A multivalent bcr-abl fusion peptide vaccination trial in patients with chronic myeloid leukemia

Kathleen Cathcart, Javier Pinilla-Ibarz, Tatyana Korontsvit, Joseph Schwartz, Victoriya Zakhaleva, Esperanza B. Papadopoulos, and David A. Scheinberg

A tumor-specific, bcr-abl–derived fusion peptide vaccine can be safely administered to patients with chronic myelogenous leukemia (CML) and can elicit a bcr-abl peptide–specific T-cell immune response. In the present phase 2 trial, 14 patients with CML in chronic phase were vaccinated with 6 fusion peptides mixed with Quillaja saponaria (QS-21). No significant toxic effects were observed. In 14 of 14 patients, delayed-type hypersensitivity (DTH) and/or CD4 proliferative responses developed after beginning vaccinations, and 11 of 14 patients showed interferon-gamma (IFN-gamma) release by CD4 enzyme-linked immunospot (ELISPOT) at one or more time points. These responses were CD4⁺CD45RO⁺. A peptide-specific CD8⁺ interferon-gamma ELISPOT was found in 4 patients. Four patients in hematologic remission had a decrease in Philadelphia chromosome (Ph) percentage (3 concurrently receiving interferon-alpha and 1 on imatinib mesylate), and 3 patients in molecular relapse after allogeneic transplantation became transiently polymerase chain reaction (PCR) negative after vaccination; 2 of these patients received concurrent donor lymphocyte infusion (DLI). All 5 patients on IFN-alpha ultimately reached a complete cytogenetic remission. In conclusion, a tumor-specific bcr-abl breakpoint peptide–derived vaccine can be safely administered and can reliably elicit measurable peptide-specific CD4 immune responses, including in patients after bone marrow transplantation, on interferon, or on imatinib mesylate. A relationship between the clinical responses and vaccination cannot be determined from this trial.

(Blood. 2004;103:1037-1042)

© 2004 by The American Society of Hematology

Introduction

Chronic myelogenous leukemia (CML) presents a unique opportunity to develop therapeutic strategies using vaccination against a truly tumor-specific antigen that is also the oncogenic protein. The chimeric fusion protein is a tumor-specific antigen because the junctional regions of p210 contain a sequence of amino acids that is not expressed in a normal cell; in addition, as a result of the codon split on the fused message, a new amino acid (lysine in bcr-abl [b3a2]) is found.¹-⁸

Fusion peptides from the junctional sequences of CML can bind to several class I and class II molecules and also elicit human leukocyte antigen (HLA)–restricted cytotoxicity and proliferation in vitro.⁹-²⁰ Recently, Clark et al²¹ have confirmed our previous supposition that peptides derived from the bcr-abl protein can be processed in the cytosol and loaded in HLA molecules that will be transported to the surface of the CML cell for potential T-cell recognition.

These features provided the immunologic basis for our initial phase 1 trial in which a bcr-abl (b3a2)–derived peptide vaccine elicited a bcr-abl peptide–specific T-cell immune response, despite the presence of active disease in these patients.²⁵

The current phase 2 trial was designed to answer the following questions in a larger cohort of patients with CML in chronic phase: (1) whether a fixed dose of peptide vaccination could safely and reliably generate specific CD4 and CD8 responses; (2) whether patients following bone marrow transplantation (BMT) or on imatinib mesylate could mount immune responses; and (3) whether donor leukocytes could be stimulated within recipient patients (“vaccination by proxy”). The trial confirmed the safety of the peptides and demonstrated reproducible T-cell immune responses from all patients, regardless of HLA type or concurrent therapy, including 7 patients who had experienced a relapse after allogeneic BMT. While clinical responses were seen in half of the patients, the contribution of the vaccine to these effects is unclear owing to the concurrent therapy in most of the patients.

Patients, materials, and methods

Study drug

Each of the peptides used in this study was shown to be 98% pure, sterile, and endotoxin free. The 5 CML class I peptides, 9 and 10 amino acids long, and the single CML class II peptide, 25 amino acids long, were synthesized by the Memorial Sloan-Kettering Cancer Center (MSKCC) Microchemistry Core Facility Laboratory by 9-fluorenylmethoxy-carbonyl (F-MOC) solid-phase synthesis and purified by high-pressure liquid chromatography. The amino acid sequences are as follows: for HLA-A0201, SSKALQRPV; for HLA-A3, KQSSKALQR; for HLA-A11, ATGFKQSSK; for HLA-A3/11, HSATGFKQSSK; and for HLA-B8, GFKQSSKAL. The class II peptide is...
IVHSATGFKQSSKALQRPVASDFEP. To prepare the vaccine, 100 µg each peptide (600 µg total peptide) were mixed with 120 µg QS-21 (a purified complex amphiphilic lipid extracted from the bark of the south American tree Quillaja saponaria molina), the immunologic adjuvant, and vailed in 0.5 mL phosphate-buffered saline (PBS) (pH 7.4). The vaccine was stored frozen at ~80°C. The vaccine was prepared according to a Food and Drug Administration (FDA)—approved investigational new drug (IND) application. The QS-21 was provided by Antigenics (Framingham, MA) and was used with reference to their master file.

**Trial design**

This protocol was approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board (IRB) and conducted under an FDA IND application held by Sloan-Kettering with written informed consent.

Patients with chronic myelogenous leukemia of any HLA type with a 10:22 translocation or bcr/abl transcript and a b3a2 breakpoint were required to have measurable active disease and to be in at least a partial or complete hematologic remission induced by interferon-alpha, hydroxyurea, a BM transplant, or imatinib mesylate, and to have a white blood count lower than 20 × 10^9/L (lower than 20,000/mm^3). The patients who had received a previous allogeneic bone marrow transplant were allowed on study only after 6 months following the procedure.

**Treatment plan**

Patients received 5 vaccinations over a 10-week period, on days 0, 7, 21, 35, and 54. Patients received a total of 600 µg peptide (100 µg each of 6 peptides) per dose. All vaccinations were administered subcutaneously, with vaccination sites rotated between arms. Delayed-type hypersensitivity (DTH), unprimed ex vivo autologous proliferation (H-thymidine incorporation), as well as primed CD4^+ and CD8^+ interferon-gamma enzyme-linked immunospot (ELISPOT) assay and intracellular cytokine detection responses were measured before the first vaccination, after the third vaccination, and 2 weeks after the last vaccination (details appear in the next section). If immunologic responses were seen, patients were allowed to receive 3 additional monthly vaccinations followed by immunologic evaluations 2 weeks later. With continued response, patients received another 3 bimonthly vaccinations, again followed by immunologic evaluations 2 weeks after the last vaccination (a total of 11 vaccinations over 11 months). Peripheral blood and bone marrow aspirates were examined for differential, morphology, cytogenetics, and reverse-transcription polymerase chain reaction (RT-PCR) for bcr/abl (as described)\(^2^6\) at the same time points as the immunologic studies.

**Evaluation of immunologic responses**

Delayed-type hypersensitivity tests and unprimed proliferation tests using a modified \(^3\)H-thymidine incorporation assay were performed as described.\(^2^5\) CD4 and CD8^+ positively selected T cells were stimulated with autologous CD14^- as antigen-presenting cells (APCs) in the presence of interleukin 2 (IL-2) (20 U/mL), IL-7 (5 ng/mL), and IL-12 (0.5 ng/mL) and b3a2 long peptide or the corresponding short peptide according to the patient's HLA. Control irrelevant peptides (ras) were used in all experiments; these experiments performed with the same number of cells. Twelve out of 10 patients had peripheral blood cell responses (background spot forming cells [SFCs]) per 1 × 10^6 plated cells represented mean values of 2 to 4 replicates. To calculate the number of CD4^+ or CD8^+ T cells responding to the antigen by interferon-gamma release, background was subtracted.

Intracellular detection of interferon-gamma by flow cytometry on CD4^+ cells was done as per manufacturer's instructions (PharMingen, San Diego, CA) and with the use of fluorescent targeted antibodies to CD3, CD4, and CD45RO or CD45RA. Cells were acquired by means of a FACSCAN (Becton Dickinson Immunocytometry Systems, San Jose, CA).

**Results**

**Patient characteristics**

A total of 14 evaluable patients were enrolled (Table 1). All patients received at least the initial series of 5 immunizations followed by 3 vaccination boosts because of demonstrable evidence of proliferative T-cell response. Four patients also received the 3 additional booster vaccinations (for a total of 11 vaccinations each) after documentation of a positive response in either DTH or proliferative assays following the eighth vaccination. Because the remaining patients had progressed or were enrolled in the study at a time when only 8 vaccinations were allowed, these patients did not receive additional vaccinations. Concurrent treatment is described in Table 1. Seven patients had received an allogeneic bone marrow transplant previously. Four patients had received prior DLI. Three other patients received DLI concomitantly with the first vaccination. Six patients started the protocol with minimal disease as documented by complete cytogenetic remission, but with bcr/abl cells detectable with RT-PCR in BM (sensitivity, 1 in 1 × 10^6 to 1 × 10^6 cells) (Table 1).

**Safety and toxicity**

Definite related toxicities were minimal (all below grade 2), and generally consisted of grade 1 local irritation, swelling, redness, tenderness, or itching at the site of vaccine administration, for 1 to 3 days. Two patients had grade 1 systemic side effects (chills or dizziness), and 7 patients had grade 1 possibly related adverse reactions in their liver function tests.

**Induction of DTH responses**

Tests for DTH reactions against the administered peptides were used for detecting induction of antigen-specific CD4^+ T-cell immunity. The administered peptides would have to be presented in the context of major histocompatibility complex (MHC) molecules in order to be recognized by effector T cells during DTH reactions. Whereas there were no positive DTH reactions before vaccination, all the patients enrolled in this trial became positive after 3 vaccinations (10 patients) or 5 vaccinations (4 patients).

**Induction of CML peptide-specific CD4^+ proliferation**

To directly test the ability of fresh T cells from CML patients undergoing vaccinations to proliferate in the presence of the b3a2 class II long peptide or a mixture of short class I and long peptides, we modified our proliferation assay to detect a response directly from the blood without additional expansion in vitro. The populations of cells used in the proliferation assay were magnetically isolated directly from peripheral blood mononuclear cells (PBMCs) to minimize the bias of high levels of myeloid progenitors in PB from some CML patients and also to more accurately compare experiments performed with the same number of cells. Twelve out
of 14 patients developed a positive proliferative response after vaccination (defined as an increase of more than twice the stimulation index [SI]: counts per minute in the test sample divided by counts per minute in the control). In 10 patients, this response occurred after 3 vaccinations (SI range, 5-25) and persisted until after 8 vaccinations (SI range, 4-299). Peak responses were seen at 10 weeks in 6 patients, and at 5 or 24 weeks in 4 patients each. Three of these patients, who had received 3 extra booster vaccinations for a total of 11, showed a persistent positive proliferative response (SI range, 6-50) until the end of the vaccine program (more than 50 weeks). Two additional patients had a positive proliferative response beginning after 5 vaccinations. In almost all the conditions, the median intensity of the response was higher after 5 vaccinations than after 8 vaccinations. In addition, the later responses were more variable (Figure 1). Remarkably, in 9 patients we also noted the presence of CD4 T-cell proliferative responses against the short class I peptides included in the vaccine (data not shown).

### CML peptide-specific CD4\(^+\) ELISPOT for interferon-gamma

Twelve patients were evaluated for the presence of specific secretion of interferon-gamma on CD4\(^+\) cells (SFCs) in response to the presence of b3a2 long peptide. (Patients 1 and 2 were not evaluated owing to technical problems with the assay.) In 9 patients, we did not detect SFCs before vaccination. All of these patients became positive in the ELISPOT assay after 3 vaccinations (n = 8) (range, 35-430 SFCs per 1 \(\times 10^5\) CD4\(^+\) cells) or 5 immunizations (n = 1) (274 SFCs per 1 \(\times 10^5\) CD4\(^+\) cells) (Figure 1). The response was heterogeneous, but there was clearly a trend toward increased frequency of SFCs after 3 vaccinations with a subsequent decrease in the numbers after 5 or 8 immunizations. In the remaining 3 patients (9, 10, and 13), there was evidence of interferon secretion by CD4\(^+\) cells before the start of the vaccine protocol: in 2 cases, at low frequency (38 and 20 SFCs per 1 \(\times 10^5\) CD4\(^+\) cells over background), and in 1 case at high frequency (251 SFCs per 1 \(\times 10^5\) CD4\(^+\) cells over background). The first 2 patients (9 and 10) had an increased frequency of interferon-secreting CD4\(^+\) cells after 3 vaccinations (430 and 225 SFCs per 1 \(\times 10^5\) CD4\(^+\) cells). The third patient had a decrease in the frequency (159 SFCs per 1 \(\times 10^5\) CD4\(^+\) cells), although comparisons are difficult to evaluate because the assays were not performed at the same time.

### Flow cytometric analysis of the CD4\(^+\) peptide-specific response

Eight patients were analyzed for the presence of intracellular secretion of interferon-gamma by CD4\(^+\); we also included staining for CD45RO and CD45RA to address the frequency of naive T cells versus memory-phenotype T cells. Magnetically isolated CD4\(^+\) cells were stimulated in vitro for 1 or 2 rounds before assay. In addition, the third patient had a decrease in the frequency (159 SFCs per 1 \(\times 10^5\) CD4\(^+\) cells), although comparisons are difficult to evaluate because the assays were not performed at the same time.

### CML peptide-specific CD8\(^+\) ELISPOT for interferon-gamma

Three patients who expressed HLA-A3 and one patient who expressed HLA-A11 showed evidence of CD8\(^+\) responses. In 3 cases, some response could be detected before vaccination, showing some pre-existing CD8\(^+\)-based immunity to peptides that bind to A3 or A11. Two of these patients had received a previous BM transplant, raising the possibility of sensitization during donor T-cell recovery in the recipient. In patient 5, we were only able to detect this CD8 T-cell immune response before vaccination. The CD8\(^+\) cell responses were against CML-A3 and CML-A3/11 peptides after one in vitro stimulation with either CML-A3 or A11. Two of these patients had received a previous BM transplant, raising the possibility of sensitization during donor T-cell recovery in the recipient.

---

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age/y/sex</th>
<th>Time from diagnosis, mo</th>
<th>Current treatment</th>
<th>HLA class I</th>
<th>HLA class II</th>
<th>CD4/CD8 responses(^+)</th>
<th>Baseline cytogenetics or molecular status</th>
<th>Clinical outcome at end of vaccinations†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63/M</td>
<td>11</td>
<td>IFN-α</td>
<td>A2/3, B14/37</td>
<td>DR01/13</td>
<td>+/–</td>
<td>45% Ph(^+) by karyotype</td>
<td>No change</td>
</tr>
<tr>
<td>2</td>
<td>44/M</td>
<td>70</td>
<td>IFN-α</td>
<td>A0201/2301, B18/41</td>
<td>DR1104/1001</td>
<td>+/–</td>
<td>5% Ph(^+) by FISH</td>
<td>No change</td>
</tr>
<tr>
<td>3</td>
<td>55/M</td>
<td>8</td>
<td>IFN-α</td>
<td>A0202/2902, B15/49</td>
<td>DR1303/0701</td>
<td>+/–</td>
<td>100% Ph(^+)</td>
<td>Cytogenetic response to 25% Ph(^+)</td>
</tr>
<tr>
<td>4</td>
<td>47/M</td>
<td>3</td>
<td>IFN-α</td>
<td>A1/65, B8</td>
<td>DR17/52</td>
<td>+/–</td>
<td>95% Ph(^+)</td>
<td>Cytogenetic response to 15% Ph(^+)</td>
</tr>
<tr>
<td>5</td>
<td>52/F</td>
<td>60</td>
<td>None</td>
<td>A0201/0301, B8/7</td>
<td>DR0301/0104/04</td>
<td>+/+</td>
<td>PCR(^+)</td>
<td>Transient PCR(^+)</td>
</tr>
<tr>
<td>6</td>
<td>51/M</td>
<td>138</td>
<td>IFN-α</td>
<td>A0301/2402 B57/39</td>
<td>DR0701/1602</td>
<td>+/+</td>
<td>31% Ph(^+)</td>
<td>Cytogenetic response to 3% Ph(^+)</td>
</tr>
<tr>
<td>7</td>
<td>25/F</td>
<td>84</td>
<td>None</td>
<td>A2/2,2, B13/61</td>
<td>DR1406/07</td>
<td>+/+</td>
<td>PCR(^+)</td>
<td>No change</td>
</tr>
<tr>
<td>8</td>
<td>29/F</td>
<td>102</td>
<td>None</td>
<td>A1/2, B7/44</td>
<td>DR15/7</td>
<td>+/+</td>
<td>PCR(^+)</td>
<td>Cytogenetic relapse</td>
</tr>
<tr>
<td>9</td>
<td>64/M</td>
<td>1</td>
<td>None</td>
<td>A11/32 B14/52</td>
<td>DR01/13</td>
<td>+/+</td>
<td>60% Ph(^+)</td>
<td>No change</td>
</tr>
<tr>
<td>10</td>
<td>59/M</td>
<td>11</td>
<td>Hydroxyurea anagrelide, imatinib mesylate</td>
<td>A03/72, B15/35</td>
<td>DR07/13</td>
<td>+/+</td>
<td>100% Ph(^+)</td>
<td>Cytogenetic response, 14% Ph(^+)</td>
</tr>
<tr>
<td>11</td>
<td>52/M</td>
<td>10</td>
<td>DLI</td>
<td>A0101/3001, B1503/1302</td>
<td>DR1001/0701</td>
<td>+/–</td>
<td>Cytogenetic relapse</td>
<td>Cytogenetic progression</td>
</tr>
<tr>
<td>12</td>
<td>44/M</td>
<td>13</td>
<td>DLI</td>
<td>A0201/3001, B4402/1801</td>
<td>DR1001/0301</td>
<td>+/+</td>
<td>PCR(^+)</td>
<td>Transient PCR(^+)</td>
</tr>
<tr>
<td>13</td>
<td>50/M</td>
<td>126</td>
<td>DLI</td>
<td>A02/32, B35/47</td>
<td>DR04/15</td>
<td>+/+</td>
<td>PCR(^+)</td>
<td>Transient PCR(^+)</td>
</tr>
<tr>
<td>14</td>
<td>53/M</td>
<td>9</td>
<td>Imatinib mesylate</td>
<td>A02/2301, B1402/5801</td>
<td>DR0102/1302</td>
<td>+/+</td>
<td>PCR(^+)</td>
<td>No change</td>
</tr>
</tbody>
</table>

UPN indicates unique patient number; M, male; IFN, interferon; Ph, Philadelphia chromosome; FISH, fluorescence in-situ hybridization; F, female; and DLI, donor lymphocyte infusion.

*CD4 response measured by DTH, proliferation, interferon-gamma ELISPOT, and flow cytometry. CD8 response measured by interferon-gamma ELISPOT.

†Patients 7, 10, 12, and 13 got 11 vaccinations; all others received 8 vaccinations.
Figure 1. Immune responses after vaccination. (A-C) Unprimed ex vivo proliferation assays of fresh CD4+ T cells from vaccinated patients. The assays were conducted as described in "Patients, materials, and methods." The number of weeks are shown on the x-axis. Before indicates before vaccination; +5, after 5 vaccinations; +24, after 8 vaccinations; and +50, after 11 vaccinations. The stimulation index is shown on the y-axis. Panel A shows stimulation at a 2:1 ratio with b3a2 long peptide at 20 μg/mL; panel B, stimulation at a 4:1 ratio with b3a2 long peptide at 20 μg/mL, and panel C, stimulation using total CD4+ cells and b3a2 at 20 μg/mL. Medians are shown as horizontal bars. (D) Interferon-gamma ELISPOT on CD4+ T cells from vaccinated patients at different time points (see panels A-C for the time points in weeks). (E-F) CD8+ interferon-gamma ELISPOT from vaccinated patients. Panel E shows patient 5 before immunization. Black bars show A3 peptide stimulation; white bars, A3/A11 peptide stimulation. The peptide with the APCs in the assay is shown on the x-axis. Panel F shows cells from patient 6 stimulated with CML-A3/A11 peptide before and after 5 vaccinations. The peptide used with the APCs in the assay are as follows: black bars, no peptide; hatched bars, CML-A3 peptide; gray bars, CML-A3/A11; and white bars, A24 negative control. Error bars indicate SEM.

CML-A3/11 peptides. In patient 6, we detected a low frequency of CD8+ precursors against CML-A3/11 peptide before vaccination and an increase in CD8+ SFCs against CML-A3 and CML-A3/11 after vaccination (Figure 1E-F). Patient 10 had a similar frequency of CD8+ precursors at baseline and, after 3 vaccinations, against peptides CML-A3 and CML-A3/11. In patient 9 (HLA-A11) after 3 immunizations, we detected a modest increase in the frequency of CD8+ SFCs against CML-A3 peptide that had not been detected before immunization (data not shown). This response may reflect modest immunization. The response was not maintained, however, at the next time point. We did not detect a positive response in 9 patients with HLA-A2 using a new peptide recently described as a weak binder to this HLA molecule.9

Clinical outcomes

Clinical outcomes as a consequence of vaccinations were difficult to assess as most of the patients were continued on stable therapy while receiving the vaccine. Five patients were simultaneously treated with interferon-alpha. Three of them experienced a significant reduction on the percentage of Ph chromosome after 8 vaccinations. (Table 1). Two of the patients had a complete cytogenetic remission after the end of the vaccine program while on combined treatment with IFN and cytarabine (ara-c).

Three patients with a previous allogeneic BM transplant were treated with concomitant donor leukocyte infusions 48 hours after vaccination with the intention of stimulating an immune response in the naive donor cells inside the recipient against residual CML cells (“vaccination by proxy”). Two of these patients were in persistent molecular relapse after the transplantation and one was in cytogenetic relapse. Patient 12 received his first dose of vaccine followed by concomitant DLI (3 × 10^7 cells per kilogram). Between 5 and 14 months after beginning vaccination, the patient became intermittently negative in the BM (3 out of 4 tests) and PB (2 out 4 tests) by RT-PCR assay for bcr-abl expression. At 3 months after completing vaccination, the patient became RT-PCR positive in PB and BM. At this time, he underwent a new dose of DLI (at 1 × 10^7 cells per kilogram), which did not affect his molecular status. Patient 13 also received a dose of DLI (at 5 × 10^7 CD3+ cells) followed by vaccine. At 1 month later (after 4 immunizations), the patient became RT-PCR negative and continued to be persistently negative after 11 immunizations. At 2 months later, patient became again RT-PCR positive.

Four patients did not receive concomitant treatment: 3 of them had received an allogeneic bone marrow transplant in the remote past, and 2 were treated in the remote past with donor leukocyte infusions. At the time of entry in the study, 3 patients were in complete cytogenetic remission with molecular relapse (persistently positive by RT-PCR). Patient 5 showed intermittent RT-PCR negativity in the BM (3 out of 6 tests), but not in the PB, over the next 18 months. Patients 7, 8, and 9 had no apparent clinical benefit from the vaccinations.

Discussion

In this phase 2 trial, we chose a safe and active vaccine dose on the basis of our previous experience; we implemented newer methods for monitoring the specific CD4 and CD8 immune responses to the vaccine in patients after BMT, on interferon, on imatinib mesylate, or receiving concomitant donor leukocyte infusions.

The vaccine consistently generated a peptide-specific CD4+ response as evidenced by DTH, unprimed proliferation assay, interferon-gamma ELISPOT assay, as well as detection of intracellular interferon-gamma secretion by flow cytometry. On the basis of our analysis, we found the CD4+ T cells to be CD45RO+ and T-helper 1 (Th1) phenotype as others have described for CD4+
b3a2-specific clones.30 These CD4 responses were independent of other treatments administered and were reproducibly observed in patients following BMT. Remarkably, most of the patients had received a T-cell–depleted BM transplant, and in spite of the profound immunosuppression, these patients developed equally robust immune responses to the peptides. Responses weakened as the interval between vaccinations increased. Interestingly, responses were independent of the HLA type of the patients. However, without control peptides containing only the native sequences of the bcr and abl proteins, CD4 specificity for the amino acids of the fusion region itself cannot be proven.

Recognition of fresh CML cells by these CD4 T cells was not shown. However, several reports support the immunogenicity of the bcr-abl fusion class II peptides. Human T cells derived from PBMCs stimulated with the bcr-abl fusion peptide region can recognize p210-b3a2+ marrow cells, CML blasts and APCs exposed to p210-b3a2-containing cell lysates, or CML cell lines transfected with class II molecules.10,19,31 Moreover, antigen-specific T cells have shown the ability to inhibit CML clonogenic precursors in a colony-forming assay in vitro.32 However, other authors have not supported the importance of p210-specific T cells in CML. Zorn et al33 generated a b3a2-specific clone in a patient with CML after DLI who reached a complete cytogenetic remission. This clone failed to recognize or suppress autologous tumor cells or dendritic cells derived from the patient’s CML blasts.

In spite of the more sensitive methods used in this trial, we did not detect CD8 responses in patients using a peptide that weakly binds to this molecule. In contrast, we detected a weak CD8 activity in all patients with HLA-A0301 or HLA-A1101. This is consistent with the work of Clark et al,24 who recently described the presence of CML-A3 peptide on the surface of CML blasts. However, in this trial, we did not demonstrate that the immune response was capable of recognizing native CML blasts or progenitors.

One strategy to circumvent the poor immunogenicity of the class I peptides is to design synthetic analog peptides that will be more immunogenic. Such peptide analogs could generate an immune response that recognizes both the immunizing epitopes and the original native peptides; this is known as a heteroclitic response. We have demonstrated such peptides in vitro. More potent vaccines are also under investigation, including the use of dendritic cells derived from patients with CML or dendritic cells transfected by a recombinant adeno-associated virus that contains a p210 bcr-abl fusion domain DNA.30,34,35

We observed significant reductions in the percentage of Ph+ in 3 of the 5 patients treated with interferon while in the trial; ultimately, all 5 of these patients reached a complete cytogenetic remission. This remarkable finding is difficult to interpret because of the concomitant and continued therapy with other drugs, but these data raise the question of an interaction between the interferon-alpha and the vaccine.

Patients on imatinib mesylate showed proliferation responses in this trial; this finding is consistent with a preserved immune response seen in patients with CML. Amplification of the bcr/abl gene has been described as a mechanism of resistance to imatinib mesylate.36 Such a response could enhance the processing and presenting of the fusion protein on the surface of the target cell. Also, recent evidence suggests that imatinib mesylate could have immunoregulatory properties on antigen-presenting cells, enhancing antigen presentation.37 This could represent a potential advantage for future vaccination trials in patients on imatinib mesylate.

Acknowledgments

We thank Susan Shaed, Miguel Perales, Richard O’Reilly, Phil Livingston, and Alan Houghton for helpful advice and discussions and Shantal Ward for data management assistance.

References

22. ten Bosch GJ, Kessler JH, Joosten AM, et al. A BCR-ABL oncoprotein p210b2afusion region sequence is recognized by HLA-DR2 restricted cytotoxic T lymphocytes and presented by
HLA-DR matched cells transfected with an Ii(b2a2) construct. Blood. 1999;94:1038-1045.


A multivalent bcr-abl fusion peptide vaccination trial in patients with chronic myeloid leukemia

Kathleen Cathcart, Javier Pinilla-Ibarz, Tatyana Korontsvit, Joseph Schwartz, Victoriya Zakhaleva, Esperanza B. Papadopoulos and David A. Scheinberg