Therapeutic intervention in leukemias that express the activated fms-like tyrosine kinase 3 (FLT3): opportunities and challenges

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Purpose of review

The fms-like tyrosine kinase 3 (FLT3) receptor tyrosine kinase is now recognized to be a critical mediator in the pathogenesis of myeloid and some lymphoid leukemias. This article reviews recent efforts to disrupt FLT3 signaling in acute myelogenous leukemia and to identify potential therapeutic challenges posed by the acquisition of resistance mutations in these malignancies.

Recent findings

Several broad classes of FLT3 protein tyrosine kinase inhibitors are undergoing evaluation in clinical trials. Although the agents are well tolerated by patients, clinical responses in relapsed or refractory acute myelogenous leukemia (AML) are limited and transient. Nevertheless, these agents may hold promise when combined with traditional chemotherapy. Use of tyrosine kinase inhibitors for AML therapy is hindered by the acquisition of mutations in the kinase catalytic domain, and in the case of BCR-ABL, these mutations confer resistance to imatinib. In anticipation of this problem, FLT3 mutations that might confer resistance to kinase inhibitors in the clinical setting have already been identified in the laboratory. Strategies to overcome such resistance are currently under development. New efforts focus on blocking the binding of FLT3 ligand to its receptor as a means of inhibiting autocrine stimulation in leukemogenesis.

Summary

FLT3 is widely expressed in AML and some cases of acute lymphocytic leukemia. Activating mutations in FLT3 confer a poor risk in patients with AML. The development of FLT3 small molecule kinase inhibitors follows from research efforts to understand signal transduction and profiles of gene expression in leukemia pathogenesis. Thus, FLT3 is a promising target for therapeutic intervention. Research priorities will include (1) identification of other groups of patients likely to benefit from FLT3 inhibition, (2) the optimal use of FLT3 inhibitors in combination with other agents, and (3) development of molecules that overcome resistance to FLT3 inhibitors that arise as a result of further acquired mutations in the receptor.

Keywords

acute myelogenous leukemia, FLT3, tyrosine kinase, drug resistance

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Abbreviations

ALL	acute lymphocytic leukemia
AML	acute myelogenous leukemia
APL	acute promyelocytic leukemia
CML	chronic myelogenous leukemia
CXCR-4	CXC chemokine receptor 4
FL	fms-like tyrosine kinase 3 ligand
FLK	fetal liver tyrosine kinase
FLT3	fms-like tyrosine kinase 3
ITD	internal tandem duplication
MLL	mixed lineage leukemia

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Introduction

The fms-like tyrosine kinase 3 (FLT3), also known as fetal liver tyrosine kinase [FLK] 2 or stem cell tyrosine kinase 1) is now known to be a critical element in the pathogenesis of many instances of acute myelogenous leukemia (AML) and mixed lineage leukemia (MLL). The FLT3 tyrosine kinase is commonly overexpressed both at the mRNA and protein levels in most cases of AML and in a large proportion of acute lymphocytic leukemia (ALL), and activating mutations of FLT3 are a poor prognostic indicator in AML. Animal models have confirmed the role of activated FLT3 in the pathogenesis of myeloid leukemias. These findings have spurred the development of strategies to inhibit the activity of the FLT3 kinase, and many of the resulting molecules are undergoing evaluation in clinical trials.

The role of FLT3 in normal and malignant hematopoiesis has been reviewed in detail [1–8]. The FLT3 kinase is a membrane-spanning protein with an extracellular ligand-binding domain and a cytoplasmic split tyrosine kinase domain characteristic of class III receptor tyrosine kinases [9,10]. The addition of its cognate FLT3 ligand (FL) [11,12] triggers receptor dimerization, autophos-

phorylation, activation of kinase catalytic activity, and phosphorylation of substrates that transduce cell survival and proliferation signals. In approximately 30% of patients with AML, the FLT3 gene is mutated and encodes a receptor containing duplications within the cytoplasmic juxtamembrane domain (termed FLT3-internal tandem duplication [ITD] mutations) [13]. In AML cells, FLT3 is also activated through mutations in the activation loop of the kinase domain [14,15]. Through either mechanism, the FLT3 kinase is activated independent of FL binding to the extracellular domain. The biologic role of these mutations is evident in cell culture models, and Ba/F3 or 32D murine hematopoietic cells that are normally dependent on interleukin 3 are able to survive and proliferate in the absence of this cytokine when engineered to express activated FLT3 [16]. Moreover, expression of activated FLT3 in murine hematopoietic progenitor cells is sufficient to generate a lethal myeloproliferative disease in recipient mice [17].

This abundant epidemiologic and preclinical evidence for the role of activated FLT3 in leukemia stimulated considerable effort to target this kinase with small molecule inhibitors to inactivate its enzymatic activity. The initial clinical experience of the agents has been followed by the use of these kinase inhibitors in combination with conventional antileukemic therapies. Guided by experience with the use of imatinib in chronic myelogenous leukemia (CML) and in other malignancies, acquisition of resistance to FLT3 inhibitors over time is quite probable. Therefore, forward-looking research efforts have already identified such mutations in the laboratory even before they have appeared in patients, and these mutant proteins are now being used to identify other agents that could inhibit FLT3. A complementary strategy is also underway to block FL binding to the FLT3 receptor and thereby disrupt an autocrine or paracrine stimulatory loop in the pathogenesis of leukemias. Principal challenges will consist of (1) the identification of patients who are likely to benefit from therapies that disrupt FLT3 activity and (2) the design of strategies to salvage patients who have leukemia in relapse or disease that is refractory to such interventions.

Small molecule FLT3 inhibitors

Efforts to target FLT3 have followed the paradigm used by Drucker *et al*. [17a] in the development of imatinib mesylate for CML. Cells engineered to express the FLT3-ITD protein and hence able to grow in the absence of interleukin 3 were used to screen candidate molecules for their ability to halt growth of these cells in the absence but not presence of interleukin 3. The first such compounds to be reported were the tyrphostin compounds AG1295 and AG1296 [18,19]. However, the clinical utility of these compounds was limited by their poor solubility [5].

Another agent, PKC412 (N-benzoylstaurosporine), inhibits the proliferation of Ba/F3-FLT3-ITD cells with an IC_{50} less than 10 nM [20]. In addition to inhibiting FLT3, PKC412 also potently inhibits other class III receptor tyrosine kinases including PDGFRα, PDGFRβ, c-KIT, and VEGFR-2. In a murine bone marrow transplant model of myeloproliferative disease induced by FLT3-ITD, oral administration of PKC412 reduced splenomegaly and peripheral blood leukocytosis and significantly prolonged the survival of recipient mice [20]. In a phase I clinical trial, dose-limiting toxicities (grade 3 lethargy/fatigue or nausea/vomiting) occurred only at the oral dose of 225 or 300 mg/d [21]. The efficacy of PCK412 in relapsed or refractory AML or advanced MDS was assessed in a phase II clinical trial [22••]. Twenty patients received orally administered PKC412 at 75 mg TID. In most patients, the drug was well-tolerated, although 13 patients experienced grade 1 or 2 nausea/vomiting. One patient experienced a grade 4 elevation in cardiac troponin, and three patients succumbed to progressive pulmonary failure; one of these adverse events was in the setting of progressive leukocytosis, and in the other patients the relation of these events to the administration of PKC412 was not clear. A greater than 50% decrease in the peripheral blast count was achieved in 14 of the patients, and in seven of these patients, the reduction in blast count was greater than two logs compared with baseline values. ALL cells that contain *MLL* gene rearrangements frequently overexpress wild-type FLT3 or contain FLT3-activating mutations, and these cells are susceptible to growth arrest by PKC412 [23]. Collectively, these findings support the assertion that inhibition of FLT3 may be effective in the treatment of some patients with AML.

The utility of FLT3 inhibition in AML was also evaluated using small molecules structurally unrelated to PKC412. SU5416 is a indolinone compound that is a competitive inhibitor of adenosine triphosphate binding and that selectively inhibits FLT3 as well the fms-related kinase FLK2 and c-KIT [24–27]; the structurally-related compound SU5614 also inhibits FLT3 kinase activity [27]. Cell lines that express activated FLT3 undergo cell cycle arrest and apoptosis when treated with SU5614 (IC_{50} 100–300 nM) [27,28], and those treated with SU5416 undergo a proliferation arrest with an IC_{50} of 100 to 1000 nM [27]. In a phase I trial of SU5416, 55 patients with AML (33 patients) or advanced MDS (22 patients) in relapse or patients older than 70 years who declined conventional chemotherapy were treated with 4-week cycles of a twice-weekly intravenous infusion of 145 mg/m^2 [29]. Four patients had an objective response as determined by bone marrow evaluation, and three others had a partial response. Grade 3 or 4 toxicities

included headache, dyspnea, fatigue, thromboembolic events, bone pain or abdominal pain, or gastrointestinal events. Translational studies showed that 62% of the AML samples evaluated demonstrated a reduction in FLT3 phosphorylation [30•]. In a second study, 43 patients with refractory AML were enrolled to receive twice-weekly SU5416 (145 mg/m^2 intravenous infusion) [31••]. One patient underwent remission of AML as determined by bone marrow evaluation, and seven patients had a partial response. Major adverse events were for the most part related to the patients' underlying AML. A third indolinone inhibitor of FLT3, SU11248, also inhibits the FLK1, PDGFRβ, and FGFR1 receptor tyrosine kinases [32,33]. In a phase I trial, 29 AML patients received orally administered SU11248 in an escalating dose schedule [34•]. A robust decrease in FLT3 phosphorylation in AML samples was seen in 10 of 13 evaluable patients who received a dose of 200 mg/d dose or higher. A minority of patients (>10%) experienced grade 1 or 2 gastrointestinal symptoms at the 250 to 350 mg/d dose levels, and two patients experienced either transient grade 3 hypertension or transient cardiac dysrhythmia.

A third class of compounds is defined by the FLT3 inhibitor MLN518 (CT53518), a piperazinyl quinazoline compound that also inhibits the c-KIT receptor tyrosine kinase [35,36]. MLN518 inhibits the proliferation and survival of FLT3-ITD-Ba/F3 cells (IC_{50} 10–30 nM). In a nude mouse leukemic allograft model and in a murine bone marrow transplant model of FLT3-induced myeloproliferative disease, oral administration of MLN518 prolonged the survival and reduced both splenomegaly and leukocytosis in recipient mice [35]. At concentrations of MLN518 that markedly attenuate FLT3-induced leukemia in mice and that impair colony formation of FLT3-ITD-positive AML blasts from patients, there is no significant effect on colony formation of normal human hematopoietic progenitor cells [37••]. This finding suggests that the therapeutic index of this drug relative to normal hematopoiesis might be substantial in patients with AML.

Also in clinical trials is the CEP-701 small molecule inhibitor of FLT3 [38]. This compound is an indolecarbazole derivative that also inhibits the TRKA tyrosine kinase with an IC_{50} of 3 nM [39]. CEP-701 inhibits the growth of FLT3-ITD-Ba/F3 with an IC_{50} of 5 nM, and subcutaneous injection of this agent prolongs the survival of mice inoculated with these cells [38]. Moreover, culture of the 32D murine myeloid cell line with CEP-701 can overcome an arrest in differentiation caused by expression of a FLT3-ITD transgene [40]. In a phase I/II clinical trial in adults with relapsed, poor risk, or primary refractory AML, 17 patients received oral CEP-701 at a dose of 40 to 80 mg BID. Five patients obtained a clinical response with a duration of 2 weeks to 3 months, one patient achieved a decrease in bone marrow blasts to less than 5%, and 4 patients showed a reduction in peripheral blast counts [41••]. Grade 3 or 4 toxicities possibly related to the drug were infrequent and included weakness/fatigue, arthralgia/myalgia, melana, epistaxis, dyspnea, or congestive heart failure. Leukemia cells may become dependent, or addicted, to the expression of an activated oncogene [42], and these cells might be particularly sensitive to the inhibition of FLT3. In support of this hypothesis, primary AML cells from pediatric patients with the highest level of FLT3-ITD expression are also the most susceptible to treatment with CEP-701 [43•].

FLT3 expression is common in B-precursor ALL, childhood ALL with MLL gene rearrangements, and childhood ALL with high hyperdiploidy [44,45]. Furthermore, activating FLT3 mutations are a recurrent finding in childhood ALL with hyperdiploidy, ALL with high hyperdiploidy, infant and childhood ALL with *MLL* gene rearrangements, and adult T-cell ALL [46••–48••]. As noted, these findings have indicated the potential utility of the PKC412 inhibitor in the setting of childhood and infant ALL. Similarly, childhood ALL cells with elevated contents of FLT3 phosphotyrosine (an indicator of FLT3 activation) are differentially susceptible to cytotoxicity by the CEP-701 inhibitor [49••].

Together, these findings indicate that FLT3 inhibition by small molecule kinase inhibitors can produce both biochemical and clinical responses in patients with AML. However, the responses with single-agent therapy were of limited frequency and duration. As in the case of other anticancer agents, their initial use in the highest risk population may underestimate their utility in other clinical settings or in combination with other agents. These findings nonetheless provide evidence that FLT3 inhibition might be an effective component of chemotherapy for some leukemias.

Combination therapy using small molecule inhibitors of FLT3

Several efforts are underway to determine the utility of FLT3 inhibition in the setting of multiagent chemotherapy. One such strategy would be to combine different classes of FLT3 small molecule inhibitors. Although there is not yet information regarding the clinical efficacy of this approach, one preliminary study showed that treatment of FLT3-ITD-Ba/F3 cells with the combination of SU5614 and PKC412 yielded an additive but not a synergistic effect [50•]. Nevertheless, such combined therapy might hinder the development of kinase resistance mutations.

Levis *et al.* [51••] recently reported the *in vitro* use of the CEP-701 FLT3 inhibitor with cytotoxic agents that are in common use for the treatment of AML. CEP-701 syn-

ergizes with the simultaneous use of cytarabine, daunorubicin, etoposide, or mitoxantrone in impairing the growth of FLT3-ITD-Ba/F3 cells or human AML cell lines with FLT3-activating mutations. Similar results were obtained when chemotherapy was followed by treatment with CEP-701. Importantly, previous treatment of leukemia cells with CEP-701 diminishes the toxicity of subsequent incubation with the S-phase specific antimetabolite cytarabine as a result of the cell cycle arrest triggered by CEP-701. These findings indicate that the combined use of FLT3 inhibitors with conventional agents will critically depend on the timing of their administration. In a similar study, the small molecule FLT3 inhibitor SU11248 in combination with daunorubicin or cytarabine synergistically killed human cells lines harboring activated FLT3 [52•]. This result confirms, with a structurally distinct kinase inhibitor, that FLT3 inhibition might augment the efficacy of conventional therapeutic agents in patients with AML.

FLT3–activating mutations in AML frequently coexist with other cytogenetic events. The high frequency of FLT3-ITD mutations in patients with acute promyelocytic leukemia (APL; as much as 36%) stimulated detailed studies of the interaction of FLT3 signaling with the fusion proteins of APL [53]. In a murine bone marrow transplant model, the PML-RARα fusion of APL cooperates with FLT3-ITD in the generation of an APL-like disease [54]. FLT3-ITD expression diminishes the association of PLZF (which is fused to the RARα retinoid receptor in some patients with retinoid-resistant APL) with the SMRT nuclear corepressor of transcription [55••]. Together, these studies indicate that targeted FLT3 inhibition might be effective in some patients with APL. Sohal *et al.* [56] provided compelling preclinical evidence to support this conjecture further. In a murine bone marrow transplant model using transgenic mice that express PML-RARα, the SU11657 FLT3 inhibitor cooperated with all-*trans* retinoic acid to induce remission of leukemias. The efficacy of APL therapy with a FLT3 small molecule inhibitor combined with all-*trans* retinoic acid, particularly in the setting of relapsed or chemotherapy-resistant disease, remains to be determined [2].

FLT3 mutations that confer resistance to small molecule inhibitors

Imatinib has served as the vanguard for the therapeutic use of small molecule protein tyrosine kinase inhibitors. Although this agent yields dramatic clinical and cytogenetic responses in CML and in other malignancies, enthusiasm has been tempered by the occurrence of resistance mutations within the BCR-ABL kinase catalytic domain [57,58]. Different activating mutations of FLT3 confer varying sensitivities to FLT3 inhibitors [59•,60•]. Cools *et al.* [61••] reported the results of an *in vitro* screen for FLT3-ITD mutants that are resistant to inhibition by PKC412. In the FLT3-ITD variants resistant to PCK412, one of four amino acids was substituted. It is not yet known whether similar substitutions will be found in clinically resistant AML patients, but the experience with imatinib resistance in CML suggests that some of these mutations will be found in AML samples resistant to PKC412. Using structural modeling of the FLT3 kinase in a complex with PKC412, each of the point mutations in the FLT3-ITD coding sequence was predicted to disrupt contacts between the protein and the kinase inhibitor. One of the mutations (G697R) conferred a high degree of resistance not only to PKC412 but also to the structurally unrelated FLT3 inhibitors SU5614, K-252a (similar to CEP-701), and six other small molecule inhibitors of FLT3. The potential therapeutic consequences of this finding are considerable, because clinicians have hoped to limit the emergence of clinical resistance through the use of a cocktail of small molecule inhibitors. The finding that a single point mutation can confer resistance to a broad spectrum of structurally unrelated inhibitors poses a substantial challenge to such a strategy.

Bagrintseva *et al.* [50•] characterized mutations within the FLT3-ITD gene that confer resistance of the kinase to the inhibitor SU5614. They showed that sensitivity to SU5614 varied depending on the specific amino acid substitution at residue D835. In a Ba/F3 assay for interleukin 3 independence, the FLT3-D835Y variant remained sensitive to SU5614 with an IC_{50} = 0.1 μM; in contrast, the FLT3-D835H mutant was resistant to this inhibitor with an IC_{50} greater than 10 μM. Moreover, this group characterized Ba/F3-FLT3-ITD cells that developed resistance to SU5614. They described two types of FLT3-ITD mutations that conferred resistance to this inhibitor: D835N and Y842H, both within the kinase catalytic domain. Interestingly, although both FLT3-ITD resistance variants conferred resistance to SU5614, both mutants were sensitive to the unrelated FLT3 inhibitor PKC412 in the Ba/F3 assay of interleukin 3 independence.

In addition to point mutations that confer intrinsic resistance of activated FLT3 variants to inhibition by kinase inhibitors, cells can develop inhibitor resistance through amplification of the target gene and overexpression of the respective kinase. Chronic exposure of p210BCR-ABL–expressing cells to imatinib selects for amplification of the BCR-ABL oncogene, resulting in overexpression of the activated kinase [62], and such amplification is observed also in patients with CML resistant to imatinib [63]. Similarly, Weisberg *et al.* [20] demonstrated that FLT3-ITD-Ba/F3 cells cultured in the presence of the PKC412 kinase inhibitor can develop resistance associated with overexpression of the FLT3-ITD protein. Thus, either overexpression or mutation of the FLT3

kinase can produce cell clones that are resistant to small molecule kinase inhibitors, and it is likely that these mechanisms will underlie clinical relapse in some AML patients. Other resistance mechanisms such as drug export, although not yet demonstrated for these inhibitors, may also be responsible for relapse or primary resistance in AML.

The ability of cells to become resistant to FLT3 inhibitors argues for the development of novel salvage strategies. One approach is to combine FLT3 inhibitors with agents that target signaling molecules downstream of this activated tyrosine kinase. Rapamycin, which is an inhibitor of the kinase mTOR, has an extensive history of use as an immunosuppressant. When combined with the FLT3 inhibitor PKC412, rapamycin markedly decreases the proliferation of FLT3-ITD-Ba/F3 cells [64••]. Significantly, rapamycin can also inhibit the growth of Ba/F3 cells that express the F691I mutant of the FLT3-ITD kinase that is resistant to PKC412 inhibition, and rapamycin and PKC412 synergistically attenuate the outgrowth of cells that express this resistance mutant. These findings suggest that inhibition of other pathways downstream of FLT3, such as the Ras-MAP kinase pathway and the JAK/STAT pathway, should be explored as well.

FLT3 ligand and mechanisms of autocrine stimulation

Although mutation of *FLT3* in AML can result in ligand-independent activation of this receptor tyrosine kinase, recent evidence indicates that FL may itself be the culprit in activating FLT3 in acute leukemia. Surprisingly, FL is commonly expressed in primary AML cells that express either wild-type nonmutated FLT3 or in cells that carry FLT3 activating mutations. Zheng *et al.* [65•] reported that FL was detected by reverse-transcription polymerase chain reaction in 90 primary AML specimens; 55 of these samples contained wild-type FLT3, 10 samples carried a D835 mutation in the activation loop of FLT3, and 25 samples carried FLT3-ITD mutations. These samples were further evaluated by immunofluorescence to detect FL protein, and cells from 25 patients demonstrated positive (albeit variable) expression of this protein. The ubiquitous coexpression of FLT3 and FL in this series suggests that these molecules might participate in an autocrine or paracrine signaling pathway. To support this conjecture, the authors further demonstrated that conditioned media from the EOL-1 cell line stimulates autophosphorylation of FLT3, and the autocrine activation of this kinase could be attenuated either with an anti-FLT3 antibody that blocks FL binding or by depleting the ligand from the medium with an anti-FL antibody. The potential functional role of FL/FLT3-dependent signaling in these cells is of particular interest, because the EOL-1 cell line also carries the recently discovered FIP1L1-PDGFRα fusion tyrosine kinase [66,67].

Such findings have stimulated interest in the development of a neutralizing antibody that will block ligand stimulation of FLT3. IMC-EB10 is a fully human monoclonal antibody that inhibits phosphorylation of wild-type FLT3 stimulated by FL [68••]. Importantly, IMC-EB10 attenuates the proliferation of leukemia cells that express wild-type FLT3 or hematopoietic cells that express a FLT3-ITD mutant, and infusion of this antibody in a murine xenograft model of leukemia significantly prolongs the survival of these mice.

In addition to the role of FL in triggering cell survival and proliferative signaling through FLT3, the CXC chemokine receptor 4 (CXCR-4) cell surface protein was recently implicated in the pathogenesis of AML with unfavorable prognosis [69••]. CXCR-4 is the receptor for stromal cell-derived factor 1α. Rombouts *et al.* [69••] evaluated a series of 90 patients with AML and assessed both the overall expression of CXCR-4 and the proportion of $CD34^+$ cells that expressed CXCR-4. Although the overall expression of CXCR-4 in these cells was of marginal prognostic value, the proportion of $CD34^+$ cells that also expressed this chemokine receptor correlated significantly with poor overall survival and relapse-free survival in these patients. Interestingly, the presence of a FLT3-ITD mutation had no additional prognostic value beyond that provided by the expression of CXCR-4. The percentage of $CD34^+$ cells that expressed CXCR-4 was significantly increased in patients with a FLT3-ITD mutation; there were too few samples to assess the correlation of CXCR-4 expression with the FLT3/D835 activation loop mutation. Based on these findings, the authors speculate that the elevated expression of CXCR-4 might underlie the poor prognosis of AML patients with activating FLT3 mutations. The study suggests implicitly that interruption of a SDF-1/CXCR-4 autocrine stimulatory loop might be of value in the therapy of AML.

Conclusion

Activation of the FLT3 receptor tyrosine kinase, either through ligand binding or through characteristic mutations at the juxtamembrane or kinase domains, is widespread in AML and in some cases of ALL. The critical role of FLT3 in many of these malignancies has elicited the development of a battery of small molecule kinase inhibitors and one antibody that blocks FL binding to the receptor. Initial trials in high-risk AML populations provide some conceptual evidence that FLT3 inhibition may be a valuable component of emerging therapeutic strategies. Ongoing efforts will lead to the identification of those patients who are most likely to benefit from FLT3 inhibition, and we can expect the design of combinatorial strategies that incorporate the inhibition of FLT3. The emergence of FLT3 mutations that confer resistance to inhibitors will likely limit the efficacy of these agents in some patients. The development of a

second generation of FLT3 inhibitors or the blockade of signaling molecules downstream of FLT3 might provide opportunities to salvage remissions in such patients.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • Of special interest
- •• Of outstanding interest

1 Levis M, Small D: FLT3: It does matter in leukemia. Leukemia 2003, 17:1738–1752.

2 Gilliland DG: FLT3-activating mutations in acute promyelocytic leukaemia: a rationale for risk-adapted therapy with FLT3 inhibitors. Best Pract Res Clin Haematol 2003, 16:409–417.

3 Gilliland DG, Griffin JD: The roles of FLT3 in hematopoiesis and leukemia. Blood 2002, 100:1532–1542.

4 Kottaridis PD, Gale RE, Linch DC: Flt3 mutations and leukaemia. Br J Haematol 2003, 122:523–538.

5 Brown P, Small D: FLT3 inhibitors: a paradigm for the development of targeted therapeutics for paediatric cancer. Eur J Cancer 2004, 40:707–721.

6 Griffin JD: FLT3 tyrosine kinase as a target in acute leukemias. Hematol J 2004, 5(suppl 3):S188–S190.

7 Stirewalt DL, Radich JP: The role of FLT3 in haematopoietic malignancies. Nat Rev Cancer 2003, 3:650–665.

8 Reilly JT: FLT3 and its role in the pathogenesis of acute myeloid leukaemia. Leuk Lymphoma 2003, 44:1–7.

9 Matthews W, Jordan CT, Wiegand GW, et al.: A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations. Cell 1991, 65:1143–1152.

10 Rosnet O, Marchetto S, deLapeyriere O, et al.: Murine Flt3, a gene encoding a novel tyrosine kinase receptor of the PDGFR/CSF1R family. Oncogene 1991, 6:1641–1650.

11 Lyman SD, James L, Vanden Bos T, et al.: Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. Cell 1993, 75:1157–1167.

12 Lyman SD, James L, Johnson L, et al.: Cloning of the human homologue of the murine flt3 ligand: a growth factor for early hematopoietic progenitor cells. Blood 1994, 83:2795–2801.

13 Nakao M, Yokota S, Iwai T, et al.: Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia 1996, 10:1911–1918.

14 Yamamoto Y, Kiyoi H, Nakano Y, et al.: Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood 2001, 97:2434–2439.

15 Abu-Duhier FM, Goodeve AC, Wilson GA, et al.: Identification of novel FLT-3 Asp835 mutations in adult acute myeloid leukaemia. Br J Haematol 2001, 113:983–988.

16 Hayakawa F, Towatari M, Kiyoi H, et al.: Tandem-duplicated Flt3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. Oncogene 2000, 19:624–631.

17 Kelly LM, Liu Q, Kutok JL, et al.: FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. Blood 2002, 99:310–318.

17a Druker BJ, Talpaz M, Resta DJ, et al.: Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001, 344:1031–1037.

18 Tse KF, Novelli E, Civin CI, et al.: Inhibition of FLT3-mediated transformation by use of a tyrosine kinase inhibitor. Leukemia 2001, 15:1001–1010.

19 Levis M, Tse KF, Smith BD, et al.: A FLT3 tyrosine kinase inhibitor is selectively cytotoxic to acute myeloid leukemia blasts harboring FLT3 internal tandem duplication mutations. Blood 2001, 98:885–887.

20 Weisberg E, Boulton C, Kelly LM, et al.: Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. Cancer Cell 2002, 1:433–443.

21 Propper DJ, McDonald AC, Man A, et al.: Phase I and pharmacokinetic study of PKC412, an inhibitor of protein kinase C. J Clin Oncol 2001, 19:1485–1492.

22 •• Stone RM, DeAngelo DJ, Klimek V, et al.: Acute myeloid leukemia patients with an activating mutation in FLT3 respond to a small molecule FLT3 tyrosine kinase inhibitor, PKC412. Blood 2004 [prepublished online September 2, 2004].

These investigators present the results of a phase II clinical trial of the PKC412 FLT3 inhibitor in the setting of relapsed/refractory AML and high-grade MDS. Seven patients obtained a decrease in peripheral blast counts of a greater than two log magnitude.

23 Armstrong SA, Kung AL, Mabon ME, et al.: Inhibition of FLT3 in MLL: validation of a therapeutic target identified by gene expression based classification. Cancer Cell 2003, 3:173–183.

24 Fong TA, Shawver LK, Sun L, et al.: SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. Cancer Res 1999, 59:99–106.

25 Mendel DB, Schreck RE, West DC, et al.: The angiogenesis inhibitor SU5416 has long-lasting effects on vascular endothelial growth factor receptor phosphorylation and function. Clin Cancer Res 2000, 6:4848–4858.

26 Sukbuntherng J, Cropp G, Hannah A, et al.: Pharmacokinetics and interspecies scaling of a novel VEGF receptor inhibitor, SU5416. J Pharm Pharmacol 2001, 53:1629–1636.

27 Yee KW, O'Farrell AM, Smolich BD, et al.: SU5416 and SU5614 inhibit kinase activity of wild-type and mutant FLT3 receptor tyrosine kinase. Blood 2002, 100:2941–2949.

28 Spiekermann K, Dirschinger RJ, Schwab R, et al.: The protein tyrosine kinase inhibitor SU5614 inhibits FLT3 and induces growth arrest and apoptosis in AML-derived cell lines expressing a constitutively activated FLT3. Blood 2003, 101:1494–1504.

29 Giles FJ, Stopeck AT, Silverman LR, et al.: SU5416, a small molecule tyrosine kinase receptor inhibitor, has biologic activity in patients with refractory acute myeloid leukemia or myelodysplastic syndromes. Blood 2003, 102:795–801.

30 • O'Farrell AM, Yuen HA, Smolich B, et al.: Effects of SU5416, a small molecule tyrosine kinase receptor inhibitor, on FLT3 expression and phosphorylation in patients with refractory acute myeloid leukemia. Leuk Res 2004, 28:679–689.

The authors assess the response of FLT3 phosphorylation as a surrogate marker for response to the SU5416 kinase inhibitor in AML.

31 •• Fiedler W, Mesters R, Tinnefeld H, et al.: A phase 2 clinical study of SU5416 in patients with refractory acute myeloid leukemia. Blood 2003, 102:2763–2767.

A phase 2 clinical trial using the SU5416 FLT3 kinase inhibitor in patients with refractory AML or elderly patients declining chemotherapy is presented. Responses to monotherapy with this agent are described.

32 Mendel DB, Laird AD, Xin X, et al.: In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 2003, 9:327–337.

33 O'Farrell AM, Abrams TJ, Yuen HA, et al.: SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. Blood 2003, 101:3597–3605.

34 • O'Farrell AM, Foran JM, Fiedler W, et al.: An innovative phase I clinical study demonstrates inhibition of FLT3 phosphorylation by SU11248 in acute myeloid leukemia patients. Clin Cancer Res 2003, 9:5465–5476.

The authors present results of a phase I trial for the use of the SU11248 FLT3 inhibitor in relapsed or refractory AML.

35 Kelly LM, Yu JC, Boulton CL, et al.: CT53518, a novel selective FLT3 antagonist for the treatment of acute myelogenous leukemia (AML). Cancer Cell 2002, 1:421–432.

36 Corbin AS, Griswold IJ, La Rosee P, et al.: Sensitivity of oncogenic KIT mutants to the kinase inhibitors MLN518 and PD180970. Blood 2004, 104:2912–2918.

37 •• Griswold IJ, Shen LJ, LaRosee P, et al.: Effects of MLN518, A dual FLT3 and KIT inhibitor, on normal and malignant hematopoiesis. Blood 2004 [prepublished online July 8, 2004].

This manuscript provides reassuring data to indicate that a FLT3 inhibitor does not substantially attenuate normal hematopoiesis. Such findings suggest that FLT3 inhibition might be combined with chemotherapy or stem cell transplantation without hematopoietic toxicity to normal progenitor cells.

38 Levis M, Allebach J, Tse KF, et al.: A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells in vitro and in vivo. Blood 2002, 99:3885–3891.

39 George DJ, Dionne CA, Jani J, et al.: Sustained in vivo regression of Dunning H rat prostate cancers treated with combinations of androgen ablation and Trk tyrosine kinase inhibitors, CEP-751 (KT-6587) or CEP-701 (KT-5555). Cancer Res 1999, 59:2395–2401.

40 Zheng R, Friedman AD, Small D: Targeted inhibition of FLT3 overcomes the block to myeloid differentiation in 32Dcl3 cells caused by expression of FLT3/ITD mutations. Blood 2002, 100:4154–4161.

41 •• Smith BD, Levis M, Beran M, et al.: Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. Blood 2004, 103:3669–3676.
The authors describe a phase 1/2 clinical trial for the use of the CEP-701 FLT3 inhibitor in AML. Clinical responses are described, and the agent is well tolerated.

42 Weinstein IB: Cancer: addiction to oncogenes–the Achilles heal of cancer. Science 2002, 297:63–64.

43 • Brown P, Meshinchi S, Levis M, et al.: Pediatric AML primary samples with FLT3/ITD mutations are preferentially killed by FLT3 inhibition. Blood 2004, 104:1841–1849.
In childhood ALL, sensitivity of cells to the CEP-701 inhibitor is closely tied to the expression of FLT3 in the cells.

44 Armstrong SA, Staunton JE, Silverman LB, et al.: MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet 2002, 30:41–47.

45 Yeoh EJ, Ross ME, Shurtleff SA, et al.: Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002, 1:133–143.

46 •• Taketani T, Taki T, Sugita K, et al.: FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with MLL rearrangements and pediatric ALL with hyperdiploidy. Blood 2004, 103:1085–1088.
See [49••].

47 •• Armstrong SA, Mabon ME, Silverman LB, et al.: FLT3 mutations in childhood acute lymphoblastic leukemia. Blood 2004, 103:3544–3546.
See [49••].

48 •• Paietta E, Ferrando AA, Neuberg D, et al.: Activating FLT3 mutations in CD117/KIT(+) T-cell acute lymphoblastic leukemias. Blood 2004, 104:558–560.
See [49••].

49 •• Brown P, Levis M, Shurtleff S, et al.: FLT3 inhibition selectively kills childhood acute lymphoblastic leukemia cells with high levels of FLT3 expression. Blood 2004 [prepublished online September 16, 2004].
The finding of FLT3 mutations in childhood and infant ALL, and adult T-ALL, suggests that inhibitors of FLT3 might be effective for some patients with these malignancies. CEP-701 is shown to be cytotoxic to childhood ALL cells that express high levels of FLT3.

50 • Bagrintseva K, Schwab R, Kohl TM, et al.: Mutations in the tyrosine kinase domain of FLT3 define a new molecular mechanism of acquired drug resistance to PTK inhibitors in FLT3-ITD-transformed hematopoietic cells. Blood 2004, 103:2266–2275.
Activated FLT3 variants that are resistant to the SU5614 FLT3 inhibitor are described.

51 •• Levis M, Pham R, Smith BD, et al.: In vitro studies of a FLT3 inhibitor combined with chemotherapy: sequence of administration is important to achieve synergistic cytotoxic effects. Blood 2004, 104:1145–1150.
These authors provide important information from cell culture studies to indicate that the sequence of combining a FLT3 inhibitor with conventional chemotherapy can markedly affect the efficacy of such regimens.

52 • Yee KW, Schittenhelm M, O'Farrell AM, et al.: Synergistic effect of SU11248 with cytarabine or daunorubicin on FLT3-ITD positive leukemic cells. Blood 2004 [prepublished online August 10, 2004].
The article provides additional evidence that a FLT3 inhibitor might be effectively integrated into a conventional chemotherapeutic regimen.

53 Kottaridis PD, Gale RE, Frew ME, et al.: The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 2001, 98:1752–1759.

54 Kelly LM, Kutok JL, Williams IR, et al.: PML/RARalpha and FLT3-ITD induce an APL-like disease in a mouse model. Proc Natl Acad Sci USA 2002, 99:8283–8288.

55 • Takahashi S, McConnell MJ, Harigae H, et al.: The Flt3 internal tandem duplication mutant inhibits the function of transcriptional repressors by blocking interactions with SMRT. Blood 2004, 103:4650–4658.
FLT3 activation may signal through transcriptional repressors to modulate the differentiation of granulocytic leukemia cells.

56 Sohal J, Phan VT, Chan PV, et al.: A model of APL with FLT3 mutation is responsive to retinoic acid and a receptor tyrosine kinase inhibitor, SU11657. Blood 2003, 101:3188–3197.

57 Shah NP, Nicoll JM, Nagar B, et al.: Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2002, 2:117–125.

58 Azam M, Latek RR, Daley GQ: Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL. Cell 2003, 112:831–843.

59 • Clark JJ, Cools J, Curley DP, et al.: Variable sensitivity of FLT3 activation loop mutations to the small molecule tyrosine kinase inhibitor MLN518. Blood 2004, 104:2867–2872.
See [60•].

60 • Grundler R, Thiede C, Miething C, et al.: Sensitivity toward tyrosine kinase inhibitors varies between different activating mutations of the FLT3 receptor. Blood 2003, 102:646–651.
The sensitivity of FLT3 activation loop mutants to a small molecule inhibitor varies depending on the particular activating mutation within FLT3.

61 •• Cools J, Mentens N, Furet P, et al.: Prediction of resistance to small molecule FLT3 inhibitors: implications for molecularly targeted therapy of acute leukemia. Cancer Res 2004, 64:6385–6389.
Using a library of FLT3 mutants generated *in vitro*, these authors are able to anticipate resistance mutations that are likely to be found in patients who relapse or are resistant to FLT3 small molecule inhibitors.

62 Weisberg E, Griffin JD: Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood 2000, 95:3498–3505.

63 Gorre ME, Mohammed M, Ellwood K, et al.: Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001, 293:876–880.

64 •• Mohi MG, Boulton C, Gu TL, et al.: Combination of rapamycin and protein tyrosine kinase (PTK) inhibitors for the treatment of leukemias caused by oncogenic PTKs. Proc Natl Acad Sci USA 2004, 101:3130–3135.
The use of rapamycin combined with a FLT3 kinase inhibitor can overcome resistance to inhibition by the FLT3 kinase inhibitor alone.

65 • Zheng R, Levis M, Piloto O, et al.: FLT3 ligand causes autocrine signaling in acute myeloid leukemia cells. Blood 2004, 103:267–274.
The authors provide evidence for an autocrine loop composed of FLT3 and FL in AML.

66 Cools J, Quentmeier H, Huntly BJ, et al.: The EOL-1 cell line as an in vitro model for the study of FIP1L1-PDGFRA-positive chronic eosinophilic leukemia. Blood 2004, 103:2802–2805.

67 Griffin JH, Leung J, Bruner RJ, et al.: Discovery of a fusion kinase in EOL-1 cells and idiopathic hypereosinophilic syndrome. Proc Natl Acad Sci U S A 2003, 100:7830–7835.

68 •• Li Y, Li H, Wang MN, et al.: Suppression of leukemia expressing wild-type or ITD-mutant FLT3 receptor by a fully human anti-FLT3 neutralizing antibody. Blood 2004, 104:1137–1144.
An antibody that blocks FL binding to FLT3 can impair both ligand-stimulated proliferation of cells that express wild-type FLT3 and the proliferation of cells driven by FLT3 activated through mutation.

69 •• Rombouts EJ, Pavic B, Lowenberg B, et al.: Relation between CXCR-4 expression, Flt3 mutations, and unfavorable prognosis of adult acute myeloid leukemia. Blood 2004, 104:550–557.
The proportion of $CD34^+$ hematopoietic progenitor cells that express the CXCR-4 chemokine receptor correlates significantly with a poor overall survival and relapse-free survival in AML patients. The expression of a FLT3-ITD activating mutation in AML specimens is associated with expression of CXCR-4, and this study suggests that expression of this chemokine receptor might underlie the poor prognosis of AML with FLT3-activating mutations.