Brief report

Relevance of the Immunoglobulin $V_H$ somatic mutation status in patients with chronic lymphocytic leukemia treated with fludarabine, cyclophosphamide, and rituximab (FCR) or related chemoimmunotherapy regimens

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Although immunoglobulin $V_H$ mutation status ($IgV_H$ MS) is prognostic in patients with chronic lymphocytic leukemia (CLL) who are treated with alkylating agents or single-agent fludarabine, its significance in the era of chemoimmunotherapy is not known. We determined the $IgV_H$ somatic mutation status (MS) in 177 patients enrolled in a phase 2 study of fludarabine, cyclophosphamide, and rituximab (FCR) and in 127 patients treated with subsequent chemoimmunotherapy protocols. $IgV_H$ MS did not impact significantly on the complete remission (CR) rate of patients receiving FCR or related regimens. However, CR duration was significantly shorter in patients with CLL that used unmutated $IgV_H$ than those whose CLL used mutated $IgV_H$ (TTP 47% vs 82% at 6 years, $P < .001$). In a multivariate model considering all baseline characteristics, $IgV_H$ MS emerged as the only determinant of remission duration (hazard ratio 3.8, $P < .001$). Our results suggest that postremission interventions should be targeted toward patients with unmutated $IgV_H$ status. (Blood. 2009;113:3168-3171)

Introduction

Immunoglobulin $V_H$ somatic mutation status ($IgV_H$ MS) is an important prognostic marker in patients with chronic lymphocytic leukemia (CLL). In the chemotherapy era, patients with cells that used an unmutated $IgV_H$ gene (UM-CLL) had inferior rates of survival compared with those that used a mutated $IgV_H$ gene (M-CLL).1,2 However, it was unclear whether this difference was because of inferior treatment response, increased risk of relapse from remission, or both. Furthermore, it is not known whether $IgV_H$ MS remains relevant in patients treated with combinations of chemotherapy and monoclonal antibodies (chemoimmunotherapy).3,4 To address these questions, we analyzed the impact of $IgV_H$ MS on the outcome of patients treated with frontline chemoimmunotherapy at our center.

Methods

The University of Texas M. D. Anderson Cancer Center Institutional Review Board approved the studies included in this report, and informed consent was obtained in accordance with the Declaration of Helsinki. The main analysis was based on 300 patients treated with the phase 2 study of the fludarabine, cyclophosphamide, rituximab (FCR) regimen, which had a mature median follow-up of 6 years. The complete remission (CR) rate was 72%, and the time to progression (TTP) for complete responders was 85 months; 6-year overall survival (OS) for all patients was 77%.3,4 Subsequent to the FCR study, our center evaluated several other chemoimmunotherapy regimens: FCR3 (FCR with 3 rituximab doses per cycle, n = 56),5 FCMR (FCR with mitoxantrone, n = 24),6 FCR plus granulocyte macrophage colony-stimulating factor (GMCSF; n = 21), and CFAR (FCR with alemtuzumab, n = 26).7 These protocols were single-arm studies with differences in baseline characteristics; however, they were conducted in patients with CLL commencing initial chemotherapy according to uniform criteria,8 and patients underwent response staging in an identical manner.8 Therefore, studies subsequent to FCR were included to provide additional insight into the impact of $IgV_H$ MS on CR achievement. The impact of $IgV_H$ MS on TTP and OS was not evaluable in these studies because of the short median follow-up (35, 26, 12, and 8 months for FCR3, FCMR, CFAR, and FCR + GMCSF, respectively).

For patients treated with FCR, $IgV_H$ MS was determined retrospectively in 101 patients from pretreatment formalin-fixed, paraffin-embedded bone marrow aspirate clot section samples and prospectively in 76 patients from fresh peripheral blood or bone marrow samples. The remaining 123 patients had insufficient specimens for analysis. All patients in protocols subsequent to FCR had their $IgV_H$ mutation status (MS) determined prospectively from fresh tissue. The technique for testing in fresh tissue was according to published methods.9

The technique for paraffin-embedded tissue was as follows: DNA was extracted from paraffin-embedded tissue section with the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) and amplified with HotStar Taq DNA Polymerase (QIAGEN) and FRc1 primer set, as described previously.10 Amplified monoclonal bands were detected on 1.5% agarose gel, excised, and extracted with use of the QIAquick Gel Extraction Kit (QIAGEN). The purified DNA was subjected to sequence analysis. The mutation status was designated as unmutated (UM-CLL) if we detected fewer than 2.0% mutations (> 98.0% homology to germline sequences) or as mutated (M-CLL) if we detected 2.0% or greater mutations.


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Results and discussion

*IgVH* MS was available in 177 FCR patients, with 59% having UM-CLL and 41% having M-CLL (Table 1). Their baseline characteristics and treatment outcome were not significantly different from that of FCR patients whose *IgVH* MS was not determined (*P > .20 for all comparisons, data not shown). Clinical data from 127 patients treated on protocols subsequent to FCR were pooled (Table 1) to examine for differences between patients with UM-CLL (69%) and M-CLL (31%). In general, patients with UM-CLL were more likely to have a high white cell count, elevated 2-microglobulin, and/or abnormal conventional karyotypic or adverse fluorescence in situ hybridization findings (Table 1).

Among FCR patients, CR rates were similar between UM-CLL and M-CLL patients, being 73% and 83%, respectively (*P = .12). The proportions of patients achieving flow cytometry–negative CR (defined as < 1% CD5/19 coexpressing cells in the marrow lymphoid gate, with normal light chain ratio) were 57% and 67%, respectively (*P = .21). This result was reproduced in patients treated on protocols subsequent to FCR, where the CR rates were similar between UM-CLL and M-CLL patients both as a group (71% vs 78%, respectively; *P = .46), and within individual regimens (Table 1). Within this group, the proportions of UM-CLL and M-CLL patients achieving flow cytometry–negative CR were 54% and 69%, respectively (*P = .16). Thus,
**IgVH** MS did not substantially influence the probability of achieving CR.

In the analysis of CR duration in FCR patients, however, UM-CLL patients who entered CR were found to have a significantly inferior TTP compared with M-CLL patients (Figure 1A). At 6 years, the proportions of complete responders remaining progression free were 47% and 82% for UM-CLL and M-CLL patients, respectively (P < .001). Among M-CLL patients, those using the VH3-21 gene were at an increased risk of relapse (3 relapses in 6 patients) compared with those using other VH subfamilies (7 relapses in 54 patients, P = .05). This effect of IgVH MS on TTP was maintained when the analysis was restricted only to complete responders with confirmed flow cytometric negativity (Figure 1B). Multivariate analysis was performed to determine whether the significance of IgVH MS was independent of prognostic factors previously established in the FCR population. In this analysis, IgVH MS status was strongly and independently significant for TTP (hazard ratio 3.8, P < .001), whereas established factors (including age, B2m, cytogenetic abnormalities, white cell count, marrow CD38 positivity, and interval between diagnosis and treatment) failed to reach statistical significance.

Considering all patients treated with FCR, 6-year OS was significantly inferior in UM-CLL compared with M-CLL patients (71% and 82%, respectively; P = .05). However, on multivariate analysis, IgVH MS was not independently associated with inferior OS (P = .10), whereas advancing age (P = .001) and high B2m (P = .006) were significant.

This study demonstrated that the prognostic impact of IgVH MS in patients receiving chemoimmunotherapy regimens was not related to differences in CR rates but was caused by aggressive relapse kinetics in patients with UM-CLL. The reason for this effect is uncertain and may be related to differential responses to B-cell receptor signaling.12 These data are highly relevant to clinical investigators because they suggest that studies of postremission interventions (eg, maintenance therapy, early relapse detection strategies, use of novel agents in remission) should be targeted toward those patients with UM-CLL, because these are the patients at the greatest risk of early relapse. Patients with CLL which use the VH3-21 gene should be managed as if they have UM-CLL.

**Authorship**

Contribution: K.I.L. performed the IgVH mutation determination in paraffin-embedded tissue and wrote the paper; C.S.T. analyzed the data and coauthored the manuscript; M.J.K. and L.V.A. designed the study and provided supervision and advice in data analysis and manuscript preparation; T.J.K. and L.R. performed the IgVH mutation determination in fresh tissue and provided advice and oversight in manuscript preparation; K.R.C. performed the sample size analysis and provided statistical oversight; E.S. and L.L.B. assisted in the sample preparation and provided technical advice; W.G.W., S.O., A.F., S.F., and H.K. contributed and verified the accuracy of patient data and provided advice and oversight in manuscript preparation; and S.L. collected the patient data and coordinated the verification of data integrity.

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