Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions

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The presence of p53 mutation or deletion predicts for poor response to conventional therapy in chronic lymphocytic leukemia (CLL). We sought to determine whether the humanized anti-CD52 antibody alemtuzumab was effective in this patient group. Thirty-six patients with flu-darabine-refractory CLL were treated with alemtuzumab, 15 (42%) of whom had p53 mutations or deletions. Clinical responses in patients with p53 mutations, deletions, or both were noted in 6 (40%) of 15 versus 4 (19%) of 21 of patients without. The median response duration for this subset of patients was 8 months (range, 3-17 months). These data suggest that alemtuzumab may be an effective therapy for patients with CLL with p53 mutations or deletions. (Blood. 2004;103:3278-3281)

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Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common types of leukemia observed in the Western Hemisphere. Although the natural history of CLL is quite varied, patients with p53 gene deletions [del(17)(p13.1)] or p53 point mutations become symptomatic soon after diagnosis and have an inferior survival.[1-5] The effect of this abnormality on treatment is quite relevant, as several studies have demonstrated that chlorambucil, fludarabine, and rituximab therapy is ineffective in patients who have del(17)(p13.1).[2,6-8] Identifying therapies that are effective against this genetic subtype of CLL, therefore, would represent a major advance for the treatment of CLL.

Alemtuzumab is a humanized anti-CD52 monoclonal antibody that recently was approved for clinical use in fludarabine-refractory CLL whereby an overall response rate of 33% was noted.[9] No molecular studies were performed as part of this trial or others performed with alemtuzumab to ascertain its effectiveness in CLL with p53 mutations, deletions, or both. Only one case report has noted that alemtuzumab might be effective in CLL with p53 mutations, deletions, or both.[10] Herein, we examine a large series of patients treated with alemtuzumab and demonstrate clinical activity.

Patients, materials, and methods

Patient samples and cell processing

The patients represent 36 consecutive patients with CLL as defined by the modified National Cancer Institute (NCI) 96 criteria[11] who received alemtuzumab at our institutions as prescribed for whom pretreatment cryopreserved samples were available for assessment of p53 mutation or deletions. Prior therapies as denoted in Table 1 refer to treatments given prior to alemtuzumab. Patients classified as having fludarabine-refractory disease either did not respond to fludarabine according to the NCI 96 criteria[11] or relapsed within 6 months of completing such therapy. Alemtuzumab was administered as previously published[9] with patients receiving stepped-up dosing (3 mg day 1, 10 mg day 2, and 30 mg day 3 intravenously, followed by 30 mg 3 times weekly for a total of 12 weeks). Support with granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor was given according to the specific institutional protocols. All patients received trimethoprim and sulfamethoxazole (double strength) twice daily on Monday, Wednesday, and Friday and acyclovir 800 mg orally 3 times daily (or equivalent if intolerant) during and 6 months after therapy for *Pneumocystis carinii* pneumonia and herpes virus prophylaxis, respectively. Written informed consent as part of an institutional review board (IRB)–approved protocol was obtained from all patients prior to procurement of cells immediately before beginning therapy with alemtuzumab. Patients were assessed with a detailed clinical evaluation (physical examination with lymph node, liver, and spleen measurement and complete blood count [CBC] with differential) 2 months after completing therapy. For patients attaining a clinical complete remission (CR), a bone marrow biopsy and aspirate were also performed at these times. Criteria for response used the Revised 1996 NCI-sponsored Working Group Guidelines.[11] As specified by those guidelines, a response had to be maintained for a period of 2 months. CLL cells were obtained prior to alemtuzumab treatment, and mononuclear cells were isolated from peripheral blood by using density-gradient centrifugation (Ficoll-Paque Plus; Pharmacia Biotech, Piscataway, NJ). The cells were then viably cryopreserved in 10% DMSO (dimethyl sulfoxide), 40% fetal calf serum, and 50% RPMI media.

Fluorescence in situ hybridization

Cells from 36 patients with CLL were thawed rapidly, washed twice in phosphate buffered saline (PBS), diluted to 1×10^6 cells/mL, and treated with 0.075 M KCl for 15 minutes at 37°C. The cells were fixed in 3:1

Table 1. Clinical features of patients treated with alemtuzumab

Characteristic	Patients
Median age, y (range)	61 (47-74)
No. men (%)	29 (81)
Rai stage, no. (%)	
Intermediate risk	9 (25)
High risk	27 (75)
Median no. prior therapies (range)	3 (1-12)
No. fludarabine refractory (%)	29 (81)
Nonprioritization, no. (%)	
p53 mutation	11 (31)
Patients with del(17)(p13.1)	12 (33)
Patients with del(11)(q22.3)	16 (44)
Patients with trisomy 12	3 (8)
Patients with del(13)(q14)	23 (64)
Patients with del(6)(q21)	4 (11)
Patients with normal interphase cytogenetics	3 (8)
Prioritization, no. (%)*	
Patients with p53 mutation/del(17)(p13.1)	15 (42)
Patients with del(11)(q22.3)	11 (31)
Patients with trisomy 12	3 (8)
Patients with del(13)(q14)	4 (11)
Patients with normal interphase cytogenetics	3 (8)

*Prioritization performed as follows: del(17)(p13.1) or p53 mutation > del(11)(q22.3) > trisomy 12 > del(13)(q14) > normal.

methanol/acetic acid, and slides for fluorescence in situ hybridization (FISH) were made by hybridizing probes for del(17)(p13.1), del(13)(q14.3), del(11)(q22.3), del(6)(q21), and centromere 12. Four of these probes are commercially available from Vysis (Downers Grove, IL). The LSI p53 17(p13.1) is 145 kilobase (kb); LSI D13S319 13(q14.3) is approximately 130 kb and is hybridized with a probe at 13(q34) used as an internal control for nullisomy; LSI ATM spans a 500-kb region surrounding 11(q22.3); and CEP 12 for centromere 12 probes the alpha satellite region at 12(p11.1-q11). All are labeled in SpectrumOrange except 13(q34) which is SpectrumGreen (Vysis).

The probe that identifies del(6)(q21) is approximately 725 kb and is not commercially available. The slides were viewed by 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 as previously published by our group.[8] A control sample was run concurrently with each test run. When several cytogenetic abnormalities were present in a given patient, data were categorized by using the hierarchical classification described by Doehner et al[12] with modifications. Specifically, abnormalities were categorized in the following order: del(17)(p13.1) and/or p53 mutation > del(11)(q22.3) > +12 > del(6)(q21) > del(13)(q14). By 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.

p53 mutational analysis

Mutations of the p53 gene were assessed by extracting DNA by using the QIAamp kit according to the manufacturer's instructions (Qiagen, Valencia, CA). Each p53 exon (5-9) was amplified individually from genomic DNA, using the primer sequences and conditions specified.[13] All cases with identified p53 mutations were repeated with identical results.

Results

The demographic data for the 36 patients treated with alemtuzumab in this study are summarized in Table 1. The patient median age was 61 years with 81% being men. The great majority (75%) of patients were in advanced stage (Rai III or IV), having received a median of 3 prior therapies (range, 1-12) before treatment with alemtuzumab. Of the 36 patients, 29 (81%) were refractory to their last course of fludarabine-based therapy. Interphase cytogenetic studies demonstrated abnormalities in 92% of patients examined. The del(13)(q14) was the most common abnormality (64%), followed by del(11)(q22.3) (44%), del(17)(p13.1) (33%), del(6)(q21) (11%), and trisomy 12 (8%). Mutational studies for p53 demonstrated mutations in 11 (31%) patients. By using a prioritization schema, 15 (42%) of the patients had p53 mutations or del(17)(p13.1). These patients included 8 with both a point mutation and deletion 17(p13.1), 4 del(17)(p13.1), and 3 p53 point mutations. The specific details of these mutations are summarized in Table 2. None of the p53 mutations noted in this patient group were silent mutations or known polymorphisms of p53. Clinical and treatment features among patients with and without p53 mutations or deletions were similar (data not shown).

Of the 36 patients included in this study, 2 (6%) of the patients attained a complete response and 9 (25%) a partial response to alemtuzumab by using the NCI 96 criteria. The median remission duration for patients responding to therapy was 10 months (range, 3-36 months). Of the 2 patients attaining a CR to alemtuzumab therapy, one went onto an autologous stem cell transplantation, and the second remained in remission for 13 months.

By using the prioritization schema outlined in Table 1 for molecular aberrations present at the time of alemtuzumab therapy, partial response was noted in 6 (40%) of 15 patients with p53 mutations or del(17)(p13.1) deletions. Among the patients with p53 mutation/deletions, the median duration of response was 8 months (range, 3-17). Clinical responses were noted in patients with both presence of mutation and deletion (4 of 8 patients responding) versus those with a deletion or mutation (2 of 7 patients responding). In 3 patients with del(17)(p13.1), posttreatment bone marrow

Table 2. P53 gene mutations detected by DGGE and sequencing

Patient no.	del(17)(p13.1) (%)	Exon	Sequence alteration
1	Yes (77.0)	5, 8	TGC>TTT, Cys>Phe, bp13206-7 + CGT>CAT, Arg>His, bp 14487
2	Yes (51.5)	6	CTT>CGT, Leu>Arg, bp 13341
3	Yes (85.5)	6	CGA>CAA, Arg>Gln, bp 13398
4	Yes (80.5)	7	GGC>AGC, Gly>Ser, bp14057
5	Yes (61.0)	7	AGG>AGT, Arg>Ser, bp 14110; at splice site
6	Yes (14.5)	7	AGG>AAA, Glu>Lys, bp 14099
7	No	7	CGG>TGG, Arg>Trp, bp 14069
8	Yes (97.0)	7	GGC>AGC, Gly>Ser, bp 14060
9	No	7	ATG>GTG, Met>Val, bp 14063
10	Yes (91.0)	8	26 bp deletion; splice site deleted
11	No	8	GAG>TAG, Glu>Stop, bp 14522

DGGE indicates denaturing gradient gel electrophoresis.

evaluation included repeated interphase cytogenetics. In 2 of these patients, an NCI 96 CR was present with exception of residual cytopenias, and there was no evidence of residual del(17)(p13.1) in the bone marrow as assessed by interphase cytogenetics. The other patient with del(17)(p13.1) before treatment had residual lymphadenopathy at the posttherapy evaluation but had no evidence of residual del(17)(p13.1) in the bone marrow as assessed by interphase cytogenetics.

For the other interphase cytogenetic groups, 3 (27%) of 11 patients with del(11)(q23) and 1 patient each in the del(13)(q14) (25%) and trisomy 12 (33%) groups responded to alemtuzumab therapy. Assessment for the presence or absence of interphase cytogenetic abnormalities following alemtuzumab therapy occurred only in one of the patients with del(11)(q22.3), demonstrating complete loss of this clone. None of the 3 patients without identifiable interphase cytogenetic abnormalities responded to alemtuzumab therapy. Table 3 summarizes clinical response (partial response [PR] or CR) to alemtuzumab on the basis of clinical and laboratory features known to be a prognostic factor for response to other therapies in CLL. Although the subgroups are small, there was no obvious difference in response to alemtuzumab on the basis of age, stage, number of prior therapies, or genetic subgroup. Of the 7 patients with prior response to fludarabine, 4 (57%) responded as compared with 7 (24%) of those who were resistant to their last course of fludarabine-based therapy.

Discussion

The data presented herein represent to our knowledge the first large series of previously treated patients with CLL, demonstrating a high frequency of p53 mutations, deletions, or both (42%). In this patient group we demonstrate that alemtuzumab is clinically effective in producing NCI 96 partial responses in patients with CLL with either aberrant p53 function from a mutation or gene deletion. In 3 of the patients in whom serial interphase cytogenetic assessment of the bone marrow occurred after therapy, we demonstrated complete loss of the del(17)(p13.1) clone. The remissions observed with alemtuzumab lasted 3, 6, 7, 10, 14, and 17 months in

these patients. This finding is quite relevant to the therapy of CLL, given both the high frequency (42%) of p53 dysfunction that we have demonstrated exists in fludarabine-refractory CLL and the inability of other therapies, including chlorambucil, fludarabine, and rituximab, to work in this setting.[2,6-8] A similarly high frequency of p53 mutations has been noted by others[1,5] in previously treated patients with CLL, including Sturm et al[5] who noted a 29% frequency in patients exposed to prior alkylating therapy as compared with a 5% frequency in previously untreated patients. Sturm et al[5] and others[14-16] have also demonstrated that significant in vitro resistance to both ex vivo treatment with irradiation, fludarabine, chlorambucil, and other alkylator-based therapies is present in the subset of patients with p53 mutations.

In addition to demonstrating a high frequency of p53 mutations or deletions in this patient group, we also demonstrated an increased frequency of other high-risk genetic abnormalities. Specifically, the del(11)(q23.2) was noted in 16 (44%) patients studied in our series, whereas 3 other series noted it in 18%,[12] 15%,[17] and 10%[18] of patients who were recently diagnosed with CLL. Eleven of the 16 patients with del(11)(q22.3) also had del(13)(q14) abnormalities, a finding that is consistent with the report of Cuneo et al[19] who noted clonal acquisition of del(11)(q22.3) with disease progression in a cohort of serially assessed CLL patients initially bearing del(13)(q14). Other abnormalities such as del(6)(q21) that are uncommon in previously untreated patients with CLL[12,17] were noted in 4 (11%) of the patients, and all but one were associated with del(17)(p13.1). Although the deletion at 13(q14) was noted in 23 (64%) patients, only 4 had this as a sole abnormality.

Given the overlap of interphase cytogenetic abnormalities observed in patients in advanced stage, it is difficult to ascertain the significance of other abnormalities on response. Outside of patients with del(17)(p13.1) or p53 mutations, only those patients with del(11)(q22.3) had sufficient numbers to examine the effect of alemtuzumab on response. When prioritized to exclude co-association with del(17)(p13.1) or p53 mutation, 11 patients had del(11)(q22.3) of which 3 (27%) responded to alemtuzumab. Of the remaining prioritized interphase cytogenetic groups, 1 of 3 patients in the trisomy 12 group and 1 of 4 patients in the del(13)(q14) group responded to alemtuzumab. Other clinical features, as demonstrated in Table 3, including age, stage, and number of prior therapies, did not influence the frequency of response. These findings suggest that alemtuzumab mediates its biologic effect through a pathway different from other therapeutic agents used in CLL.

How can these results be applied to the treatment of patients with CLL? Although recently identified prognostic factors such as VH mutational status and associated ZAP-70 expression are predictive of disease progression and inferior survival,[4,20,21] one preliminary study did not relate this resistance to conventional CLL therapies.[22] This finding contrasts with del(17)(p13.1)/p53 abnormalities that become increasingly common with disease progression and are associated with resistance to most conventional therapies used in the treatment of CLL.[6-8,12] The data described support the case report of Stilgenbauer and Dohner[10] who demonstrated a complete response in a single patient with CLL with del(17)(p13.1) and p53 mutation. Similar to the results reported in that single case report, several patients included in our series had durable remissions that ranged from 3 to 17 months with 3 having complete eradication of the del(17)(p13.1) clone in the bone marrow after therapy. If our collective findings are confirmed in

Table 3. Clinical or laboratory features predicting response to alemtuzumab

Clinical or laboratory feature	No. CR or PR (%)
Age, y	
Younger than 60, n = 17	5 (29)
60 or older, n = 19	6 (32)
Rai stage	
Intermediate risk, n = 9	4 (44)
High risk, n = 27	7 (26)
Prior therapies	
3 or less, n = 18	6 (33)
More than 3, n = 3	5 (28)
Fludarabine refractory	
Yes, n = 29	7 (24)
No, n = 7	4 (57)
Genetic prioritization*	
p53 mutation/del(17)(p13.1), n = 15	6 (40)
del(11)(q22.3)*, n = 11	3 (27)
Trisomy 12, n = 3	1 (33)
del(13)(q14), n = 4	1 (25)
Normal interphase cytogenetics, n = 3	0 (0)

*Prioritization was performed as follows: del(17)(p13.1) or p53 mutation > del(11)(q22.3) > trisomy 12 > del (13)(q14) > normal.

larger prospective cohorts of patients, it would appear that alemtuzumab, as opposed to fludarabine, chlorambucil, or rituximab, would be a more rational initial treatment choice for patients with p53 mutations, del(17)(p13.1), or both. In addition, these data would provide preliminary evidence for screening all patients at time of initial and subsequent therapies for the presence of del(17)(p13.1) and p53 mutations to avoid administration of otherwise ineffective therapy for this disease.

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