New Agents in Chronic Myelogenous Leukemia

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Key Words
Chronic myelogenous leukemia, chemotherapy, new agents, imatinib, bcr-abl, intracellular pathways

Abstract
Multiple new agents are currently being developed in chronic myelogenous leukemia (CML). Most of these agents are now being investigated in patients who have developed resistance to imatinib. Their mechanisms of action are diverse and many may be synergistic with imatinib. These agents will be used soon in different combinations, most likely including imatinib, with the hope of obtaining a complete blockade of the intracellular pathways that are triggered by Bcr-Abl. If this is successful, complete eradication of disease may become a reality for the majority of patients with CML. (JNCCN 2003;1:501-512)

The introduction of imatinib mesylate (Gleevec, Novartis Pharmaceuticals, East Hanover, NJ) to the therapeutic armamentarium has changed the approach to chronic myelogenous leukemia (CML). As we continue to gather more information from the ongoing trials with imatinib, it is evident that it has become the building block in the treatment of CML. It also is evident that there is a need to continue to investigate new therapies for CML as we discover patients who become resistant to therapy with imatinib. The focus now is to investigate therapeutic modalities with specific targets that could eventually lead to a sequential blockade of intracellular pathways. This section will discuss novel approaches currently being investigated in CML, and an outline of some of these options is presented in Table 1.

Troxacitabine
Troxacitabine is a novel nucleoside analogue that differs from other dideoxycytidine analogs by being in the L-configuration. L-enantiomers of nucleosides were originally not considered as potential antineoplastic agents as they were thought to be poor substrates for activating enzymes. Some of these L-enantiomers (e.g., lamivudine) were found to be potent antiviral agents, and others were then investigated as antineoplastic agents. Troxacitabine has shown significant antineoplastic activity against several solid tumor cell lines and animal models. It also has shown significant activity in patients with relapsed acute myelogenous leukemia (AML). A phase I study in patients with acute leukemias and CML in blast phase identified hand-foot syndrome and mucositis as the dose-limiting toxicity, and the maximum tolerated dose (MTD) was 8 mg/m² daily for 5 days. Evidence of activity was found in AML and myelodysplastic syndromes (MDS) and the one patient with CML in blast phase treated on this study achieved a second chronic phase. In a recent phase II study, 17 patients with CML in myeloid blast phase were treated with the dose identified in the previous study. Six of these patients had CML failure to ima- tinib therapy, and 9 patients were in the second or later relapse. Six of 16 evaluable patients (37%) returned to a chronic phase. One patient relapsed after 20 months, one died in second chronic phase from sepsis, and 4 continue in second chronic phase after a follow-up of 2 to 11 months. Further investigation of troxacitabine in CML blast phase is warranted.

Homoharringtonine
Homoharringtonine (HHT) is a plant alkaloid derived from an evergreen tree found in China. The antileukemia activity of HHT in acute myeloid leukemia has been known for several years, and more recently activity in
acute promyelocytic leukemia and MDS has been sug-
ggested. Significant activity has been reported in CML.\(^6\)–\(^8\) Patients in late chronic phase treated with this agent after interferon-based therapy failed and with a median time from diagnosis of 3 years had a complete hematologic response (CHR) rate of 67%, cytogenetic response (CGR) rate of 33% (major cyto-
genetic response [MCR] in 15%).\(^9\) Similar results were obtained using a combination of homoharringtonine and low-dose cytarabine (CHR 72%, CGR 32%, MCR 15%), with a suggestion of improved sur-
vival with the latter combination compared with sin-
gle-agent HHT.

Based on this synergy, HHT has been com-
bined with interferon, and with IFN-\(\alpha\) and ara-C in treating patients with early chronic phase CML.\(^6\) The combination of HHT and IFN-\(\alpha\) in patients in this population resulted in a MCR rate of 27% with a significantly shorter median time to achievement of an MCR compared to IFN-\(\alpha\) alone (MCR at 6 months 27% with HHT + IFN-\(\alpha\) vs. 11% with IFN-\(\alpha\)).\(^10\) In addi-
tion, the median daily dose delivered in the first 12 months of therapy with the combination was 2.4 MU/m\(^2\) compared with 5 MU/m\(^2\) with IFN-\(\alpha\) alone. This resulted in a significant reduction in IFN-\(\alpha\)-re-
lated toxicities.\(^11\) New formulations of HHT are now available that allow for a subcutaneous administra-
tion of this agent. HHT is also synergistic with ima-
tinib mesylate in vitro. Therefore, HHT remains a very attractive therapeutic option for patients with CML after imatinib failure or in combination with imatinib. Such clinical studies are being developed.

### Demethylating Agents and Histone Deacetylase Inhibitors

Deoxyribonucleic acid (DNA) methylation is an epigenetic phenomenon that can greatly affect gene expression. In mammalian tissues, methylation occurs in sequences of cytosine followed by guanosine, known as CpG islands.\(^12\) Methylation of the promoter of affected genes results in altered chromatin organization that leads to suppressed gene transcription. Hypermethylation of the promoter of multiple genes is a common phenomenon in cancer and hematologic malignancies.\(^13\) Such hypermethylation-associated gene-silencing has been shown for several genes regu-
lating the growth and differentiation of cells, including the estrogen receptor (ER) gene, P15, P16, and others.\(^14\) In CML, increased methylation of se-
veral genes is seen with disease progression. The Pa pro-
moter of ABL1 is frequently hypermethylated, with increasing levels of methylation as the disease pro-
gresses.\(^15\) Hypermethylation of this gene has been re-
ported to correlate with a poor outcome, although other studies do not support this observation.\(^16\)–\(^19\) In con-
trast, p15 methylation was associated with disease progressio-
in one report.\(^16\) The methylation pattern of the breakpoint cluster region (Bcr) gene has been re-
ported to be different in the lymphoid versus myeloid

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**Table 1. New Therapies in Chronic Myelogenous Leukemia**

<table>
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© Journal of the National Comprehensive Cancer Network | Volume 1 | Number 4 | October 2003
blast phase CML. More recently, hypermethylation of cadherin-13 was identified in chronic phase and was associated with high-risk features and a lower probability of response to IFN-α. There are currently several inhibitors of DNA methylation, including 5-azacytidine (AZA) and 5-aza-2’-deoxycytidine (decitabine, DAC), and the cardiovascular drugs hydralazine and procainamide. AZA and DAC have demonstrated anti-leukemic activity in myeloid malignancies, including AML and MDS. In CML, hypomethylating agents have been mostly used after transformation. A combination regimen of AZA, with etoposide or mitoxantrone resulted in responses in 25% to 60% of patients. In a recent study, 130 patients in different phases of CML (123 with Ph-positive CML [64 blastic, 51 accelerated, 8 chronic]; 7 with Ph-negative CML) received DAC 100 mg/m² over 6 hours every 12 hours × 5 days (1000 mg/m² per course) in the first 13 patients, 75 mg/m² in the subsequent 33 patients, and 50 mg/m² in the remaining 84 patients; a total of 552 courses were given to the 130 patients. Only four patients (3%) died during the first course from myelosuppressive complications (3 patients) or progressive disease (1 patient). Of 64 patients in CML blastic phase, 18 patients (28%) achieved objective responses. Of these 18 patients, 6 achieved CHR, 2 achieved partial hematologic responses (PHR), 7 achieved hematologic improvements (HI), and 3 returned to the second chronic phase (second CP). Five patients (8%) had cytogenetic responses. Among 51 patients in the accelerated phase, 28 patients (55%) achieved objective responses (12 CHR, 10 PHR, 3 HI, and 3 second CP). Seven patients (14%) had cytogenetic responses. Among eight patients treated in the chronic phase, 5 (63%) had objective responses. Of 7 patients treated for Ph-negative CML, 4 (57%) had objective responses. There was no evidence of a dose-response effect. The estimated 3-year survival rate was less than 5% in the blastic phase and 27% in the accelerated phase. The only significant toxicity reported was severe myelosuppression, which was delayed, prolonged, and dose dependent. With decitabine 50 to 75 mg/m², the median time to granulocyte recovery above 0.5 × 10⁹/L was about 4 weeks. Myelosuppression-associated complications included febrile episodes in 37% and documented infections in 34%.

Issa et al. have investigated the minimal effective dose of DAC in a phase I study. Preclinical observations suggest that optimal hypomethylation activity is achieved with low concentrations of DAC, and higher concentrations only result in cytotoxicity. Significant activity with minimal toxicity was reported in patients with AML, MDS and CML, with the most effective dose being 15 mg/m² daily for 10 days. This schedule is now being investigated in patients with imatinib-resistant CML in chronic, accelerated and blast phase. In addition, DAC has been reported to be synergistic with imatinib in pre-clinical models. The combination of imatinib and DAC is currently being investigated in the clinic.

Another epigenetic phenomenon involved in regulation of transcription is acetylation of histones. Histone deacetylases mediate the deacetylation of the core of nucleosomal histones. Several histone deacetylase inhibitors are being investigated currently in the clinic. One of these agents, suberoylanilide hydroxamic acid (SAHA) has shown to down regulate the expression of the Bcr/Abl protein and induce apoptosis in blast cells derived from patients who develop resistance to imatinib. In addition, SAHA was synergistic with imatinib in inducing apoptosis. Depsipeptide, another histone deacetylase inhibitor, has shown similar effects. Thus, interfering with these epigenetic phenomena, through inhibition of methylation or of histone deacetylation, may have a role in the management of CML.

Farnesyl Transferase (FTase) Inhibitors (FTI)

One of the best recognized downstream events resulting from the tyrosine kinase activity of Bcr/Abl is the activation of Ras. Ras is synthesized as an inactive precursor and remains in the cytoplasm. To become active, several post-translational modifications are required. The first of these changes is achieved through a prenylation process that provides the necessary anchor for attachment to the cellular membrane. Two enzymes are responsible for this prenylation process: the most important one is FTase that attaches a farnesyl group to the C-terminal cysteine of Ras. Prenylation also can be mediated by geranylgeranyl protein transferase. Inhibition of FTase thus has been pursued as a therapeutic strategy to block Ras. However, multiple other intracellular proteins are activated through farnesylation and it is possible that the antineoplastic activity of the FTI is mediated through their effects on...
proteins such as RhoB or the centromeric proteins CENP-E and CENP-F. Several FTI, mostly non-peptidomimetics, are being investigated in the clinic. Some of these drugs have demonstrated clinical activity in solid tumors and hematologic malignancies such as AML and MDS. There is significant preclinical evidence of the efficacy of FTI in CML. SCH66336 (lonafarnib), induced a dose-dependent inhibition of colony formation and proliferation of BaF3 cells that had been transformed with Bcr/Abl. A similar effect was achieved in cells from patients with CML. Significant activity was demonstrated also in two different Bcr/Abl leukemia mouse models. More important, SCH66336 inhibits the proliferation of imatinib-resistant Bcr/Abl positive cell lines and the colony formation of cells from imatinib-resistant CML patients, and sensitizes imatinib-resistant cells to the apoptotic effect of imatinib.

Three studies have reported clinical activity of FTI in CML. In one study, 22 patients with CML (10 in chronic, 6 in accelerated, and 6 in blast phase) were treated with R115777 (tipifarnib) 600 mg PO twice daily. All patients had failed prior therapy, including interferon alpha (91%), and imatinib (77%). Seven patients (32%; 6 in chronic phase and 1 in accelerated phase) responded: 5 had a complete and 2 a partial hematologic response. Four of the responders had a transient minor cytogenetic response. The median duration of response was 9.4 weeks. In addition, 3 patients with CML in blast phase (only 2 with the Philadelphia chromosome –Ph-) were included in a phase I study of R115777 in high-risk acute leukemias. Both patients with Ph-positive CML achieved a partial hematologic response. A third study investigated the efficacy of SCH66336 administered at a dose of 200 mg PO twice daily to 12 patients with CML (8 chronic phase, 4 accelerated phase), all of them previously refractory or intolerant to imatinib. Two patients (17%; 1 chronic, 1 accelerated) had a hematologic response. Although the responses as single agent are modest for these agents, these studies demonstrate clinical activity. Based on the preclinical data showing synergy with imatinib, ongoing studies are currently investigating the combinations of FTI with imatinib.

**Arsenic Trioxide**

The interest in arsenicals for the treatment of hematologic malignancies dates to the end of the 19th century. Since 1931, Forkner and Scott reported on the use of arsenicals for the treatment of chronic myeloid leukemia. However, issues related to toxicity and other practical aspects affected the enthusiasm on pursuing these agents. Recently, arsenic trioxide (As$_3$O$_3$) has been found to be an effective and safe agent for the management of acute promyelocytic leukemia, and preclinical and early clinical data suggest activity in other hematologic disorders such as MDS and multiple myeloma.

There is significant preclinical data on the effect of As$_3$O$_3$ on CML. As$_3$O$_3$ at concentrations that can be achieved clinically (0.5 to 2.0 μM/L) induced a decline in Bcr/Abl protein levels, but not other proteins such as Bcl-xL, Bax or Fas. Bcr/Abl-positive cell lines underwent apoptosis after incubation with As$_3$O$_3$. The induction of apoptosis by As$_3$O$_3$ on Bcr/Abl-positive cell lines appeared to be independent on Bcr/Abl kinase activity. In addition, As$_3$O$_3$ reduced proliferation of blasts from patients with CML, but had no effect on peripheral CD34+ progenitors. Most important, co-treatment of Bcr/Abl-positive cell lines with As$_3$O$_3$ and imatinib induced significantly more apoptosis and resulted in greater reduction of levels of Bcl-xL, XIAP, and Akt, and inhibition of Akt kinase activity, suggesting potential synergy of this combination. Based on this reported synergy, early results of a trial combining As$_3$O$_3$ and imatinib were reported by Mauro et al. Six patients with imatinib-refractory CML in chronic phase were treated with imatinib 400mg per day together with As$_3$O$_3$ 0.25 mg/kg/day for 5 days as a loading dose followed by a maintenance dose of 0.25 mg/kg twice weekly. No significant toxicity was reported and at the time of the report, all patients had increased their dose of imatinib. Another ongoing study is evaluating the efficacy of this combination in accelerated and blast phase.

**Proteasome Inhibition**

The ubiquitin-proteasome pathway is the main intracellular pathway responsible for protein degradation. This system plays both a “housekeeping” role by disposing of damaged proteins, as well as a regulatory role by degrading proteins that play a role in functions such as cell cycle regulation and tumor growth. Inhibition of proteasomes results in antineoplastic effects in several tumor models. One important substrate of proteasomes is IκB, the inhibitor of nuclear factor...
When stimulated, NF-κB is translocated to the nucleus where it activates the transcription of several genes. Thus, one important consequence of proteasome inhibition is the inhibition of NF-κB through decreased inactivation of its inhibitor IκB. Thus, the antineoplastic effect of proteasome inhibitors may be due, at least in part, to NF-κB inhibition. Although the role of NF-κB in the pathogenesis of CML is not clear, it has been suggested that Bcr/Abl activates NF-κB–dependent transcription, and NF-κB may be required for Bcr/Abl mediated transformation, possibly mediated by the RhoGEF domain of Bcr. Proteasome inhibition with N-carbobenzoxy-L-leucyl-L-leucyl-norvalinal (LLnV), a proteasome inhibitor, results in a significant reduction in Bcr/Abl protein expression in K562 cells with no change in the expression of p145Abl or p160Bcr. In addition, proteasome inhibition induces apoptosis in this system. PS341 (bortezomib, Velcade) is a potent and selective proteasome inhibitor that has significant activity against a broad range of human tumor cells and cell lines, and clinical activity in multiple myeloma and possibly other lymphoproliferative disorders. Gatto et al. showed that PS-341 inhibits growth and induces apoptosis of several Bcr/Abl positive cell lines, both imatinib-sensitive and imatinib-resistant. This is associated with accumulation of cells in the G2/M phase of the cell cycle, down regulation of NF-κB DNA binding activity, and Bcl-x1 expression, and the sequential exposure to the two drugs (PS-341 followed by imatinib) was synergistic. Clinical studies of bortezomib in imatinib-resistant CML patients are ongoing.

**Antiangiogenic Agents**

Evidence of the significance of angiogenesis and angiogenic factors in leukemia is growing. Vascular endothelial growth factor (VEGF) is the central proangiogenic molecule involved in tumor-related neovascularature. VEGF regulates critical endothelial cell functions, including mitogenesis, permeability, and the production of vasoactive molecules, involved in vessel budding and tube formation. It is also a survival factor required for the maintenance of new blood vessels. VEGF acts by binding to 3 receptor tyrosine kinases (RTK): VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flik-1), and VEGFR-3 (Flt-4). Endothelial cell proliferative and mitogenic responses to VEGF, as well as vascular permeability, are mediated primarily by binding to VEGFR-2. Patients with chronic myelomonocytic leukemia (CMML) or CML-BP have elevated levels of urinary and leukemia blast levels of VEGF. Increased bone marrow VEGF levels are associated with reduced CR rates, disease free (post-CR) survival, and overall survival in patients with CMML or other MDS. These data suggest that the VEGF/VEGFR-2 pathway affects the biological behavior of the hematologic malignancies and may be a therapeutic target in patients with these diseases.

Elevations of plasma VEGF levels have been reported in patients with CML compared with control subjects (76.3 vs. 26.7 pg/mL) and these levels were the highest among all leukemias tested (including CLL, MDS, AML, ALL, and CMML). Plasma levels of basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and tumor necrosis factor-α (TNFα) were also significantly elevated. There is a significant increase in bone marrow vascularity as determined by the number of blood vessels and area of vascularity. Patients with CML had a median of 21.4 blood vessels (11.2 for controls, P = .003) and the relative vascular area was 6.2% (compared with 2.8%; P = .02). These values were also the highest among all leukemias. High VEGF plasma levels were associated with a significantly shorter survival in AML. VEGF also has been shown to suppress the function of dendritic cells, which have been shown to stimulate autologous anti-leukemia T-cell response, particularly in CML. Antibodies to VEGF may enhance the function of dendritic cells. Recently, increased cellular expression of VEGF has been associated with shorter survival in a multivariate analysis in 148 CML patients in chronic phase. Therefore, suppression of VEGF might prove beneficial in CML, possibly through enhancing a specific anti-CML immune reaction. Specific anti-VEGF monoclonal antibodies (i.e., bevacizumab) are currently in clinical trials in patients with CML, as are a number of receptor tyrosine kinase (RTK) inhibitors, including SU5416, PTK787, and PKC412.

VEGF directly stimulates the production of several hematopoietic growth factors from human umbilical vein endothelial cells, including granulocyte macrophage-colony stimulating factor (GM-CSF) and stem cell factor (SCF). Subsets of patients with CMML show hyperproliferative responses to GM-CSF. SU5416 (Z-3- [(2,4-dimethylpyrrol-5-yl)- methylidenyl] -2-indolinone) is a small, lipophilic,
highly protein-bound synthetic receptor tyrosine kinase (RTK) inhibitor (RTKI) of VEGFR-2. It inhibits VEGFR-2 and other RTK targets by binding to the conserved adenosine triphosphate binding site within the kinase domain of the receptor. It thus inhibits the autophosphorylation induced by ligand binding to its receptor. SU5416 inhibits VEGF-dependent endothelial cell proliferation in vitro and in animal models. SU5416 has no direct cytotoxic properties, yet produces a dose-dependent inhibition of tumor growth in xenograft models, including those of malignant melanoma, glioma, fibrosarcoma, and carcinomas of the lung, breast, prostate, and skin. As well as inhibiting VEGFR-2, SU5416 is a RTKI for both c-kit and the FLT3 (fms-related tyrosine kinase Flk2) receptor. In both human myeloid leukemia cell lines and blasts from patients that express c-kit, SU5416 inhibits SCF-induced tyrosine phosphorylation, reduces cell proliferation, or induces apoptosis. FLT3 is expressed also on hematopoietic progenitors, and dysregulation of FLT3 signaling is associated with acute myeloid leukemia (AML). Internal tandem duplication (ITD) mutations in FLT3 result in constitutive FLT3 tyrosine kinase activity, and are associated with a poor prognosis in patients with AML. FLT3-ITD is the most frequently observed molecular defect in AML (25%–30% of patients). Recent in vitro studies have shown that SU5416 is a potent inhibitor of both wild type and mutant forms of FLT3. Therefore, SU5416 may directly target bone marrow angiogenesis via VEGFR-2 and blast cell proliferation via FLT3 and c-kit in patients with CML. Initial data on this agent in other hematologic malignancies have recently been published.

PTK787, an oral aminophthalazine, inhibits VEGFR, especially VEGFR-2 and, to a lesser degree, platelet-derived growth factor (PDGF) receptor, bFGF receptor, c-kit, and c-fms. PTK787 effectively inhibits proliferation of human endothelial cells, and caused tumor growth delay in several xenograft models. Clinical studies of single agent PTK787 are ongoing, and combination studies with chemotherapy or bevacizumab are planned. PKC412 has been identified as an inhibitor of FLT3 and, as with other FLT3 inhibitors such as CEP-701, is rapidly undergoing clinical investigation in patients with hematologic malignancies.

The RTK c-kit, essential for normal hematopoietic cell development, has a functional role in some patients with the MPD. Binding of the c-kit ligand, SCF, initiates a signal transduction cascade that includes receptor autophosphorylation with subsequent phosphorylation of numerous intracellular substrates. Increased tyrosine phosphorylation of c-kit and proliferation on SCF stimulation occurs in most blasts expressing c-kit. SCF-induced blast proliferation is synergistic with that induced by GM-CSF or interleukin-3. A number of specific c-kit inhibitors are under development.

**Peptide Vaccines**

Several lines of evidence suggest that CML may be amenable to immune recognition and elimination. There is clear evidence of graft-versus-leukemia effect in CML after stem cell transplantation and donor lymphocyte infusion. In addition, immune modulation may be at least partially responsible for the antileukemia effect of interferon alpha in CML. Thus, considerable interest has been generated in developing a “vaccine” for patients with CML that could stimulate a T-cell-mediated antitumor effect. Three potential sources for antigens for such a vaccine exist: tumor specific, tissue specific, and nonspecific. The chimeric p210\(^{bc-
abla}\) protein generated by the fusion of Abl and Bcr is tumor specific because it contains a sequence of amino acids not expressed in nonleukemic cells. Several short peptides containing 8 to 12 amino acids have been identified that bind to the major histocompatibility complex (MHC). Immunization of mice with these peptides can elicit a peptide-specific CD4+ T-cells response. These T-cells proliferated after exposure to the intact p210\(^{bc-
abla}\) protein but not when exposed to related proteins with a different junction sequence (e.g., p185\(^{bc-
abla}\)) or unrelated proteins. Several peptides have been identified that can bind to class 1 and 2 human lymphocyte antigen (HLA) molecules and elicit HLA-restricted cytotoxicity in vitro from lymphocytes of healthy volunteers or CML patients. Pinilla-Ibarz et al. used a combination of several peptides with binding motifs to various HLA molecules to vaccinate patients with chronic phase Ph-positive CML. The patients included in this study had been treated with IFN-\(\alpha\) or hydroxyurea and were in partial or complete remission. After vaccination, 2 patients developed significant DTH reactivity, and 3 patients developed peptide-specific T-cell proliferation. This was a dose-finding study, and all responses occurred at one of the
2 highest doses tested. However, no cytotoxic T-lymphocytes were identified in these patients after vaccination. Interestingly, one patient had a transient molecular response and one a partial cytogenetic response (transient). These investigators have recently reported results of a phase II trial with this vaccine. All 12 evaluable patients developed a DTH and CD4 proliferative response after vaccination, and 6 had IFN-α release by CD4 ELISPOT. Three of the patients had a decrease in the percentage of Ph-positive metaphases, although all 3 were receiving IFN-α concurrently with the vaccine. One patient treated for recurrence after stem cell transplantation had a transient molecular response. Thus, this approach appears to be safe and shows promising efficacy.

One example of a tissue-specific antigen is PR1, a nonapeptide derived from proteasome 3. Proteinase 3 is a serine protease induced during differentiation, stored in azurophilic granules, and overexpressed in myeloid leukemias. PR1 was selected as a potential vaccine because of its ability to bind to HLA-A2.1, which is expressed in nearly half of the population. Cytotoxic T lymphocytes (CTL) specific for PR1 selectively inhibited colony formation of HLA-A2.1 CML targets. PR1-specific CTL also have shown significant lytic activity against fresh leukemia cells from patients with HLA-A2.1+ CML or AML. In addition, CTL generated through stimulation with PR1 inhibited colony forming units from HLA-A2.1+ CML patients, but not HLA-A2.1+ bone marrows or HLA-A2.1− CML, demonstrating the specificity of these lymphocytes. These specific T lymphocytes play a role in the elimination of CML, as shown by the fact that 11 of 12 patients who responded to IFN-α and in 6 of 8 who responded to an allogeneic BMT had PR1-specific CTL. In contrast, these were undetectable in untreated patients, patients treated with chemotherapy only, or patients who did not respond to IFN-α. Based on these observations, a study was initiated for patients with myeloid malignancies who were treated with one of 3 dose levels of PR1 vaccine administered subcutaneously with incomplete Freund’s adjuvant. Four patients had AML, 1 MDS, and 4 CML. Three patients experienced induction into complete remission, and one additional patient sustained a complete remission; 2 patients experienced a cytogenetic remission. All 4 patients in complete remission had evidence of PR1-specific CTL after the vaccine. Thus, it is clear that this vaccine has significant potential for the treatment of patients with myeloid malignancies.

**New ABL Kinase Inhibitors**

The major clinical success of imatinib in CML has triggered increased enthusiasm for the investigation of other agents that could inhibit tyrosine kinases. An alternative approach at tyrosine kinase inhibition is the use of molecules that could prevent the binding of peptide substrates rather than adenosine 5'-triphosphate (ATP) binding. A group of agents called tyrphostins have been found to inhibit tyrosine kinase. A member of this family, AG957, can inhibit Bcr-Abl autophosphorylation, but is not specific for Bcr-Abl and is not as active as imatinib. Adaphostin is the adamantany ester of AG957 with greater in vitro potency than AG957. Imatinib-resistant cell lines are sensitive to adaphostin, and a combination of these 2 agents may be synergistic. Inhibitors of other kinases are being investigated also. AG490 is potent inhibitor of JAK2, another tyrosine kinase and is synergistic with STI571. Src kinase inhibitors are being investigated. Although these molecules are earlier in their development, they may prove valuable in the near future either in combination with imatinib or for patients who have developed resistance to imatinib.

**Interferon-alpha**

The efficacy of IFN-α in CML is unquestionable. Patients treated in early chronic phase CML with IFN-α achieved MCR rates of 20% to 40%, complete cytogenetic response (CCR) rates of 5% to 30%. Combining IFN-α with cytarabine may increase the rate of CCR to 25% to 35%. Most of the CCR obtained with IFN-α are durable and result in molecular remissions in some patients. The introduction of imatinib has changed the therapeutic algorithm of CML. Thus, the use of IFN-α as first line therapy in CML is likely to disappear soon. However, it is still a valuable option and in coming years there will be an increasing number of patients whose CML may have become resistant to imatinib and who would have not been exposed to IFN-α. New formulations of IFN-α may improve tolerance and efficacy. Attachment of IFN-α to polyethylene glycol (PEG) prolongs the half-life of IFN-α allowing a weekly administration. In a phase I study on patients with CML that failed to re-
spond or were intolerant to IFN-α, significant activity was observed. Seven of 19 patients with active disease achieved a complete hematologic response and 2 (11%) had a CCR. Seven of 8 patients treated in CHR improved their response to CCR (n = 4) or partial (n = 3). All 6 patients intolerant to IFN-α tolerated PEG-IFN and 4 improved their cytogenetic response. A different approach to sustained-release formulations of IFN-α is Albuferon, composed of recombinant human albumin fused to recombinant human IFN-α. Because the median half life of albumin is approximately 20 days, it prolongs the half life of IFN-α and allows for administration every 2 weeks. This drug is currently being investigated in CML.

Conclusions
Multiple new agents are currently being developed in CML. Most of these agents are being investigated now in patients who have developed resistance to imatinib. Their mechanisms of action are diverse and many may be synergistic with imatinib. These agents will also be used soon in different combinations, frequently including imatinib, with the hope of obtaining a complete blockade of the intracellular pathways that are triggered by Bcr-Abl. If this is successful, complete eradication of disease may become a reality for the majority of patients with CML.

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