



Donna Duke Morrison. *Rosy*. Watercolor, 22" × 30".

Interactions between tyrosine kinase inhibitors and the immune system are key issues for patients with CML

The Biological Basis for Immunotherapy in Patients With Chronic Myelogenous Leukemia

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Background: Chronic myelogenous leukemia (CML) has long been recognized as an entity responsive to immunotherapeutic interventions. Despite the success of the tyrosine kinase inhibitors (TKIs) in this disease, CML remains incurable. Only allogeneic bone marrow transplantation can provide long-term eradication of CML.

Methods: This review summarizes the recent advances in the field of immunology in CML, specifically in tumor antigen discovery, that have been incorporated into the design of new clinical trials.

Results: Multiple vaccine approaches are currently under clinical investigation. Recent laboratory and clinical data also point to a unique interaction of TKIs with the immune system.

Conclusions: A better understanding of these interactions combined with advances in the field of immunotherapy will likely lead to incorporation of TKIs in future therapeutic interventions to develop a cure for this disease.

Introduction

Clinical interest in immunotherapy for hematologic malignancies, and more specifically for chronic myelogenous leukemia (CML), has grown due to the fact that allogeneic donor-derived T cells can exhibit a potent

graft-vs-leukemia effect in hematopoietic stem cell transplantation (HSCT) compared to syngeneic or T-cell-depleted grafts. Even after the recent development of molecular-targeted therapies such as tyrosine kinase inhibitors (TKIs), HSCT remains the only curative treatment for CML. The differences in minor histocompatibility antigens between donor and recipient as well as the presence of effector cells directed at specific leukemic antigens contribute to the eradication of disease by HSCT.¹⁻⁶ Even after the incorporation of targeted drugs such as TKIs, HSCT remains the only curative treatment for CML to date. Further evidence for an immunologic component to remission comes from allogeneic donor lymphocyte infusions that demonstrate significant and durable responses in relapsed leukemias after HSCT, particularly in relapsed CML cases.^{4,7-9} Approximately 80% of these CML responders would achieve reverse transcrip-

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Abbreviations used in this paper: CML = chronic myelogenous leukemia, TKI = tyrosine kinase inhibitor, HSCT = hematopoietic stem cell transplantation, RT-PCR = reverse transcription polymerase chain reaction, MHC = major histocompatibility complex, CTL = cytotoxic T lymphocyte, PR3 = proteinase 3, WT1 = Wilms' tumor antigen 1, AML = acute myeloid leukemia, GM-CSF = granulocyte-macrophage colony-stimulating factor, DC = dendritic cell.

tion polymerase chain reaction (RT-PCR) negativity for the bcr-abl translocation.¹⁰ Therefore, data from HSCT underscore the therapeutic potential of generating T-cell-mediated immunity to CML treatment.

CML, a clonal disorder of pluripotent hematopoietic stem cells, is characterized by a chromosomal translocation between chromosomes 9 and 22. The abl proto-oncogene on chromosome 9 juxtaposes the breakpoint cluster region (bcr) gene on chromosome 22, resulting in formation of the bcr-abl fusion protein.¹¹⁻¹³ Virtually all cases of CML exhibit this distinctive genetic abnormality. The t(9;22) mRNA is translated to a chimeric protein of molecular weight 210kd. However, there are variations in the fusion transcripts, and different breakpoint areas in the bcr gene have been identified. The most common mRNA transcripts expressed in CML are the b3a2 and b2a2 transcripts (e14a2 and e13a2, respectively), encoding the p210 bcr-abl protein.¹⁴ The generation of this unique neoantigen is tumor-specific since it contains a new sequence of amino acids in the junctional region of p210 that are not expressed in normal hematopoietic stem cells.

Antigen-Specific Targets in CML

Bcr-abl Junctional Peptides

CML presents a unique opportunity to develop active immunotherapeutic strategies using a vaccine approach against a truly tumor-specific antigen (bcr-abl), which is also the oncogenic protein that drives the disease. With a constitutively activated tyrosine kinase activity, bcr-abl is a tumor-specific antigen because the junctional regions of p210 contain a sequence of amino acids uniquely expressed in CML.^{15,16} In addition, a new amino acid is formed (lysine in b3a2 and glutamic acid in b2a2) as a result of a codon split during translocation.¹⁷⁻¹⁹ Our group and several other investigators have demonstrated the immunogenicity of the fusion region-derived peptides of p210 in the context of major histocompatibility complex (MHC) class I and II.^{20,31} By screening large numbers of junctional sequences of these peptides, p210/b3a2-derived fusion protein amino acid sequences were shown to bind to different class I (A0201, A3, A11, and B8) HLA antigen molecules. The observation that CML cells can present endogenous b3a2 peptides in the context of HLA supports the potential of these peptides as targets for class I HLA-restricted T-cell cytotoxicity. Clark et al³² demonstrated that the b3a2 junctional peptide KQSSKALQR could be eluted from HLA-A3/b3a2-positive clinical samples. This peptide (and others) also can elicit a restricted cytotoxic response in vitro.^{21,23,30} Furthermore, bcr-abl-specific cytotoxic T cells have been demonstrated in patients with CML as well as in those following HSCT.³³⁻³⁶ Interestingly, the CML-specific T-cell response was inversely proportional to the leukemic burden, indicating a correlation between immune recognition and possible clinical outcome. However, presentation of other bcr-abl junctional peptides has not been

established in other HLA types, currently limiting the clinical applicability of class I peptide vaccines to subpopulations with specific HLA alleles. Alternative bcr-abl splice variants have been investigated as a source of potential class I immunogenic peptides, and HLA-A2 and HLA-A3 peptides were identified.²⁷ Peptides derived from the reciprocal abl-bcr fusion protein have been shown to bind to even a broad repertoire of HLA class I molecules, including HLA-A1, -A2, -B27, and -B35, but only the HLA-A0201 candidate was able to elicit peptide-pulsed specific cytotoxicity.²⁰ More recently, Wagner et al^{28,29} reported that T cells generated in the presence of a different HLA-A0201 peptide from the abl-bcr reciprocal translocation previously described were able to recognize HLA-matched primary CML cells.

A negative association between HLA-A3, HLA-B8, and HLA-DR4 with incidence of CML has been reported, as well as a negative correlation between HLA-A68 and HLA-B61 haplotypes and bcr-abl transcript levels.³⁷⁻³⁹ These reports suggest a natural immunity and cytotoxic T lymphocyte (CTL)-mediated immunosurveillance against bcr-abl peptides presented by certain HLA class I molecules.

Other bcr-abl translocations such as b2a2 and e1a2 are also sources of potential immunogenic peptides. In this case, the codon disruption that occurs (Asp altered to Glu) is not a novel amino acid and may not be as immunogenic since it is also present in normal a1a2 junction. One peptide with high affinity binding to HLA-A0201 has been described and confirmed by two groups.^{20,40} However, there are no reports to date showing the immunogenicity of this peptide in vitro. HLA-B61 and HLA-A68 binding peptides also have been reported to generate specific immune response in vitro.⁴⁰ The p210-e1a2 protein also has generated much interest as it is the predominant transcript found in Ph+ acute lymphoblastic leukemias. An HLA-B61 epitope has been characterized by the same group.⁴⁰ This peptide was found to generate cytotoxicity toward peptide-pulsed-specific cell lines as well as e1a2 cell lines transfected with HLA-A61, pointing to the endogenous production and natural presentation of this peptide.

To overcome the poor immunogenicity of bcr-abl peptides, our group designed a new strategy to improve the binding to HLA class I molecules by amino acid substitutions at key binding residues.⁴¹ Using computer-algorithm-based predictive analysis, synthetic peptides were derived from the junctional sequences of CML (p210/b3a2 and p210/b2a2) in which amino acid substitutions were introduced into the peptides at key HLA-A0201 binding positions (heteroclitic peptides). Peptide candidates derived from previously described native peptides were improved in terms of immunogenicity and in their capacity to bind HLA-A0201 molecules. The two peptides derived from b3a2 and b2a2 were also able to generate a strong cytotoxic CD8+ response in vitro. More importantly, T cells spe-

cific to these heteroclitic peptides were able to cross react with the native sequences, thus opening the door to new immunotherapeutic strategies.

Because of their limitation on the broad clinical applicability of class I peptides, interest has developed in the class II bcr-abl-specific peptides. Although less is known about the interaction of bcr-abl peptides with HLA class II molecules, support for the immunogenicity of these peptides has been accumulating as well. Peptides corresponding to the b3a2 fusion sequences were shown to bind DR3 (DRB1*0301), DR4 (DRB1*0402), and DR11 (DRB1*1101). B3a2 peptides were also shown to induce HLA-DR1 (DRB1*0101), DR2 (DRB1*1501), DR4 (DRB1*0401), DR9 (DRB1*0901), and DR11 (DRB1*1101) restricted proliferative responses from CD4+ T lymphocytes and cytotoxic T-cell responses associated with DRB1*0901.⁴²⁻⁴⁵ Processing of endogenous bcr-abl protein and presentation in the context of class II molecules by CML cells have not yet been proven biochemically, but indirect evidence has been described in the past.⁴⁵⁻⁴⁷ A b2a2 class II peptide was also identified in a specific proliferative and cytotoxic HLA-DR2a (DRB5*0101) restricted fashion. However, allogenic HLA-DR-matched CML cells were not recognized.⁴⁸

Selectively Expressed and Overexpressed Antigens

Antigens that are selectively expressed or overexpressed in tumor cells, such as proteinase 3 (PR3)^{49,50} and Wilms' tumor antigen 1 (WT1),⁵¹ are potential targets for immunotherapy.

WT1, a zinc finger transcription factor that is overexpressed in most human leukemias (acute myeloid leukemia [AML], CML, and acute lymphoblastic leukemia [ALL]) and in solid malignancies, is an attractive target for vaccine immunotherapeutic approaches.^{52,53} Several class I restricted epitopes have been identified to date. Ohminami et al⁵⁴ reported the generation of CD8+ CTLs that recognized HLA-A24-restricted WT1 peptides and were capable of selectively killing WT1 leukemic cells. In addition, at least four different HLA-0201-restricted epitopes from WT1 have been identified.^{54,61} Again, CTLs generated in the presence of some of these peptides were able to selectively lyse WT1-expressing leukemic cells while sparing normal progenitors.^{56-58,60,62} More recently, an HLA-A1 epitope capable of efficiently killing autologous WT1-expressing tumors has been described.⁵⁹

Animal models have also been used to demonstrated the ability of peptide- or DNA-based WT1 vaccines to facilitate the rejection of WT1-expressing tumor cells. Mice immunized with either WT1 peptide or DNA-encoding WT1 elicited CTL responses specific for WT1 and rejected a challenge from WT1-expressing tumor cells.^{63,64} Importantly, histopathologic studies performed in the immunized animals did not show any evidence of autoimmunity. Finally, nonobese diabet-

ic/severe combined immunodeficiency (NOD/SCID) mouse models have been used to demonstrate the activity of human T-cell lines or clones in the eradication of clonogenic cells capable of transferring leukemia.^{57,65} In humans, WT1 has been shown to be naturally immunogenic with detectable T-cell responses in patients with leukemia.^{3,35,66-68}

A recent report describes WT1-DNA vaccination in a humanized transgenic mice model expressing chimeric HLA-A0201.⁶² The vaccine induced WT1-specific CTLs without affecting hematopoietic stem cells.

Our group used a strategy to circumvent a possible immunologic tolerance to this antigen by designing synthetic immunogenic analog peptides that could cross-react to the native peptides (heteroclitic response). A number of the synthetic analogs derived from native WT1 sequences were obtained by single amino acid substitutions at the HLA-0201 MHC binding residues. Several of the new synthetic analogs better stabilized A0201-HLA molecules, and some were able to elicit WT1-specific T-cell recognition and CTLs more effectively than native sequences. Importantly, T cells stimulated with the new analogs cross-reacted with the native WT1 peptide sequence and were able to kill HLA-matched CML cell lines.⁶⁹

WT1-specific antibodies directed against the N-terminus portion of the WT1 protein were found in the sera of 15% to 30% of patients with AML but in only 2% of healthy donors.⁷⁰⁻⁷² This implies that WT1-specific CD4 T-helper responses should be present in patients with myeloid malignancies. WT1 class II peptide candidates specific for HLA-DRB1*0401, HLA-DP-5, DR53 (DRB4*0101), and DRB1*0405 have been identified and can elicit peptide-specific responses.^{68,73-77} A CD4+ specific T-cell clone for HLA-DRB1*0405 was able to recognize WT1-expressing transformed hematopoietic cells and autologous dendritic cells (DCs) pulsed with apoptotic-induced WT1-expressing cells, indicating that this peptide was a naturally processed helper epitope.⁶⁸ More recently, our group also described the capacity of three class II peptides to generate T-cell recognition of WT1+ expressing tumor cells in multiple DRB1 settings as well as the natural process and presentation of these peptides. Interestingly, one peptide was designed to incorporate a class I heteroclitic peptide within the class II sequence. This peptide was able to induce both CD4+ and CD8+ cytotoxic WT1-specific responses that could recognize the native WT1 epitope presented on the surface of WT1+ cancer cells.⁷⁷

PR3 is a neutral serine protease stored in primary neutrophilic granules and is overexpressed in leukemic progenitors as well as AML and CML populations (in approximately 50% and 75% of patients, respectively). PR3-specific cytotoxic T cells preferentially lysed human myeloid cells, and inhibited colony-forming units granulocyte-macrophage (CFU-GM) in an HLA-A0201-restricted

manner were identified.^{49,50} Interestingly, the cytotoxicity and colony inhibition were proportional to PR3 overexpression in leukemic cells compared with normal HLA-matched marrow progenitors.⁷⁸ CD8+ T cells specific for PR3 were also identified in CML patients in remission following HSCT and correlated with cytogenetic remission.⁷⁹ These CTLs from patients in remission were able to kill HLA-matched CML cells but not normal bone marrow cells, demonstrating that PR3 self-antigen was recognized by allogeneic CTLs. These intriguing results have underscored the promising role of PR3 as a target in immunotherapy.⁸⁰

Several other antigens have been described as overexpressed in CML and other acute myeloid or lymphoid leukemias.^{81,82} Antigens such as hyaluronan-mediated motility receptor (RHAMM)/CD168,⁸²⁻⁸⁴ human telomerase reverse transcriptase (hTERT),⁸⁵ preferentially expressed antigen of melanoma (PRAME),^{86,87} CML28,⁸⁸⁻⁹⁰ CML66,⁹¹⁻⁹³ and survivin⁹⁴ are the subject of active investigations. HLA class I peptides have been described, and in some cases they were able to generate specific CTL responses against CML.

The differential and sequential expression of several tumor antigens in different phases of CML is also important in the development of novel immunotherapeutic strategies. Thus, RHAMM/CD168, PR3, and PRAME are upregulated in accelerated and blast-phase CML.⁸² A more detailed analysis on different stem cell subpopulations suggests that WT1 and PRAME are expressed in higher amounts in patients with advanced-phase CML, while PR3 is more limited to chronic phase.^{95,96} This fact emphasizes the relevance of combining several antigens in the design of future vaccines (Table 1 and Figure).

Clinical Trials

Bcr-abl Peptide-Based Vaccines

The induction of in vitro class I and II CTLs by bcr-abl peptides led to an interest in the use of peptide-based vaccines to treat CML. Our group reported the first phase I study in which we used a combination of four class I peptides (A3, A11, A3/11, and B8) and a class II bcr-abl peptide in combination with the adjuvant QS-21 in vaccinations in 12 patients with CML in chronic phase with concomitant treatment that included interferon alpha (IFN- α).⁹⁷ The vaccination was safe, and 3 of 6 patients treated at the second highest dose of the vaccine demonstrated a peptide-specific proliferative response. However, no cytotoxic response was shown by a nonsensitive Cr51 release assay. Therefore, meaningful clinical responses could not be established in this pilot study.

In a subsequent phase II trial, 14 patients with CML in chronic phase received five vaccinations over a 10-week period that consisted of six peptides (the same as in the previous study except for one additional HLA-A0201 class I peptide) with QS-21.⁹⁸ With no restriction of any HLA type, all patients had measurable active disease and were treated previously or concurrently with IFN- α , hydroxyurea, stem cell transplant, or imatinib mesylate. Peptide-specific CD4+ proliferative or delayed-type hypersensitivity (DTH) response was seen in 14 out of 14 patients, and 11 of the 14 patients showed a peptide-specific CD4+ IFN- γ response by enzyme-linked immunospot (ELISPOT) assay. These responses were characterized as CD45RO+. In contrast, only weak CD8+ responses were observed in HLA-0301 patients. A decrease in the percentage of

Table 1. — T-Cell-Specific Antigens Described in CML and Other Myeloid Malignancies

Antigen	MHC Class I	MHC Class II	T-Cell Responses in CML	mRNA Expression in CML	T-Cell Responses in Other Hematologic Malignancies	References
BCR-ABL						
b3a2	A0201/A3/A11/B8	Yes	Yes	95%	N/A	18–46
b2a2	A0201	Yes				
e1a2	B61	No				
WT1	A1/A0201/A24	Yes	Yes	53%	AML, MDS	49–72
PR3	A0201	No	Yes	71%	AML, MDS	73–77
PRAME	A0201	No	Yes	62%	AML, MDS	83,86
hTERT	A0201	No	Yes	54%	CLL	82
RHAMM	A0201	No	Yes	83%	AML, CLL	79,80
Survivin	A0201	No	ND	Overexpressed in CML	AML, CLL	91
CML28	A0201	No	Yes	Yes	N/A	85–87
CML66	A2402	No	Yes	Yes	N/A	88–90

CML = chronic myelogenous leukemia, WT1 = Wilms' tumor antigen 1, AML = acute myeloid leukemia, MDS = myelodysplastic syndrome, PR3 = proteinase 3, PRAME = preferentially expressed antigen of melanoma, hTERT = human telomerase reverse transcriptase, CLL = chronic lymphocytic leukemia, RHAMM = hyaluronan-mediated motility receptor, ND = not determined. Modified from Greiner J, Schmitt M. Leukemia-associated antigens as target structures for a specific immunotherapy in chronic myeloid leukemia. *Eur J Haematol.* 2008;80(6):461-468. Epub 2008 Feb 12. Reprinted with permission by John Wiley & Sons, Ltd.

Ph+ cells was noted in 4 patients who were in previous hematologic remission; 3 were concurrently being treated with IFN- α and 1 was being treated with imatinib mesylate. Transient PCR negativity was noted in some patients who had received prior allogeneic transplant and donor lymphocyte infusions. Therefore, a relationship between clinical response and the peptide vaccination was not clearly demonstrated.

Bocchia et al⁹⁹ investigated the use of the previously described bcr-abl peptide vaccine⁹⁷ in combination with imatinib or IFN- α in patients with CML. In this approach, QS-21 with granulocyte-macrophage colony-stimulating factor (GM-CSF) was used as an adjuvant in 16 CML patients with stable cytogenetic residual disease for at least 6 months despite continuous treatment with imatinib or IFN- α and restricted HLA molecules. All of the 10 patients using imatinib displayed improved cytogenetic responses. Five of these 10 achieved complete cytogenetic remission, with 3 of the 5 having undetectable amounts of b3a2 by RT-PCR. Among the 6 patients on IFN- α treatment, 5 showed

reductions in the percentage of Ph+ cells, and 2 achieved a complete cytogenetic remission. Most of the patients displayed a CD4+ immunologic response. These results indicated that clinical responses could be induced in CML patients with residual disease.

More recently, Rojas et al¹⁰⁰ reported a clinical trial that included two class I peptides (A3/B8) previously linked to the pan DR epitope PADRE to augment CD4+ responses.¹⁰⁰ Nineteen patients treated with stable doses of imatinib received six vaccinations together with GM-CSF over 9 weeks. Fourteen of the 19 patients demonstrated bcr-abl-specific T-cell responses, again CD45RO+, indicative of a memory phenotype. Of 14 patients in major cytogenetic response (MCR) at trial entry, 13 had at least a single log decrease in bcr-abl transcripts, while no benefit was seen in the 5 patients not in MCR at entry. The molecular responses occurred several months after completing vaccination, thereby suggesting an effect at a primitive stem cell level.

Based on our observation that amino acid substitutions at or near the anchor residues may increase the

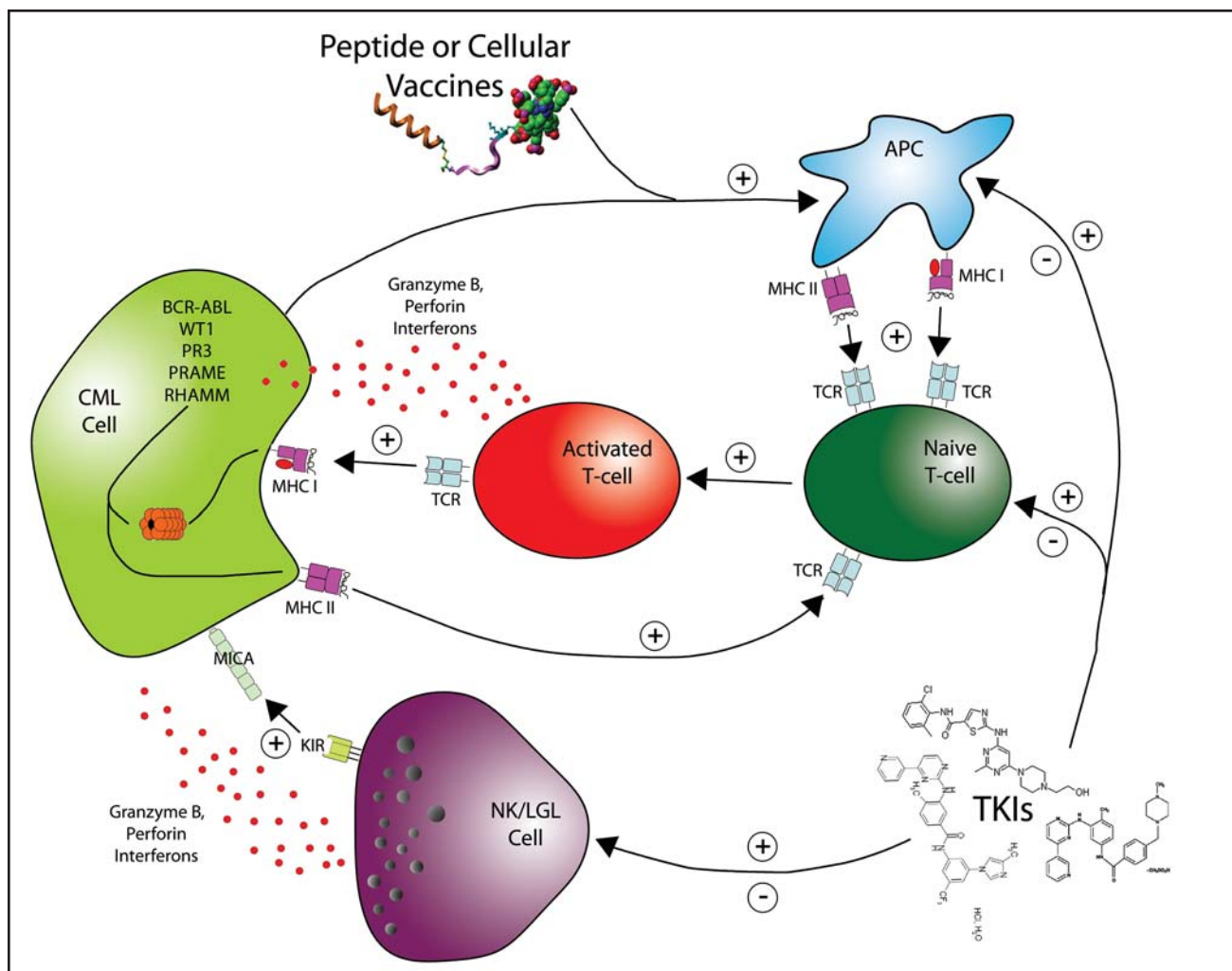


Figure. — Potential pathways for immune response against chronic myeloid leukemia, immunotherapeutic targets, and possible interactions with tyrosine kinase inhibitors.

immunogenicity of junctional bcr-abl peptides,⁴¹ Maslak et al¹⁰¹ conducted a pilot vaccine study that included 13 patients with CML who were being treated with imatinib and were in stable complete cytogenetic remission or in MCR but with measurable molecular disease. Eleven patients received vaccinations over a 12-month period. Montanide ISA 51 and GM-CSF were used as adjuvant therapy. For the first time, patients with the b2a2 breakpoint were vaccinated with a heteroclitic class I peptide that binds with high affinity to HLA-0201 and a class II peptide. Patients with b3a2 received the classic A3 and B8 class I peptides, two heteroclitic peptides that bind effectively to HLA-A0201 and a previously described and utilized class II peptide. Eleven of 13 patients completed the 11 vaccinations. Six out of 6 patients with HLA-0201 (3 patients with b3a2 and 3 with b2a2) and 1 patient with HLA-0205 (closely related to HLA-A0201) responded to the analog peptides after five vaccinations. Furthermore, T cells from 4 out of 6 HLA-0201 patients responded to the native sequence (heteroclitic response), overcoming the poor immunogenicity of the peptides. Again, we observed peptide-specific CD8+ responses against native A3 and B8 sequences as well as proliferative CD4+ responses, including for the first time in patients with the b2a2 breakpoint.

Two patients with a low level of bcr-abl positivity by fluorescence in situ hybridization (FISH) converted to negative after week 10 of the trial. Three of 5 patients who were RT-PCR-positive for bcr-abl prior to vaccination achieved RT-PCR negativity after five doses of vaccination. Both intra- and inter-laboratory baseline sensitivity and variability among the serial measurements bcr-abl transcript levels made this observation difficult to interpret because at the end of the study, all patients remained positive for the bcr-abl transcript in either peripheral blood or the bone marrow as measured with nested PCR. This trial underscored the difficulty of making any conclusions on the reduction of bcr-abl transcripts in the setting of current treatments with TKIs. The long-term impact on the improvement of the molecular response in patients with continuous treatment with imatinib will make it difficult, if not impossible, to conclude a beneficial effect of any vaccination approach if not targeting patients with complete molecular responses with an end point of imatinib interruption and time to molecular relapse.¹⁰²

Phase I/II Trial of the PR1 Peptide Vaccine

PR1, a class I HLA-A0201-restricted peptide derived from PR3, has been shown to elicit myeloid leukemia-specific CTL responses. A pilot phase I/II study evaluating PR3 vaccine in refractory or progressive myeloid leukemia, including patients who relapsed after HSCT, was reported in abstract form and has been recently updated.^{103,104} An analysis of 66 patients with AML (42 patients), CML (13 patients), or myelodysplastic syn-

drome (MDS, 11 patients) who were vaccinated with a PR1 peptide using Montanide ISA 51 and GM-CSF as adjuvant therapy exhibited an increase in PR1-specific CTLs in 25 of 53 patients with measurable disease. Clinical responses were observed in 9 of 25 immune responders vs 3 of 28 immune nonresponders. The immune response was associated with longer event-free survival. Therefore, a detectable PR1 immune response to the vaccine was associated with significantly better clinical response and prolonged event-free survival.

Trials of the WT1 Peptide Vaccine

WT1 is overexpressed in a variety of hematologic malignancies, such as CML, and the expression has been shown to directly correlate with disease progression.^{105,106} The potential efficacy of a WT1-based vaccine was studied by Oka et al¹⁰⁷ in a phase I trial. Patients with AML, MDS, breast cancer, or lung cancer were vaccinated with the HLA-A*2402-restricted native or altered WT1 peptide ligand along with Montanide ISA 51 adjuvant at 2-week intervals in a dose-escalation study. The vaccine was well tolerated; the only notable side effect was profound leukopenia in 2 patients with hypoplastic MDS, which was reversed by corticosteroid treatment that suppressed the WT1 response. Twelve of 20 evaluable patients had clinical responses, including reductions in blood or marrow leukemic blasts, tumor volume, and tumor markers. Similarly, a clinical trial using an HLA-A0201-restricted WT1 peptide in combination with keyhole limpet hemocyanin (KLH) and GM-CSF was published and updated in recent meetings.^{108,109} Of the 16 patients with AML or MDS who were vaccinated, a vaccine WT1-specific T-cell response occurred in 12 of the 16 patients, and 1 patient achieved a complete remission for 12 months. The above findings indicate that a WT1 vaccine can induce functional CTL responses associated with clinical response in myeloid malignancies; however, no CML patients were included in these trials.

A clinical trial with WT1 heteroclitic peptides has also been reported.¹¹⁰ The vaccine consisted of a heteroclitic HLA-A0201 WT1 peptide previously described to have higher binding and immunogenicity⁶⁹ and three class II peptides, including one that combined the class I and class II sequences,⁷⁷ in combination with Montanide ISA 51 and GM-CSF. Twelve patients with mesothelioma, non-small cell lung cancer (NSCLC), or AML received up to 12 vaccinations over a 9-month period. Six of 7 evaluable patients had a CD8 or CD4 response. Most notably, 1 patient with mesothelioma had evidence of stable disease after 12 vaccinations, and 2 out of 5 AML patients remain in remission. In general, the vaccine was well tolerated except for one case of grade 2 urticaria.¹¹⁰

Since the anti-leukemia immune response is often directed against multiple antigens, Rezvani et al¹¹¹ stud-

Table 2. — Peptide Vaccine Clinical Trials in CML and Other Myeloid Malignancies

Antigen	Sequences	No. of Patients With CML	HLA Type	Adjuvants	Responses	References
BCR-ABL	p210 b3a2 multipeptide vaccine ATGFKQSSK-A11 KQSSKALQR-A3 HSATGFKQSSK-A3/A11 GSKQSSKAL-B8 IVHSATGFKQSSKALQRPVASFDFE class II	12, treated with imatinib or interferon	Any	QS-21	Only safety of the study was described	93
	p210 b3a2 multipeptide vaccine Same peptides as in reference 93 plus SSKALQRPV-A0201	14, treated with imatinib or interferon	Any	QS-21	Clinical improvement of patients also treated with interferon or imatinib	94
	p210 b3a2 multipeptide vaccine Same peptides as in reference 93	16, treated with imatinib or interferon	A3/A11/B8/DR1/DR11/DR4	QS-21	Improved cytogenetic responses as well as an increase in the number of patients reaching molecular response	95
	p210 b3a2 multipeptide vaccine KQSSKALQR-A3 GSKQSSKAL-B8	19, treated with imatinib	A0201/A3/B8	PADRE	13 of 14 patients (already in major cytogenetic remission under imatinib) showed a log reduction in combination with vaccination	96
BCR-ABL heteroclitic	p210 b3a2 multipeptide vaccine YLKALQRPV-A0201 heteroclitic KLLQRPVAV-A0201 heteroclitic KQSSKALQR-A3 GSKQSSKAL-B8 IVHSATGFKQSSKALQRPVASFDFE class II p210 b3a2 multipeptide vaccine YLINKEEAL-A0201 heteroclitic IPLTINKEEALQRPVASFDFE class II	13, treated with imatinib	Any	ISA 51 and GM-CSF	3 patients reached a complete cytogenetic response	97
PR3	VLQELNVTV-A0201	Exact number not published	HLA-A2	ISA 51 and GM-CSF	Clinical and immunological responses in AML and MDS patients	99, 100
WT1	CMTWNQMNL-A0201/A24 CYTWNQMNL-A0201/A24 heteroclitic	None	HLA-A24	ISA 51	Clinical and immunological responses in AML and MDS patients	103
	RMFPNAPYL-A0201	None	HLA-A2	KLH and GM-CSF	Clinical and immunological responses in AML and MDS patients	104, 105
WT1 heteroclitic	YMFPNAPYL-A0201 RSDELVRHHNMHQQRNMTKL class II PGCNKRYFKLSHLQMSRKHTG class II SGQAYMFPNAPYLPSCLES class II	None	Any	ISA 51 and GM-CSF		106
WT1 and PR3	RMFPNAPYL-A0201 (WT1) VLQELNVTV-A0201 (PR3)	1, vaccinated in CR, MRD negative	HLA-A2	ISA 51 and GM-CSF	Immunological responses in all patients Reduction of WT1 expression in 3 of 6 AML/MDS patients	107
RHAMM	ILSLELMKL-A0201	None	HLA-A2	ISA 51 and GM-CSF	Clinical and immunological responses in AML and MDS patients	81

Bold font letters represent amino acid substitutions from the native peptide.

CML = chronic myelogenous leukemia, HLA = human leukocyte antigen, QS-21 = Quillaja saponaria, PADRE = unnatural peptide-like sequence and a viral epitope, ISA 51 = incomplete Seppic's adjuvant 51/Montanide, GM-CSF = granulocyte-macrophage colony-stimulating factor, AML = acute myeloid leukemia, MDS = myelodysplastic syndrome, WT1 = Wilms' tumor antigen 1, KLH = keyhole limpet hemocyanin, CR = complete remission, MRD = minimal residual disease, RHAMM = hyaluronan-mediated motility receptor. Modified from Greiner J, Schmitt M. Leukemia-associated antigens as target structures for a specific immunotherapy in chronic myeloid leukemia. *Eur J Haematol.* 2008;80(6):461-468. Epub 2008 Feb 12. Reprinted with permission by John Wiley & Sons, Ltd.

ied a combined PR1 and WT1 approach in an attempt to improve the probability of generating a sustained immune response against myeloid malignancies. Eight patients (7 AML or MDS, 1 CML) received a single dose of PR1 and WT1 HLA-A0201-restricted peptides in combination with Montanide ISA 51 and GM-CSF. A CD8+ T-cell response against PR1 or WT1 was detected in all patients, which was associated with a reduction in leukemic load assessed by WT1 mRNA expression. However, the responses were short-lived, suggesting the need for further manipulation (Table 2).

Trials of Other Non-Peptide–Based Immunotherapy

Modulation of host immunity, particularly the antigen-presenting cells (APCs), is an intriguing approach to overcome tumor-induced tolerance.¹¹² Strategies to augment immune function include the following: (1) attracting APCs to the vaccine site and improving antigen presentation (GM-CSF tumor cell-based vaccines),^{115,114} (2) enhancing APC functions (GM-CSF/CD40 ligand tumor cell-based vaccine),¹¹⁵⁻¹¹⁷ (3) converting tumor cells into APCs,¹¹⁸ and (4) enhancing costimulatory signals to the T-cell arm of the immune system.¹¹⁹ Regarding CML, several groups have demonstrated the generation of DCs from blood precursors of CML patients.¹²⁰⁻¹²³ These DCs displayed strong T-cell stimulation and cytotoxic activity against CML cells.¹²⁴ More recent reports have investigated the role of plasmacytoid DCs in this disorder.^{125,126}

To improve and prolong the efficacy of imatinib, Smith et al¹²⁷ reported the results of a pilot study utilizing a K562/GM-CSF vaccine in combination with imatinib in patients with persistent and measurable disease despite prolonged imatinib treatment (> 1 year). K562/GM-CSF is a tumor vaccine derived from a CML cell line that expresses several defined CML-associated antigens and has been genetically engineered to produce GM-CSF.¹¹⁴ Several clinical responses were reported among the 34 patients who had cytogenetic or molecularly positive disease, including 4 patients with molecular remissions.

Two phase I/II trials were reported that used autologous DC vaccination in patients with CML who did not achieve an adequate cytogenetic response after treatment with imatinib or IFN- α .^{128,129} The larger trial¹²⁸ described 10 patients who received the vaccine after in vitro generation of DCs from peripheral blood monocytes. Four injections of increasing numbers of DCs were administered. FISH assessment showed that 4 of 10 patients demonstrated improved cytogenetic responses that were possibly related to the vaccine. Three of these 4 patients generated a T-cell response to leukemia-associated antigens (PR3, bcr3-abl2, and abl-bcr).

Heat shock protein 70 (Hsp70), a molecular chaperone to the MHC class I and class II antigen-processing pathway, is another immunotherapeutic target. A recent

study reported the use of Hsp70 in CML patients with persistent disease on imatinib treatment.¹³⁰ Cytogenetic or molecular responses were observed in 13 out of 20 patients with minimal toxicity. However, due to concurrent imatinib treatment, it was unclear whether the Hsp70 peptide complex had contributed to the overall clinical response. A phase II study after imatinib failure showed less impressive results.¹³¹

Immunomodulation Mediated by TKIs

TKIs have dramatically changed the prognosis of CML in recent years. Their unique mechanism of action, by interfering with the ATP binding to abl and bcr-abl, makes these agents the first truly targeted therapy for CML. Much evidence points to an effect of TKIs on the immune system. The available data on the negative interaction of TKIs with the immune system is currently under debate. In vivo data show that imatinib does not substantially impair the anti-leukemic immunity, thus supporting the relative value of most of these in vitro data.^{85,132-134}

Imatinib may restore the clonality of DCs from bcr-abl+ to polyclonality with restoration of normal DC function.^{135,136} However, it may impair the differentiation of monocytes and CD34+ progenitors into functional DCs, limiting their ability to stimulate a primary T-cell response but not the immunogenicity of human myeloid DCs from healthy donors or CML patients.¹³⁷⁻¹⁴⁰

In addition, several studies have reported impaired T-cell-specific proliferation and responses as well as the inhibition of antigen-specific memory T cells,¹⁴¹⁻¹⁴⁵ possibly due to inhibition of other kinases such as Lck. These data support the hypothesis that imatinib reduces the efficacy of the graft-vs-leukemia effect or other T-cell-based immunotherapies. Conversely, imatinib-based negative effects on proliferation and function of CD4+CD25+ regulatory T cells (decreased on interleukin 10 [IL-10], transforming growth factor beta (TGF- β 1, and granzyme B) have been reported for which its negative effect on T-cell proliferation could depend on the balance between the susceptibility of CD8+ cytotoxic T cells and CD4+CD25+ regulatory T cells.¹⁴⁶

In contrast to the presumed negative immunologic effects, imatinib has been observed to elicit an increase in IFN- γ -producing T cells after 3 months of treatment, and it may even restore the function of helper T cells (Th1) even in the absence of cytogenetic remission.¹⁴⁷ However, the inhibition of antigen-specific IFN- γ , tumor necrosis factor alpha (TNF- α), or IL-2 secretion by CD4+ or CD8+ T effector cells has also been reported.^{148,149} A possible explanation for the conflicting data is that many of these in vitro studies have used far higher imatinib concentrations than serum levels achieved in vivo, even when higher doses are administered.

Tumor-antigen-specific T-cell tolerance to leukemia-associated antigens is a significant barrier to the development of effective therapeutic cancer vaccines. In a

murine model, imatinib has been shown to enhance the activation of naive antigen-specific T cells and restore the responsiveness of tolerant T cells from tumor-bearing hosts by enhancing the APC function. Furthermore, in vivo treatment with imatinib prevented the induction of tolerance in tumor-specific CD4+ T cells and resulted in an enhanced vaccine efficacy. These changes in APCs were attributed to the inhibition of c-kit kinase.¹⁵⁰

Finally in vivo antitumor T-cell immunity has been observed in several clinical trials using bcr-abl peptide vaccines⁹⁹⁻¹⁰¹ as well as in other cellular vaccines immunotherapies.¹²⁷ The use of imatinib in conjunction with donor lymphocyte infusion for relapsed CML post-allogeneic transplant has been shown to be efficacious and perhaps synergistic.¹⁵¹⁻¹⁵³ These data, as well the absence of an increasing incidence of infections in CML patients taking imatinib, suggest that the clinical effect of imatinib on the immune system is neutral if not beneficial.

Second-generation TKIs are efficacious in patients with relapse/refractory CML after imatinib therapy. These drugs are more potent than imatinib against leukemia cells in vitro, and in vitro nilotinib also has been shown to inhibit the expansion of CD8+ T lymphocytes specific for leukemia or viral antigens. The inhibitory effect caused by nilotinib was twice as strong as that generated by imatinib. These effects were thought to be mediated through the inhibition of the phosphorylation of ZAP-70, Lck, and ERK 1/2 and the NF- κ B signaling transduction pathway.^{154,155}

Dasatinib, a dual src and abl TKI far more potent than imatinib, was shown to have a profound T-cell inhibitory effect in vitro. Dasatinib inhibited T-cell receptor (TCR)-mediated signal transduction, cellular proliferation, cytokine production, and in vivo T-cell responses. This effect was seen more strongly in CD4+ T cells than in CD8+ T cells and was preferential to the naive as opposed to the memory T-cell subsets. Interestingly, this effect does not induce apoptosis, is reversible, and may be overcome by TCR-independent signals such as phorbol myristate acetate (PMA) or IL-2. Again, this observation is thought to be a cross-targeted effect in the inhibition of LCK and FYN.¹⁵⁶⁻¹⁶⁰

A recent interesting report demonstrated an in vivo expansion of lymphocytes with large granular lymphocyte morphologic features in patients being treated with dasatinib.¹⁶¹ The phenotypic characteristics of these cells are cytotoxic CD8+ T cells or NK-type cells consistent with the description of large granular lymphocytes (LGL) chronic leukemias. Even more interesting was the association of these lymphocyte proliferations in patients who developed pleural effusions, fevers, and colitis with accumulation of these cells in biopsy samples. Leukemic response in this group of patients was remarkable, even considering the advanced stage, thus suggesting the possibility of an aberrant anti-host and anti-leukemia effect mediated by these cytotoxic T/NK LGL cells.

Conclusions

With the recent advances in immunology, efforts have focused on the development of immunotherapeutic development for leukemias. The goals of effective immunotherapy are two-fold: to generate an active systemic immune response leading to elimination of residual malignant cells and to provide long-lasting immunologic surveillance to prevent disease relapse. Tumor antigens, including WT1, PR3, and the CML-specific fusion protein bcr-abl, play a vital role in leukemic vaccine development. In contrast to the antigen-specific approach, cell-based vaccine strategies rely on the modulation of immunity against unknown tumor antigens such as the GM-CSF-secreting vaccine and the DC-primed vaccine. It is hoped that a better understanding of the interaction between TKIs and the immune system will lead to the incorporation of these drugs in future immunotherapeutic clinical trials.

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No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.

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