Alemtuzumab is an Effective Therapy for Chronic Lymphocytic Leukemia with p53 Mutations and Deletions.

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Abstract

The presence of p53 mutation or deletion predicts for poor response to conventional therapy in chronic lymphocytic leukemia (CLL). We sought to determine if the humanized anti-CD52 antibody alemtuzumab was effective in this patient group. Thirty-six patients with fludarabine refractory CLL were treated with alemtuzumab, fifteen (42%) of which had p53 mutations or deletions. Clinical responses in patients with p53 mutations and/or deletions were noted in 6 of 15 (40%) versus 4 of 21 (19%) 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 CLL patients with p53 mutations or deletions.
Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common types of leukemia observed in the Western Hemisphere. While 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 impact 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 by for clinical use in fludarabine-refractory CLL where 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 and/or deletions. Only one case report has noted alemtuzumab might be effective in CLL with p53 mutations and/or deletions.10 Herein, we examine a large series of alemtuzumab treated patients and demonstrate clinical activity.

Methods

Patient Samples and Cell Processing: The patients represent 36 consecutive patients with CLL as defined by the modified NCI 96 criteria11 who received alemtuzumab at our institutions as prescribed for whom pre-treatment cryo-preserved samples were available for assessment of p53 mutation and/or deletions. Prior therapies as denoted in Table 1 refers 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 or relapsed within 6 months of completing such therapy. Alemtuzumab was administered as previously published with patients receiving stepped up dosing (3 mg day 1, 10 mg day 2, and 30 mg day 3 IV followed by 30 mg three 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/Friday and acyclovir 800 mg orally three times daily (or equivalent if intolerant) during and 6 months post-therapy for pneumocystis carini pneumonia and Herpes virus prophylaxis, respectively. Written informed consent as part of an 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 exam with lymph node, liver, and spleen measurement; and CBC with differential) two months after completing therapy. For patients attaining a clinical CR, a bone marrow biopsy and aspirate was also performed at these times. Criteria for response utilized the Revised 1996 NCI-sponsored Working Group Guidelines. As specified by these 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 using density-gradient centrifugation (Ficoll-Paque Plus, Pharmacia Biotech, Piscataway, N.J.). The cells were then viably cryopreserved in 10% DMSO, 40% fetal calf serum and 50% RPMI media.

**Fluorescence in situ hybridization:** Cells from 36 CLL patients were thawed rapidly, washed twice in phosphate buffered saline (PBS), diluted to 1x10^6 cells/ml and treated with 0.075 M KCl for 15 minutes at 37°. 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)(q22.3), del(6)(q21), and centromere 12. Four of these probes are commercially
 available from Vysis, Inc. The LSI p53 (17)(p13.1) is 145 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 500kb 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, Inc.).

The probe which identifies del(6)(q21) is approximately 725kb and is not commercially
 available. 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 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 using the
 hierarchical classification described by Doehner12 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). 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
 using the QIAamp kit according to the manufacturer’s instructions (Qiagen Inc., Valencia
 CA). Each p53 exon (5-9) was amplified individually from genomic DNA, using the
primer sequences and conditions specified. 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 male. The great majority (75%) of patients were advanced stage (Rai III or IV) having received a median of 3 prior therapies (range 1-12) prior to 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%) of patients. Utilizing a prioritization schema, 15 (42%) of the patients had p53 mutations and/or del(17)(p13.1). These 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 utilizing the NCI 96 criteria. The median remission duration for patients responding to therapy was 10 months (range 3-36). Of the two patients attaining a CR to alemtuzumab therapy, one went onto an autologous stem cell transplant and the second remained in remission for 13 months.
Using the prioritization schema outlined in Table 1 for molecular aberrations present at the time of alemtuzumab therapy, partial response was noted in 6 of 15 (40%) of patients with p53 mutations and or del(17)(p13.1) deletions. Among the patients with p53 mutation/deletions, the median duration of response was 8 (range 3-17) months. 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 three patients with del(17)(p13.1), post treatment bone marrow evaluation included repeat interphase cytogenetics. In two of these patients, a 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) pre-treatment had residual lymphadenopathy at the post-therapy 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 of 11 (27%) patients with del(11)(q23) and one patient each in the del(13)(q14) [25%] and trisomy 12 [33%] group 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 three patients without identifiable interphase cytogenetic abnormalities responded to alemtuzumab therapy. Table 3 summarizes clinical response (PR or CR) to alemtuzumab based upon clinical and laboratory features known to be a prognostic factor for response to other therapies in CLL. Although the sub-groups are small, there was no obvious difference in response to alemtuzumab based upon age, stage, number of prior therapies, or genetic
subgroup. Of the 7 patients with prior response to fludarabine, 4 (57%) responded as compared to 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 CLL patients demonstrating a high frequency of p53 mutations and/or deletions (42%). In this patient group we demonstrate that alemtuzumab is clinically effective in producing NCI 96 partial responses in CLL patients with either aberrant p53 function from a mutation and/or gene deletion. In three of the patients where serial interphase cytogenetic assessment of the bone marrow occurred post-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. A similarly high frequency of p53 mutations has been noted by others in previously treated CLL patients including Sturm and colleagues who noted a 29% frequency in those exposed to prior alkylating therapy as compared to a 5% frequency in previously un-treated patients. Sturm and colleagues and others 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%) of patients studied in our series, whereas three other series noted it in 18\textsuperscript{12}, 15\textsuperscript{17}, and 10\textsuperscript{18} percent 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 and colleagues who noted clonal acquisition of del(11)(q22.3) with disease progression in cohort of serially assessed CLL patients initially bearing del(13)(q14).\textsuperscript{19} Other abnormalities such as del(6)(q21) that are uncommon in previously untreated CLL patients\textsuperscript{12,17} were noted in 4 (11%) of the patients and in all but one were associated with del(17)(p13.1). While the deletion at (13)(q14) was noted in 23 (64%) of patients, only 4 had this as a sole abnormality.

Given the overlap of interphase cytogenetic abnormalities observed in advanced stage patients, 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) patients 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 of patients had del(11)(q22.3) of which three (27%) responded to alemtuzumab. Of the remaining prioritized interphase cytogenetic groups, 1 of 3 of the trisomy 12 and 1 of 4 of the del(13)(q14) patients 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 utilized in CLL.

How can these results be applied to the treatment of patients with CLL? While recently identified prognostic factors such as VH mutational status and associated ZAP-70 expression are predictive of disease progression and inferior survival, one preliminary study did not relate this to resistance to conventional CLL therapies. This 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. The data described support the case report of Stilgenbauer and colleagues who demonstrated a complete response in a single CLL patient with del(17)(p13.1) and p53 mutation. Similar to the results reported in this single case report, several patients included in our series had durable remissions that ranged from 3 to 17 months with three having complete eradication of the del(17)(p13.1) clone in the bone marrow post-therapy. If our collective findings are confirmed in larger prospective cohorts of patients, it would appear that alemtuzumab, as opposed to fludarabine, chorambucil, or rituximab would be a more rational initial treatment choice for patients with p53 mutations and/or del(17)(p13.1). 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.
Table 1: Clinical Features of Alemtuzumab Treated Patients

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range) age</td>
<td>61 (47-74) years</td>
</tr>
<tr>
<td>No (%) Male</td>
<td>29 (81)</td>
</tr>
</tbody>
</table>

No (%) Rai Stage
- Intermediate Risk 9 (25)
- High Risk 27 (75)

Median (range) Prior Therapies 3 (1-12)

No (%) Fludarabine refractory 29 (81)

Non-Prioritization
- p53 mutation 11 (31)
- No (%) pts with del(17)(p13.1) 12 (33)
- No (%) pts with del(11)(q22.3) 16 (44)
- No (%) pts with trisomy 12 3 (8)
- No (%) pts with del(13)(q14) 23 (64)
- No (%) pts with del(6)(q21) 4 (11)
- No (%) pts with normal interphase cytogenetics 3 (8)

Prioritization*
- No (%) pts with p53 mutation/del(17)(p13.1) 15 (42)
- No (%) pts with del(11)(q22.3) 11 (31)
- No (%) pts with trisomy 12 3 (8)
- No (%) pts with del(13)(q14) 4 (11)
- No (%) pts with normal interphase cytogenetics 3 (8)

Key: No-number; pts-patients; *prioritization performed as follows: del(17)(p13.1) or p53 mutation > del(11)(q22.3) > trisomy 12 > del(13)(q14) > normal
Table 2: P53 gene Mutations Detected by DGGE and Sequencing

<table>
<thead>
<tr>
<th>Patient #</th>
<th>del(17)(p13.1)</th>
<th>Exon</th>
<th>Sequence Alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>yes - 77.0%</td>
<td>5, 8</td>
<td>TGC&gt;TTT, Cys&gt;Phe, bp13206-7 + CGT&gt;CAT, Arg&gt;His, bp 14487</td>
</tr>
<tr>
<td>2</td>
<td>yes - 51.5%</td>
<td>6</td>
<td>CTT&gt;CGT, Leu&gt;Arg, bp 13341</td>
</tr>
<tr>
<td>3</td>
<td>yes - 85.5%</td>
<td>6</td>
<td>CGA&gt;CAA, Arg&gt;Gln, bp 13398</td>
</tr>
<tr>
<td>4</td>
<td>yes - 80.5%</td>
<td>7</td>
<td>GGC&gt;AGC, Gly&gt;Ser, bp 14057</td>
</tr>
<tr>
<td>5</td>
<td>yes - 61.0%</td>
<td>7</td>
<td>AGG&gt;AGT, Arg&gt;Ser, bp 14110; at splice site</td>
</tr>
<tr>
<td>6</td>
<td>yes - 14.5%</td>
<td>7</td>
<td>AGG&gt;AAA, Glu&gt;Lys, bp 14099</td>
</tr>
<tr>
<td>7</td>
<td>no</td>
<td>7</td>
<td>CGG&gt;TGG, Arg&gt;Trp, bp 14069</td>
</tr>
<tr>
<td>8</td>
<td>yes - 97.0%</td>
<td>7</td>
<td>GGC&gt;AGC, Gly&gt;Ser, bp 14060</td>
</tr>
<tr>
<td>9</td>
<td>no</td>
<td>7</td>
<td>ATG&gt;GTG, Met&gt;Val, bp 14063</td>
</tr>
<tr>
<td>10</td>
<td>yes - 91.0%</td>
<td>8</td>
<td>26 bp deletion; splice site deleted</td>
</tr>
<tr>
<td>11</td>
<td>no</td>
<td>8</td>
<td>GAG&gt;TAG, Glu&gt;Stop, bp 14522</td>
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</table>
### Table 3. Clinical or Laboratory Features Predicting Response to Alemtuzumab

<table>
<thead>
<tr>
<th>Clinical or Laboratory Feature</th>
<th>CR or PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 60 years (n=17)</td>
<td>5 (29)</td>
</tr>
<tr>
<td>≥60 years (n=19)</td>
<td>6 (32)</td>
</tr>
<tr>
<td><strong>Rai Stage</strong></td>
<td></td>
</tr>
<tr>
<td>Intermediate Risk (n=9)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>High Risk (n=27)</td>
<td>7 (26)</td>
</tr>
<tr>
<td><strong>Prior Therapies</strong></td>
<td></td>
</tr>
<tr>
<td>3 or less (n=18)</td>
<td>6 (33)</td>
</tr>
<tr>
<td>&gt; 3 (n=3)</td>
<td>5 (28)</td>
</tr>
<tr>
<td><strong>Fludarabine refractory</strong></td>
<td></td>
</tr>
<tr>
<td>Yes (n=29)</td>
<td>7 (24)</td>
</tr>
<tr>
<td>No (n=7)</td>
<td>4 (57)</td>
</tr>
<tr>
<td><strong>Genetic Prioritization</strong>*</td>
<td></td>
</tr>
<tr>
<td>p53 mutation/del(17)(p13.1) [n=15]</td>
<td>6 (40)</td>
</tr>
<tr>
<td>del(11)(q22.3)*[n=11]</td>
<td>3 (27)</td>
</tr>
<tr>
<td>trisomy 12 [n=3]</td>
<td>1 (33)</td>
</tr>
<tr>
<td>del(13)(q14) [n=4]</td>
<td>1 (25)</td>
</tr>
<tr>
<td>normal interphase cytogenetics [n=3]</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

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