Interphase Cytogenetic Abnormalities in Chronic Lymphocytic Leukemia May Predict Response to Rituximab

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Abstract

Select cytogenetic abnormalities such as del(17)(p13.1) and del(11)(q22-q23) predict rapid disease progression and inferior survival in chronic lymphocytic leukemia (CLL). We sought to determine the impact of the four most common interphase cytogenetic abnormalities in 28 CLL patients relative to response to three-times-a-week rituximab therapy. Abnormalities were noted in 25 of the 28 patients to include del(13)(q14.3) [n = 16 (57%)] del(11)(q22.3) [n = 10 (36%)], +12 [n = 6 (21%)], del(17)(p13.1) [n = 5 (18%)], and normal [n = 3 (11%)]. Only a minority of each of these occurred as sole abnormalities. To categorize patients into specific group, we used the hierarchical order del(17)(p13.1) > del(11)(q22.3) > trisomy 12 > del(13)(q14.3) to prioritize. Response to rituximab was noted to vary by cytogenetic group: del(17)(p13.1), 0% [n = 5]; del(11)(q22.3), 66% [n = 9]; del(13)(q14.3), 86% [n = 7]; +12, 25% [n = 4]; and normal, 0% [n = 3]. Response was significantly lower (P = 0.05) in patients with del(17)(p13.1) as compared with those with other abnormalities. These data suggest that interphase cytogenetics in CLL may be predictive of a response to rituximab therapy and provide support for additional studies validating risk-adapted therapy in this disease.

Introduction

CLL is the most common type of leukemia observed in the Western Hemisphere and has a variable natural history with respect to time to progression and response to standard cytotoxic therapies. Interphase cytogenetic analysis using FISH reveals chromosomal changes in the majority of CLL samples (1–7) and is superior to standard karyotype analysis for identifying known cytogenetic abnormalities. In the largest comprehensive FISH cytogenetic series published to date (7), the relative incidence of abnormalities occurring at a frequency of 7% or greater included del(13)(q14.3), del(11)(q22-q23), trisomy 12, and del(17)(p13.1). Several of these abnormalities were shown to have various clinical significance, with del(13)(q14) patients having a prolonged time from diagnosis to treatment (median, 92 months) and overall survival (median, 133 months) whereas those patients del(17)(p13.1) and del(11)(q22-q23) have a more rapid time from diagnosis to treatment (9 and 13 months) and an inferior survival (median, 92 months) and overall survival (median, 133 months) whereas those patients del(17)(p13.1) and del(11)(q22-q23) have a more rapid time from diagnosis to treatment (9 and 13 months) and an inferior survival

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The abbreviations used are: CLL, chronic lymphocytic leukemia; FISH, fluorescence in situ hybridization.

Comparisons of response by abnormalities used Fisher’s exact test with two-sided P values performed with SPSS version 11.0 statistical software. Our small sample size precluded a multivariate analysis to identify combinations of cytogenetic abnormalities that might have improved our impact of the more common poor-risk interphase cytogenetic abnormality del(11)(q22-q23). Similarly, the impact of these abnormalities on response to monoclonal antibodies such as campath-1H or rituximab has not been examined. Herein, we assessed the impact of the four most common interphase cytogenetic abnormalities with respect to response to rituximab in patients with B-cell CLL.

Patients and Methods

Patient Samples and Cell Processing. The patients represent 31 consecutive patients with CLL, as defined by the modified National Cancer Institution 96 criteria (8), who received three-times-a-week rituximab as described previously (9) and from whom pretreatment cryopreserved samples were available for interphase cytogenetic analysis. Written informed consent was obtained from all of the patients before the procurement of cells. Patients were assessed with a detailed clinical evaluation (physical exam with lymph node, liver, and spleen measurement, and complete blood count with differential) 2 months after completing therapy. For patients attaining a complete clinical recovery, a bone marrow biopsy and aspirate was also performed at these times. Criteria for response used the Revised 1996 National Cancer Institute-sponsored Working Group Guidelines (8). As specified by these guidelines, a response had to be maintained for a period of 2 months. CLL cells were obtained before rituximab treatment, and mononuclear cells were isolated from peripheral blood using density-gradient centrifugation (Ficoll-Paque Plus; Pharmacia Biotech, Piscatway, NJ). The cells were then viably cryopreserved in 10% DMSO, 40% FCS, and 50% RPMI medium.

FISH. Cells from 31 CLL patients were thawed rapidly, washed twice in PBS, diluted to 1 × 10^6 cells/ml, and treated with 0.075 M KCl for 15 min at 37°C. The cells were fixed in 3:1 methanol:acetic acid, and slides for FISH were made by hybridizing probes for del(17)(p13.1), del(13)(q14.3), del(11)(q22-q23), and centromere 12. Three of these probes are commercially available from Vysis, Inc. The LSI p53 (17)(p13.1) is 145 kb; LSI D13S319 (13)(q14.3) is ~130 kb; CEP 12 for centromere 12 probes the α satellite region at 12p11.1- q11. All are labeled in SpectrumOrange (Vysis, Inc.). The fourth probe for del(11)(q22.3), available for research use only (Vysis, Inc.), is ~500 kb and will hybridize to a locus from D11S1285 to D11S1294 including the ataria telangiectasia mutated gene. The slides were viewed using a Zeiss Axioskop fluorescence microscope equipped with the appropriate filters and imaging software (Perspective System Instrumentation). The number of signals was evaluated in 200 cells for each probe. Standard quality control procedures were used. A control sample was run concurrently with each test run. Before testing patient samples, appropriate specificity and sensitivity were established as specified (10) on cells isolated and cryopreserved in a manner similar to that described for the CLL cells above. The mean ± 3 SDs, considered positive for a cytogenetic abnormality in these CLL samples were 4% for centromere 12, 10% for del(13)(q14.3), 9% for del(17)(p13.1), and 10% for del(11)(q22.3). When several cytogenetic abnormalities were present in a given patient, data were categorized using the hierarchical classification described by Dohner et al. (7). In this classification, abnormalities are categorized in the following order del(17)(p13.1) > del(11)(q22.3) > +12 > del(13)(q14). Using this classification, a patient having both a del(17)(p13.1) and del(13)(q14) would be categorized to the del(17)(p13.1) group.

Statistical Analysis. Comparisons of response by abnormalities used Fisher’s exact test with two-sided P values performed with SPSS version 11.0 statistical software.
ability to predict patient response to rituximab. However, these findings should prove valuable in designing future studies.

Results and Discussion

A total of 31 consecutive CLL patients treated with rituximab for whom viably preserved CLL cells were available before therapy were studied for the presence of the four most common interphase cytogenetic abnormalities. Of these 31 patient samples, successful hybridization of all of the probes was possible in 28 (90%). The clinical characteristics of these 28 patients include a median age of 64 with 7 (25%) being female. The patients received a median of three (range 0–6) therapies, and 15 (54%) were fludarabine refractory. Advanced stage (modified Rai 3 or 4) was present in 20 (71%) of patients.

Interphase cytogenetic abnormalities were noted in 25 of the 28 patients with adequate FISH samples. The frequency of abnormalities noted were del(13)(q14.3) [n = 16 (57%), del(11)(q22.3) [n = 10 (36%)], +12 [n = 6 (21%)], and del(17)(p13.1) [n = 5 (18%)]. Three patients (11%) lacked any of these genetic lesions. The del(13)(q14.3) was monoallelic loss in 15 patients and biallelic in the remaining patient. Interphase abnormalities were noted in isolation in 7 (44%) of patients with del(13)(q14.3) abnormality, 3 (33%) with del(11)(q22.3), 3 (50%) with trisomy 12, 30%, and 1 (20%) with del(17)(p13.1). A hierarchical classification, used to stratify outcome as previously described (7), included 5 patients with del(17)(p13.1), 9 with del(11)(q22.3), 7 with del(13)(q14.3), 3 with trisomy 12, and 3 patients with no FISH abnormality.

The response to rituximab for these patients using the cytogenetic hierarchical classification is shown in Table 1. Response to rituximab varied significantly based on the prioritized cytogenetic abnormality as shown in Table 1. None of the five patients with del(17)(p13.1) responded, whereas responses were noted in 12 (52%) of 23 of patients without this abnormality (P = 0.05). Only two of these del(17)(p13.1) patients were fludarabine refractory at the time of rituximab treatment. Although patients with del(11)(q22.3) have been noted to have rapid progression and inferior survival, six (66%) of the nine patients with this abnormality responded to rituximab. The overall difference in response among the del(11)(q22.3) and the del(17)(p13.1) patients was significantly different (P = 0.03). Similarly, six (86%) of the seven patients with the del(13)(q14.3) abnormality responded to rituximab, which was significantly higher (P = 0.02) than observed with del(17)(p13.1).

The data presented herein represent, to our knowledge, the first study in CLL that examines interphase cytogenetic abnormalities and relates them to response to rituximab therapy. In this study, we have demonstrated that in patients who have the del(17)(p13.1), detected by interphase cytogenetic abnormality associated with a poor response to standard chemotherapy (2, 3, 5, 6), the del(17)(p13.1) also predicts for lack of response to dose-intensive rituximab. In contrast, patients with the two most common deletions observed in CLL, del(13)(q14.3) and del(11)(q22.3), have a high response rate to rituximab. The dichotomous response among the two high-risk interphase cytogenetic

del(17)(p13.1) and del(11)(q22.3) groups is surprising. This suggests that biological differences may exist in these two genetic subtypes of CLL that produce the divergent response to rituximab. Although both del(17)(p13.1) and del(11)(q22.3) deletions likely occur as secondary events, as demonstrated both by their frequent association with other abnormalities (7) and by their higher frequency in previously treated patients, their biological consequences on the CLL tumor cells are likely different. Because antibody-mediated signaling and apoptosis likely contribute to how rituximab exerts its cytotoxic effect in vivo, (11, 12) studies examining the difference in signaling associated with these secondary abnormalities appears warranted.

How can these results be applied to the treatment of patients with CLL? The data described herein extend the observation of others regarding the chemoresistance of del(17)(p13.1) to also include rituximab. This contrasts with preliminary findings of Stilgenbauer et al. (13), who demonstrated in a small series that CLL patients with del(17)(p13.1) had clinical responses to Campath-1H. If the findings of Stilgenbauer et al. are confirmed in larger cohorts of patients, it would appear that Campath-1H, as opposed to fludarabine, chlorambucil, or rituximab would be a more rational initial treatment choice for patients with del(17)(p13.1). This will be particularly true if new combination regimens of rituximab and fludarabine (14) or fludarabine, cyclophosphamide, and rituximab (15) cannot overcome the resistance associated with del(17)(p13.1). Studies examining this important clinical question are currently under investigation by our group.

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