations observed here were nonfunctional (Table 1).
15. These results suggest that TP53 screening could be incorporated into the design of clinical trials in FL. If mutation is validated as a high-risk genetic marker, investigational treatments could be considered in this group of patients.


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