K562/GM-CSF Immunotherapy Reduces Tumor Burden in Chronic Myeloid Leukemia Patients with Residual Disease on Imatinib Mesylate

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Abstract

Purpose: Chronic myeloid leukemia (CML) can be responsive to T-cell–mediated immunity. K562/granulocyte macrophage-colony stimulating factor (GM-CSF) is a GM-CSF producing vaccine derived from a CML cell line that expresses several CML-associated antigens. A pilot study was developed to determine if K562/GM-CSF immunotherapy could improve clinical responses to imatinib mesylate (IM) in patients with chronic myeloid leukemia.

Experimental Design: Patients with chronic phase CML who achieved at least a major cytogenetic response but remained with persistent, measurable disease despite one or more years on imatinib mesylate were eligible. Each was given a series of four vaccines administered in three-week intervals, with or without topical imiquimod, while remaining on a stable dose of imatinib mesylate. CML disease burden was measured serially before and after vaccination.

Results: Nineteen patients were vaccinated, with a median duration of previous imatinib mesylate therapy of 37 (13–53) months. Mean PCR measurements of BCR-ABL for the group declined significantly following the vaccines (P = 0.03). Thirteen patients had a progressive decline in disease burden, 8 of whom had increasing disease burden before vaccination. Twelve patients achieved their lowest tumor burden measurements to date following vaccine, including seven subjects who became PCR-undetectable.

Conclusions: K562/GM-CSF vaccine appears to improve molecular responses in patients on imatinib mesylate, including achieving complete molecular remissions, despite long durations of previous imatinib mesylate therapy. Clin Cancer Res; 16(1): 338–47. ©2010 AACR.

Chronic myeloid leukemia (CML) can be highly responsive to T-cell–mediated immunity. The curative potential of allogeneic stem cell transplantation and donor lymphocyte infusion suggests that strategies targeting the immune system may have a role in improving responses to single-agent imatinib mesylate (Novartis International AG, Basel, Switzerland). K562/granulocyte macrophage-colony stimulating factor (GM-CSF) is a vaccine derived from a CML cell line genetically modified to produce GM-CSF (1). The paracrine production of GM-CSF by irradiated tumor cells promotes the local recruitment of dendritic cells to the vaccine site, which capture liberated tumor antigen and traffic to draining lymph nodes to activate tumor-specific CD4+ and CD8+ T cells (2, 3). K562/GM-CSF expresses a number of CML-associated antigens and has the potential to generate or augment CML-specific immune responses.

This pilot study was developed to determine whether K562/GM-CSF immunotherapy could improve the molecular responses in patients with chronic phase CML on imatinib mesylate. Secondary objectives included assessing the safety and tolerability of the K562/GM-CSF vaccine in CML and characterizing its impact on the T-cells and antibodies specific for CML associated antigens.

Patients and Methods

Protocol and patients

This single-institution pilot study was approved by the Johns Hopkins Institutional Review Board and conducted under a United States Food and Drug Administration (FDA) Investigational New Drug application held by the Johns Hopkins Medical Institution and Cell Genesys...
(South San Francisco, CA). All subjects were over 18 years old with a diagnosis of chronic phase Ph+ CML. All were required to have been treated with imatinib mesylate for ≥12 months, achieved a major cytogenetic response (≤35% Ph+ hematopoiesis), and have measurable disease by either fluorescence in situ hybridization (FISH) for Ph+ chromosome or quantitative real-time, reverse transcriptase-PCR (RQ-PCR) for BCR-ABL mRNA. Figure 1 displays the trial outline and its three phases. Imatinib mesylate was continued at the patients’ enrollment doses. Tumor burden was measured at 6-week intervals. These included standard clinical measurements of BCR-ABL done at 3-month intervals as well as protocol-specified measures of BCR-ABL that were collected at 6-week intervals and stored for future testing as described below.

**K562-GM-CSF immunotherapy**

The K562 cell line was transfected with a plasmid vector encoding human GM-CSF as previously described (1). K562/GM-CSF cells were manufactured by Cell Genesys, Inc. The cell line stably expresses >1000 ng of GM-CSF/10⁶ cells/24 h (4). All patients were vaccinated with 1 × 10⁸ irradiated K562/GM-CSF cells (1.0 × 10⁷ cells per injection in 0.5 cc × 10 intradermal sites on the limbs) every 3 weeks for a total of 4 vaccinations. The 10 intradermal sites were distributed as follows: 3 each on the non-dominant upper and lower extremities and 4 on the dominant side lower extremity. Each injection was placed >5 cm from other sites and areas of previous injections were avoided. A topical lidocaine-based anesthetic cream (EMLA cream, AstraZeneca) was placed 1 hour before vaccinations at all 10 vaccine sites. Patients washed the areas with warm water and soap 4 hours following vaccinations.

**Addition of clinical adjuvant 5% imiquimod cream**

The toll-like receptor-7 agonist imiquimod (Aldara, Graceway Pharmaceuticals) was prescribed following the package insert. Briefly, the subjects applied imiquimod...
cream on 9 of the 10 vaccine sites 4 hours after the vaccinations were delivered. Subjects used one packet of the imiquimod cream (250 mg) to cover 3 vaccine sites for a total dose of 750 mg for each administration. The cream was rubbed into the skin covering a circular area of approximately 2.5 cm in diameter until no longer visible. Subjects washed the sites 8 hours after the cream was applied. One site on the leg did not receive any imiquimod cream. All subjects repeated the imiquimod application to the same sites on days 3 and 5 following their vaccinations.

**Real-time quantitative PCR**

The diagnosis of CML is based on cytogenetic detection of the Ph chromosome and/or detection of the BCR-ABL rearrangement RQ-PCR (see supplemental text for complete methodology). Due to the lack of a consensus “gold standard” baseline clinical reference available to the scientific community during the study, a Johns Hopkins standard BCR-ABL RQ-PCR was established as a reference for baseline tumor burden. This standard consisted of the initial quantitative BCR/ABL values from 19 newly diagnosed chronic phase CML patients and followed the approach piloted in the IRIS study that used 30 newly diagnosed patient samples for the same purpose (5). The mean RQ-PCR value was determined to be 1033 (SD = 80%) copies of BCR-ABL per 1000 copies of ABL. Over the course of the study, BCR-ABL was measured every six weeks. Blood was drawn and stored at 4 degrees centigrade following routine conditions to minimize RNA loss. These banked RNA samples were assayed in a single PCR run after the 36-week sample was collected to control for assay variability. This resulted in every other time point having two measurements from the same blood collection. To use all of the RQ-PCR data collected for this study, the relationship between the batched results and the contemporaneous values was modeled, enabling the conversion of the batched results to contemporaneous-scaled values as detailed in the supplement. Statistically valid inferences were made with respect to contemporaneous results (Supplementary Fig. S2; ref. 6). All available clinical measures through June 1, 2008 are reported.

**Response definitions**

**Cytogenetic response.** Patients whose tumor burden was measurable by FISH at the time of study enrollment were considered to have achieved a complete cytogenetic response (CCR) by becoming FISH negative. The cutoff for FISH positivity is 1% background for peripheral blood samples.

**Molecular response.** Molecular responses were determined by comparing the individual patients RQ-PCR result to the JH reference standard level at diagnosis as noted previously. A major molecular response (MMR) was defined as achieving a ≥3 log reduction below this standardized baseline (i.e., below 1.003 BCR-ABL transcripts per 1000 ABL transcripts), while a complete molecular response (CMR) was defined as becoming undetectable, which represents a ≥4.5 log reduction in BCR-ABL transcript number by RQ-PCR.

**Skin biopsies**

On day 0 (before the first vaccine) and day 3 following the first and fourth vaccines, elective skin biopsies were planned to evaluate the local immunologic response. Patients who were treated with imiquimod cream had an additional skin biopsy on day 3 to include the non-imiquimod site.

**Immunologic assays**

Peripheral blood mononuclear cells and serum were collected and cryopreserved at the same time points as the samples obtained for tumor burden assessment. For each subject, the full set of collected samples was tested in a single assay after completion of the study period. Details of the methods used for detecting antibodies and T cells specific for CML-associated antigens are presented in the supplemental materials.

**Statistical methods**

The primary statistical outcome for this pilot study was to detect a decrease in disease burden as measured by a change in the number of BCR-ABL transcripts at enrollment baseline versus at defined intervals following vaccination. The goal of the analysis was to detect a difference in the ratio of BCR-ABL/ABL with at least 80% power. We considered a decrease of 1 in the log₁₀ scale to be reliably

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**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Total Patients Enrolled</th>
<th>$N = 19$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male: Female</td>
<td>9 : 10</td>
</tr>
<tr>
<td>Median age</td>
<td>52 (28–76) y</td>
</tr>
<tr>
<td>Median duration disease</td>
<td>57 (16–111) mo</td>
</tr>
<tr>
<td>Previous imatinib mesylate therapy ($n = 19$)</td>
<td></td>
</tr>
<tr>
<td>Median dose</td>
<td>400 mg (300–800 mg) daily</td>
</tr>
<tr>
<td>Median duration</td>
<td>37 (13–53) mo</td>
</tr>
<tr>
<td>Previous interferon therapy ($n = 16$)</td>
<td></td>
</tr>
<tr>
<td>Dosing range</td>
<td>$3\times10^6$ units daily</td>
</tr>
<tr>
<td>Median duration</td>
<td>13.5 (5–75) mo</td>
</tr>
<tr>
<td>Best previous cytogenetic response to imatinib mesylate</td>
<td></td>
</tr>
<tr>
<td>FISH positive</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>FISH negative/PCR positive</td>
<td>15 (79%)</td>
</tr>
<tr>
<td>Best previous molecular response to imatinib mesylate</td>
<td></td>
</tr>
<tr>
<td>MMR</td>
<td>7/15 (47%)</td>
</tr>
<tr>
<td>No MMR</td>
<td>8/15 (53%)</td>
</tr>
</tbody>
</table>

Abbreviation: MMR, major molecular response.
discriminated by the RQ-PCR assay and a potentially clinically relevant change compared to the null hypothesis of a 0.5 log mean difference. A range of SDs from 0.3 to 0.8 on the log scale was chosen, the differences in the log values were assumed to be normally distributed, and a paired \( t \) test analysis was planned. Any change from baseline that resulted in the patient’s tumor burden becoming undetectable was also considered relevant. For each individual, a trajectory of PCR measures was plotted versus time. Information was combined across individuals to obtain an estimated response at each of the time points. This combined curve was used to further describe biologic changes due to the vaccine.

**Results**

**Patient characteristics.** Between January 2004 and February 2005, a total of 19 subjects were enrolled and their general characteristics are shown in Table 1. All patients had Ph+ CML in first chronic phase with a median duration of disease of 57 months (16–111). Patients were on imatinib mesylate for a median of 37 (13–53) months with a median dose of 400 mg (range 300 mg to 800 mg) daily. Sixteen (84%) of the 19 patients were initially treated with interferon-\( \alpha \) at the time of their diagnosis. Four subjects had disease measured by peripheral blood FISH testing and the remaining 15 subjects were FISH negative but RT-PCR positive for the \( BCR-ABL \) breakpoint. All subjects were evaluable for toxicity and response.

**Clinical response.** Of the 4 subjects initially FISH positive at enrollment, 2 became FISH negative following the planned immunotherapy and remain so beyond 2.5 years (Fig. 2A). Interestingly, 1 went on to become RQ-PCR undetectable and remains so for over 2 years. The remaining patient ultimately met the criteria of a MMR. In addition to the clinical milestone of becoming cytogenetically negative, responses were also defined by achieving molecular milestones demarcating significant reductions in tumor burden. With a median follow-up of 33 (range, 10–44) months postvaccination, 74% (\( n = 14 \)) of patients ultimately met the clinical benchmark of MMR and 37% (\( n = 7 \)) of patients achieved a CMR. Of the 15 FISH-negative patients, 4 became FISH negative following immunotherapy and remain so beyond 2.5 years. One patient became RT-PCR undetectable and remains so for over 2 years. The remaining patient ultimately met the criteria of a MMR. 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subjects, 8 had never achieved a MMR before undergoing immunotherapy despite a median of 25 months on imatinib mesylate. Following immunotherapy, 2 (25%) achieved a CMR, 3 (37%) achieved a MMR, and 3 remain with stable RQ-PCR measurable disease (Fig. 2B). Of the 7 patients who previously achieved a MMR, 4 (57%) achieved a CMR and 3 (43%) remained stable with an ongoing MMR (Fig. 2B). A total of 7 (37%) subjects became PCR undetectable following the initiation of immunotherapy at a median of 6 (range 3–22) months. The median duration of CMR was 22 (3–27) months and 5 patients are currently undetectable. One of the 5 subjects (Subject 001, Table 2) is currently undetectable (at 27 months post-vaccine) for the b2a2/b3a2 transcript that defined his original breakpoint but did have emergence of a low level e1a2 transcript (typically associated with the p190 gene product seen in acute lymphoblastic leukemia) suggesting persistent CML. The other two patients with transient complete responses had evidence for the re-emergence of CML, although both remain with very low RQ-PCR levels.

Studying the group as a whole, the range of tumor burden (patient 1 = 0.01, patient 6 = 0.03, and patient 7 = 0.01) was quite small (patient 3 = 0.001, patient 6 = 0.03, and patient 7 = 0.01) which likely represents stable PCR levels rather than true increases. Overall, there was a significant association between the pre- and postvaccination periods (P = 0.02, two-tailed Fisher’s exact test, Supplementary Table S1).

### Table 2. Patient clinical status pre- and post-immunotherapy

<table>
<thead>
<tr>
<th>Study #</th>
<th>Age (mo)</th>
<th>Imatinib mesylate dosing (mo on imatinib mesylate)</th>
<th>FISH status pre-vaccine</th>
<th>MMR pre-vaccine</th>
<th>Best response post vaccine</th>
<th>Time to best response (mo)</th>
<th>Duration of best response (mo)</th>
<th>Duration of post vaccine (mo)</th>
<th>Δ BCR-ABL* pre/post</th>
</tr>
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<tbody>
<tr>
<td>001†</td>
<td>60</td>
<td>800 (24)</td>
<td>+</td>
<td>+</td>
<td>CMR</td>
<td>6</td>
<td>27</td>
<td>33</td>
<td>+/−</td>
</tr>
<tr>
<td>002</td>
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<td>+</td>
<td>−</td>
<td>MMR</td>
<td>21</td>
<td>16</td>
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<tr>
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<tr>
<td>014‡</td>
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<td>−</td>
<td>No MMR</td>
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<tr>
<td>017‡</td>
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<td>−</td>
<td>MMR</td>
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<td>3</td>
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<td>No MMR</td>
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<td>CMR</td>
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<td>CMR</td>
<td>6</td>
<td>3</td>
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<td>35</td>
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<td>MMR</td>
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<td>18</td>
<td>30</td>
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<tr>
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<td>51</td>
<td>400 (51)</td>
<td>−</td>
<td>+</td>
<td>CMR</td>
<td>3</td>
<td>12</td>
<td>39</td>
<td>+/−</td>
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<tr>
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<td>NA</td>
<td>20</td>
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</tr>
<tr>
<td>019‡</td>
<td>36</td>
<td>400 (14)</td>
<td>−</td>
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<td>No MMR</td>
<td>NA</td>
<td>NA</td>
<td>22</td>
<td>−/−</td>
</tr>
</tbody>
</table>

*+ indicates an increasing RQ-PCR value, − indicates a decreasing value, and NC is no change in the slope considering the absolute (slope) < 0.01.
†Patient enrolled with FISH positive disease and did not undergo RQ-PCR before initiating immunotherapy. Batch sample testing on study completion showed RQ-PCR value consistent with a MMR.
‡Patient received Aldara cream.
and a total of 13 subjects had a progressive fall in their tumor burdens (Table 2).

**Addition of clinical adjuvant 5% imiquimod cream.** Preliminary data in animal models have shown augmented immune responses with the topical application of 5% imiquimod cream to the GM-CSF tumor vaccine injection site (7). To gain some initial clinical experience with this combination, the last 14 consecutive subjects

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**Fig. 3.** Individual trends of log$_{10}$ contemporaneous-scaled measures of molecular levels of BCR-ABL in all patients pre and post K562/GM-CSF immunotherapy. RQ-PCR measures were obtained approximately every 6 weeks while on study, including the pre-immunotherapy window and for at least 6 mo following initiation of immunotherapy. The 2 periods (pre- and post-vaccination) are separated by a vertical dashed line. The dashed lines indicate estimated trends within each of pre- and post-vaccine evaluation periods. The y-axis denotes log$_{10}$ contemporaneous-scaled BCR-ABL measures. The y-axis differs between subjects because of the large variation in tumor burden at trial enrollment (over 3 logs). The x-axis designates BCR-ABL evaluations and is ordered according to prior (indicated by first five 'tick marks') and post-vaccination (indicated by 17 'tick marks' to the right of the vertical dashed line). The patient order corresponds to Table 2.
received imiquimod as a topical vaccine adjuvant. The addition of imiquimod to the vaccine-sites was well tolerated and resulted in an increase in the area of induration compared to what was observed in sites without (mean area of 255 mm² versus 100 mm², P = 0.005). Evaluation of vaccine-site skin biopsies is on-going. Clinical responses were seen in patients immunized with or without imiquimod.

Toxicity. The K562/GM-CSF immunotherapy was well tolerated with the most common side effect being grade 1-2 local injection site reactions experienced by each of the subjects. Three subjects (16%) experienced grade 3 injection site reactions with pain, swelling, and erythema that improved with locally applied cold packs. Other symptoms possibly related to the immunotherapy included mild (grade 1-2) myalgias in 4 subjects (21%), low-grade temperatures (grade 1) in 3 subjects (16%), and 2 subjects (11%) with grade 1-2 fatigue and flu-like symptoms. There were no toxicity-related delays in administration of the immunotherapy. A total of 6 immunotherapy cycles resulted in a re-call phenomenon of swelling and erythema at previous injection sites. There was no clinical evidence of autoimmune reactions in any subject.

Correlative studies. We initially examined antibodies generated against undefined K562 antigens via Western blot. In virtually all subjects, there was an increase in the intensity of polyclonal antibodies detected against K562 lysate. This was detected as early as the first post-vaccine measurement. The intensity peaked after the final vaccine and gradually declined out to week +36 (Fig. 4A). In some subjects, this was accompanied by antibodies recognizing antigens of distinct molecular weights that were not evident in the pre-vaccine samples (arrows, Fig. 4B). Whereas these changes are consistent with vaccine priming of immunity to K562, the resolution of the immunoblots is insufficient to discriminate specific vaccine responses. Antibody and T-cell responses to specific candidate antigens shared by K562 and primary CML [BCR-ABL fusion protein, proteinase-3 (ref. 8), Wilm’s tumor-1 (WT-1) (refs. 9–14), survivin (refs. 15–18), and PRAME (ref. 19)] were examined (see supplemental text for methodology) but were not detected (data not shown).

Discussion

Treatment of patients with chronic phase CML with imatinib mesylate results in hematologic remissions in over 90% of chronic phase patients and complete cytogenetic remissions expected in over 80% of patients in upfront treatment (20). Despite these excellent clinical responses, the overwhelming majority of patients treated with imatinib mesylate remain with molecularly measurable disease. Data from the Imperial College recently reported a 5-year cumulative incidence of CMR of only 8.3% for patients treated upfront with imatinib mesylate (21). Perhaps more reflective of the subjects reported here, Palandri et al., found that only one of 277 late chronic phase patients resistant or intolerant to IFN-α had a durable CMR with a median of 72 months of follow-up (22). Data continue to accumulate that the quiescent, primitive Ph chromosome-positive CML stem cells are insensitive to imatinib in vitro and therefore render single-agent imatinib mesylate as incapable of completely eradicating the disease (23–25) and result in relapse in most patients who stop imatinib mesylate therapy (26–30).

Previous reports have shown the ability to induce T-cell responses in patients undergoing peptide-based vaccines that attempted to target the fusion sequence in the P210 protein unique to the CML. The majority of the early reports suggested the detection of peptide-specific T-cell responses.
proliferative responses and delayed-type hypersensitivity responses and not clinical responses (31). Efforts to modify the strategies through vaccine alteration or adjuvants resulted in small, but measurable changes in disease burden including several cases who achieved molecular remissions (32, 33). As with all single-antigen vaccine approaches targeting defined epitopes, these strategies are limited by the requirement for patients to express specific HLA class I or II molecules for peptide presentation. In contrast, the broad spectrum of tumor-associated antigens delivered by genetically modified tumor cell–based vaccines are not HLA class restricted. In addition, each patient selects the hierarchy of antigens that are recognized, increasing the potential for a response while the polyvalency of this formulation reduces the likelihood of tumor escape by antigen loss. The K562 cell line, originally derived from a patient with CML, expresses many potential antigens that may serve as immunologically important therapeutic targets. Candidate antigen targets shared by both the K562 cell line and primary CML include the BCR-ABL fusion protein, proteinase-3 (8), Wilm's tumor-1 (WT-1; refs. 9–14), survivin (15–18), and PRAME (19). In spite of these advantages, neither antibody nor T-cell responses were detected against these reported candidate antigens, even though they are abundantly expressed by the K562 cells (data not shown). These results mirror those reported by Lee and colleagues who identified T-cell responses to autologous leukemia cells, but not to any known leukemia-associated antigens (including those examined in our study) after imatinib mesylate–induced remission (34). We have since embarked on an unbiased screen of a cDNA expression library from K562 cells using sera from the subjects reported here. Although still preliminary, we have now identified over 25 antigens expressed by CML cells that were recognized in post-, but not pre-vaccine samples. Interestingly, although some of the antigens identified are known immune targets in CML, including hyaluronan-mediated motility receptor (RHAMM; ref. 35) and enolase alpha (36), most of the targets that have been discovered in this screen are novel. Studies are ongoing to determine the frequency of immune responses to each of these antigens in larger numbers of CML patients and to examine the correlation of clinical responses with the pattern of antigen recognition.

Although our study was not designed to test the impact of imatinib mesylate on the immune effects seen, it is possible that concurrent use of imatinib mesylate may have influenced the effectiveness of the K562/GM-CSF immunotherapy. Abelson tyrosine kinases are one of the three families of tyrosine kinases that play important roles in the development, activation, and expansion of T cells. These non–receptor kinases are activated in response to engagement of both the pre-T cell receptors and T-cell receptors and transduce signals that affect T-cell development and mature T-cell function (37, 38). Limiting the function of these signaling pathways with imatinib mesylate and other TKIs is one mechanism that could result in immunosuppressive effects (39, 40). It is just as plausible however, that imatinib mesylate enhanced the biologic activity of the K562/GM-CSF vaccines by its interactions at one or more critical junctures of the proposed T-cell activation pathways. Imatinib mesylate has been shown to enhance the stimulatory capacity of antigen-presenting cells to activate T cells (41), to promote the development and activity of interferon-producing killer dendritic cells (42), and to limit the suppressive feedback loop of regulatory T cells (43). The balance between imatinib mesylate’s influence on the opposing arms of the immune response remains unclear, however, its effects should not be discounted. These issues not withstanding, the ability of imatinib mesylate to hold the disease in a state of minimal residual disease affords a unique opportunity to intercede with immunotherapies that have mechanisms of action that are distinct from leukemia-intrinsic targeted therapies.

In general, the clinical importance of small changes in CML tumor burden, as measured by PCR, for remains unclear. Most PCR assays suggest that changes of up to 1 log may fall within the variability of the assay and trends in the PCR values over time may better reflect a true clinical effect. Our study attempted to minimize the confusion in presenting the clinical responses by not simply duplicating the measures recommended by Branford et al. (44) and Hughes et al. (45) to help analyze the IRIS study, but by presenting all of the data points to best show the trends in measures over time. The changes in tumor burden observed following immunotherapy in our study were intriguing as 13 of our patients had decreasing PCR trends following our immunotherapy, more than half of the responding patients reached the level of molecularly undetectable disease at some point during follow-up, and 5 of these 7 responses appear durable. Despite the fact that PCR is in its relative infancy as a clinical tool, downward trends and achieving molecularly undetectable levels of disease support the biologic activity of the immunotherapy. The current study did not randomize patients between immunotherapy and observation leaving the question of ongoing molecular improvement based solely on imatinib mesylate therapy unanswered. Our patient population was composed of late chronic phase patients, 12 of whom had never achieved a major molecular response despite a median of over 3 years on imatinib mesylate, who would be unlikely to show significant changes in their tumor burdens on stable imatinib mesylate alone.

This pilot study showed that the use of K562/GM-CSF immunotherapy is well tolerated with no serious adverse events for any of the 76 vaccine cycles in our 19 subjects. This intervention was associated with the reduction of tumor burden and the achievement of both major and complete molecular responses in CML patients with previous incomplete responses to single agent imatinib mesylate. Ultimately, the impact of immunotherapy will best be determined in a trial in which the persistence of undetectable disease is assessed following the planned withdrawal of imatinib mesylate. This design has been incorporated into our ongoing follow-up trial to this pilot study.
Disclosure of Potential Conflicts of Interest

K. Hege, T.C. Harding, and G.H. Tu are former employees and stockholders of Cell Genesys; H.I. Levitsky receives payment and royalties from sales of GVAX, previously was a consultant to Cell Genesys, and received NIH funding to support correlative studies. The other authors disclosed no potential conflicts of interest.

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K562/GM-CSF Immunotherapy Reduces Tumor Burden in Chronic Myeloid Leukemia Patients with Residual Disease on Imatinib Mesylate

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