Clinical Implications of *c-Kit* Mutations in Acute Myelogenous Leukemia

Muriel Malaise, PhD, Daniel Steinbach, MD, and Selim Corbacioglu, MD

Corresponding author
Selim Corbacioglu, MD
Department of Pediatrics, University of Ulm,
Eythstrasse 24, 89075 Ulm, Germany.
E-mail: selim.corbacioglu@uniklinik-ulm.de

Current Hematologic Malignancy Reports 2009, **4**:77–82
Current Medicine Group LLC ISSN 1558-8211

c-Kit is a receptor tyrosine kinase (RTK) with a pivotal role in melanogenesis, gametogenesis, and hematopoiesis. Aberrantly activated RTK and related downstream signaling partners were identified as key elements in the molecular pathogenesis of several malignancies. This finding culminated in a two-class model integrating constitutive activating and maturation arrest–inducing mutations as key elements for the pathogenesis of acute myelogenous leukemia (AML). c-Kit is expressed by myeloblasts in about 60% to 80% of patients, and the most frequently observed activating RTK mutations in AML (next to FLT3) are mutations or internal tandem duplications in *c-Kit*, with an overall incidence of 17%. The identification of small-molecule tyrosine kinase inhibitors capable of blocking key kinase switches introduced a paradigm change in the treatment of diseases like gastrointestinal stromal tumors and chronic myelogenous leukemia. Despite encouraging preclinical data, it appears that a complex clonal disease like AML will probably benefit from a synergistic approach of targeted drugs used (at least for now) in combination with conventional chemotherapy.

Introduction

Mutations in multiple genes and aberrant expression through epigenetic abnormalities are constant findings in most malignancies. A multistage mutational process is favored in cancer research, combining uninhibited growth (oncogenes) and dysregulation of growth inhibition (tumor suppressor genes) [1]. Recently Weinstein [2] coined the phrase "addiction to oncogenes," summarizing the observations of several investigators that maintenance of the malignant phenotype strictly depends on the presence of constitutive oncogenic stimuli. Inactivating these pivotal, disease-specific mutations leads to proliferation arrest, maturation, and apoptosis. Furthermore, the exquisite sensitivity of cancer cells to certain highly specific drugs indicates that our understanding of carcinogenesis as a multistep process (in which a phenotype is the sum of individual oncogenic effects) needs the addition of the apparent existence of a hierarchy of mutations with hypersensitive targets. Identification of these targets ("the Achilles heal of cancer") within the decoys of a variety of secondarily acquired mutations is crucial.

Receptor tyrosine kinases (RTKs) and their downstream effectors have been identified as key elements in the molecular pathogenesis of malignancies. Mutations at different levels of affected pathways lead to an aberrant deregulation starting at the receptor level, inducing a constitutively activated phenotype, sometimes through mechanisms of autocrine/paracrine stimulation or by activating or inhibiting downstream effectors such as signal transducers and activators of transcription (STATs), ras, and mammalian target of rapamycin (mTOR). RTK mutations have been identified in myeloid malignancies such as mastocytosis, myelodysplastic syndrome, and acute myelogenous leukemia (AML).

Functional analyses of AML generated a model of the pathogenesis of AML in which two classes of mutations were identified. Class I mutations are constitutively activated tyrosine kinase mutations, which lead through aberrant signal transduction to proliferation and survival. Class II mutations result in loss of function of transcription factors that are important for hematopoietic differentiation [3].

The only known activating mutations in RTK and related pathways in AML are mutations or internal tandem duplications (ITD) in *c-Kit* (17%) [4–6], mutations or ITD in FMS-like tyrosine kinase 3 (*FLT3*) (25%), and rare mutations observed in Janus kinases *JAK2* and *JAK3* as well as platelet-derived growth factor receptor (*PDGFR*). Furthermore, STATs and other cytoplasmic serine and tyrosine kinases, as well as related and unrelated signaling molecules, are aberrantly phosphorylated, suggesting additional mechanisms and sources of oncogenic activation that induce uninhibited proliferation, apoptosis, and radiation resistance [7].

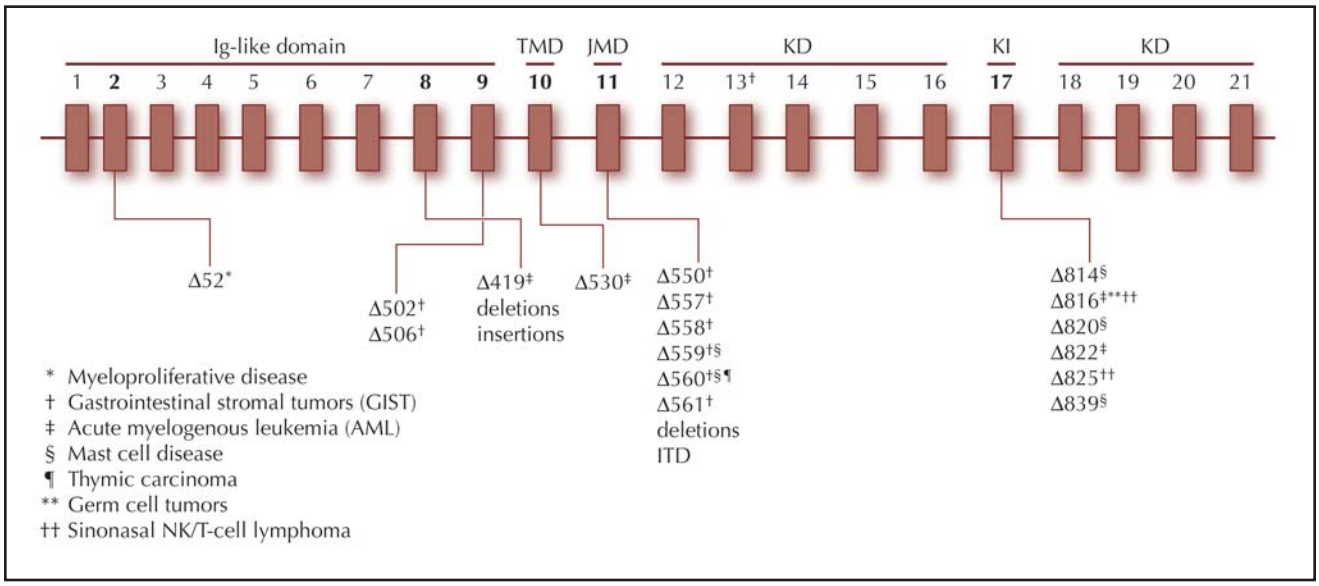

Figure 1. Molecular structure of c-kit, with the location of disease-associated *c-kit* mutations. ITD—internal tandem duplication; JMD—juxtamembrane domain; KD—kinase domain; KI—kinase insert; TMD—transmembrane domain.

Stem Cell Factor Receptor c-Kit

The proto-oncogene *c-kit*, also called stem cell factor receptor (SCFR) or CD117, was discovered and cloned more than 20 years ago. It encodes with 21 exons on chromosome 4q11-q12 the RTK c-Kit. The ligand for c-Kit is SCF or kit ligand (KL), which is encoded on chromosome 12q22-q24. c-Kit is a member of the class III RTK subfamily, structurally related to PDGFR, macrophage colony-stimulating factor receptor (M-CSFR) and FLT3. As illustrated in Figure 1, *c-kit* is characterized by five immunoglobulin-like domains, a single transmembrane helix, a cytoplasmic juxtamembrane domain (JMD), and a split kinase domain (KD) with a kinase insert (KI). This receptor ligand pair plays an elementary role in hematopoiesis, melanogenesis, gametogenesis, and cell proliferation, differentiation, survival, and migration.

c-Kit Signaling

Upon binding of SCF, c-Kit forms a homodimer, autophosphorylates tyrosine residues, and activates diverse signal transduction cascades. Phosphorylated tyrosine 567 (Tyr567) and tyrosine 569 (Tyr569) serve as docking sites for adapter proteins, tyrosine phosphatases SHP-1 and SHP-2, and Src family kinases. Tyrosine residue 721 (Tyr721) in the KI domain interacts with the regulatory subunit p85 of the phosphoinositide-3 kinase (PI3K). Activated c-Kit induces tyrosine phosphorylation of STATs, either via members of the JAK family or directly by Src family kinases.

c-Kit expression and activity is regulated by internalization, ubiquitination, and extracellular domain cleavage via proteinase C, and by the phosphatases SHP-1 and SHP-2.

Through diverse signal transduction pathways (including the JAK/STAT, PI3K/AKT/mTOR, and RAS/RAF/ERK/MAPK pathways) and tissue-specific regulation, c-Kit affects development and maintenance of several cell systems. Loss-of-function mutations of *c-Kit* lead to defects in hematopoiesis, germinal cell development, and pigmentation. Null mutations of *c-Kit* are embryonically lethal because of severe macrocytic anemia. More subtle mutations in humans produce the piebald trait (Mendelian Inheritance in Man [MIM] #172800), associated with pigmentation anomalies, deafness, and megacolon.

c-Kit Mutations and Their Implications in Diseases

Somatic mutations causing constitutive activation of c-Kit have been associated with a number of neoplastic conditions, including mast cell diseases, germ cell tumors, myeloproliferative diseases, gastrointestinal stromal tumors (GIST), and leukemias. Most mutations have been identified in relatively few exons, namely the extracellular exon 8, exon 11 encoding the JMD, and exon 17 encoding the KI. For example, the JMD mutations are responsible for most cases of GIST, with a reported prevalence of approximately 70%.

c-Kit is expressed by myeloblasts in approximately 60% to 80% of patients with AML [8]. The overall reported frequency of *c-Kit* mutations in adults and children with AML is 17% [9•,10,11], but it occurs in 52% of patients with core binding factor (CBF) AML [12]. In adult AML, the most common mutations involve exon 8 of the immunoglobulin-like domain (12%) and exon 17 of the KI domain (4%) [9•]. Mutations in the JMD of *c-kit* are rare in AML of adults, with only a few reported cases consisting of ITD. In childhood AML, the incidence of *c-Kit* mutations is about 18%: 4% involving exon 8, 7% involving exon 17, and 7% involving the JMD/KD, where

a complex ITD involving exon 11 and exon 12 was identified predominantly in CBF AML [11].

The functional consequence of all reported *c-Kit* mutations is a constitutive activation of the receptor, through either ligand-independent dimerization (exon 8) or activation of c-Kit monomers through conformational changes (exons 11 and 17), leading to aberrant signal transduction. The oncogenic potential of these single gene mutations was demonstrated in vitro in stably transfected cytokine-dependent cell lines, in which the expression of mutant *c-Kit* induced constitutive cytokine-independent growth, aberrant signaling, apoptosis, and radiation resistance. The exclusive expression of a constitutively activating mutant of *FLT3*, an RTK class III family member of *c-Kit*, induced a myeloproliferative phenotype in a knock-in mouse model [13••].

As well as contributing to the understanding of pathogenesis, RTK mutations in AML can be of prognostic significance. In adult CBF AML, *FLT3* mutations are associated with a poor prognosis [9•]. *c-Kit* exon 8 and exon 17 mutations correlate with an increased relapse rate [12] and poor prognosis [14]. In children, a poor prognosis was strongly correlated with *c-Kit* mutations rather than *FLT3* ITD [15]. c-Kit is highly conserved between species, with an up 100% amino acid sequence homology for certain exons. In addition to several mutations identified in AML, five single nucleotide polymorphisms (SNPs) of *c-Kit* are commonly accepted, only some of which lead to modifications of the amino acid sequence. Advani et al. [16•] reported that overexpression of wild-type *c-Kit* in AML is a poor prognostic factor for progression-free and overall survival in adults. Nevertheless, the way that *c-Kit* polymorphisms, aberrant expression, and epigenetics affect pathogenesis and prognosis of AML is still unclear.

Molecular Targeted Therapies

The substantial prevalence of constitutively activating genetic alterations of RTKs that seem to be crucial for the pathogenesis and maintenance of leukemia, together with the clinical impact of potential treatment options for mutations associated with poor outcome, has led to the development of promising new therapeutic strategies for AML.

Imatinib mesylate

The first molecular targeted drug developed with the understanding of these molecular mechanisms was imatinib mesylate (IM), also called STI571 or Gleevec (Novartis Pharmaceuticals, East Hanover, NJ). IM is a two-phenylaminopyrimidine tyrosine kinase inhibitor that binds to the adenosine triphosphate (ATP) binding site of inactive tyrosine kinases (ABL, PDGFR, and c-Kit). In this way, tyrosine autophosphorylation and consecutive downstream signaling and activation of aberrant pathways are blocked. IM induced impressive responses in several diseases. The excellent clinical results obtained in chronic myelogenous leukemia (CML) have completely changed the therapeutic approach to this disease; IM is now the gold standard for treatment of newly diagnosed CML. But mutations within the kinase domain of BCR-ABL1 represent the most frequent mechanism of resistance to IM, which achieves a partial response or stable disease in about 80% of patients with metastatic GIST. Most patients show disease progression at a median of 2 years because of drug resistance. Acquired resistance to IM in GIST commonly occurs via secondary gene mutations in the c-Kit kinase domain. Moreover, the primary sensitivity to IM depends on the presence of certain *c-Kit* mutations, in which, for example, mutant V560G is sensitive and D816V is resistant. Mahadevan et al. [17•] identified a novel mechanism of resistance by which an IM-resistant GIST cell line was developed from an IM-sensitive cell line by growing these cells in IM. In this in vitro GIST model, c-Kit was downregulated and replaced by AXL, another RTK. We observed a similar switch in an in vitro c-Kit ITD model of childhood AML. After continuous incubation with IM, resistance developed and a switch in the phosphorylation pattern of associated signaling partners was observed, together with downregulation of the primarily aberrant activation. IM response was re-established after treatment was paused (Malaise et al., unpublished data). These observations may be relevant for the design of therapeutic protocols.

Although the preclinical data were encouraging, the rate of clinical response to IM in AML could not confirm the unique pathogenetic relevance of *c-Kit* mutations as observed in GIST. Kindler et al. [18] used IM to treat patients with wild-type c-Kit–positive AML and observed only minor reductions in the peripheral blast count. Several studies using IM in AML patients carrying *c-Kit* mutations showed mostly minor activity [19–21].

Other kinase inhibitors

Primary or secondary resistance to IM observed in the treatment of GIST and CML propelled the development of novel RTK inhibitors. SU5416 is a multi-RTK inhibitor including activity against c-kit. In patients with refractory AML, only a partial response was observed [22,23].

Dasatinib (BMS-354825) is a potent dual Src/Abl tyrosine kinase inhibitor with activity in IM-resistant *c-Kit* mutants and patients with refractory AML [24,25]. Sorafenib is a multikinase inhibitor that has shown in vitro activity against cells carrying *c-Kit* mutations [26] and activity in AML in vivo [27,28]; it is awaiting clinical testing in patients with mutant *c-Kit*–positive AML.

Another potential substitute for IM in the treatment of AML is sunitinib (SU11248), a multitargeted kinase inhibitor with in vitro activity against AML cells expressing mutant *c-Kit* [26]. Sunitinib induced a partial remission of short duration in 15 patients with refractory AML [29]. Other RTK inhibitors, such as nilotinib, PKC412, EXEL-0862, and OSI-930, demonstrated activity against a variety of *c-Kit* mutations. Some are being tested in clinical phase 2 trials or are being evaluated in GIST and await further analysis in AML.

In the search for the best kinase inhibitors to treat AML, it must be taken into account that these substances can be affected by multidrug resistance caused by drug efflux pumps. It was recently shown that IM is a substrate for permeability glycoprotein (P-gp) [30] and for the breast cancer resistance protein (BCRP) [31]. Both drug efflux pumps are associated with resistance to cytostatic drugs in adult AML. In pediatric AML, this resistance occurs only with BCRP [32].

Other ATP-binding cassette (ABC) transporters that cause clinically relevant drug resistance in AML are ABCC3 [33] and ABCA3 [34]. The ability of these transporters to efflux kinase inhibitors remains to be investigated.

Combination Therapy and Synergism

The successes achieved with molecular targeted therapies for the treatment of chronic-phase CML and GIST encouraged the development of therapies targeting molecular abnormalities central to the pathogenesis of AML. Such therapies seem more effective—as well as more selective and therefore less toxic—than conventional chemotherapy and stem cell transplantation. Nevertheless, the swift emergence of drug-resistant mutations in CML and GIST is evidence enough that combination therapy directed to complementary targets, including conventional chemotherapy, is still required. Several recent publications have shown that combination therapy is surprisingly effective.

Conventional chemotherapy like arabinosylcytosine (cytarabine, ara-C) and cladribine were combined with IM, PKC412, and nilotinib and demonstrated synergism in vitro [35,36] and in a phase 1 study of the CLAG regimen (cladribine, ara-C, and granulocyte colony-stimulating factor) with escalating doses of IM in relapsed, refractory AML. Of 15 patients with AML and 1 with CML, 4 (25%) achieved a complete morphologic response with normal cytogenetics, 2 (12.5%) achieved a complete morphologic response only, and 1 had a complete response in the bone marrow but incomplete blood count recovery. The overall response rate was 43.8% [37].

Beyond a combination with conventional chemotherapy, other strategies that may circumvent resistance target several signaling pathways or consecutive steps of one activated pathway. Bradeen et al. [38] showed that dual combinations of IM, dasatinib, and nilotinib acted synergistically in BCR-ABL–expressing cell lines.

Point mutations of the guanosine triphosphate (GTP)-ase oncogene *Ras* occur in about 20% of patients with AML. The Ras/RAF/MAPK/ERK pathway also is downstream and is frequently involved in constitutive activation of RTK, including c-Kit and FLT3. Because Ras activation depends on posttranslational farnesylation, farnesyltransferase inhibitors (FTIs) have been investigated as a potential Ras-targeted therapy for AML. In a phase 2 study in patients with refractory or relapsed AML, the oral FTI tipifarnib prolonged survival [39] and a Children's Oncology Group phase 1 trial for children with refractory leukemias was completed successfully, demonstrating tolerability with inhibitory concentrations [40]. Furthermore, inhibition of MEK/ERK signaling synergistically potentiates histone deacetylase inhibitor–induced growth arrest and apoptosis in AML in vitro [41].

Another exquisite target for combination therapy is mammalian target of rapamycin (mTOR). mTOR is a highly conserved serine/threonine kinase with significant homology to the catalytic domain of phosphoinositide-3 kinase (PI3K). mTOR is a pivotal hub in multiple signal transduction pathways, including PI3K/AKT. The PI3K/AKT/mTOR pathway is commonly deregulated in cancer and contributes to cellular processes that are important for the formation and progression of cancer, including apoptosis, transcription, translation, metabolism, angiogenesis, and cell cycle progression. In AML, this pathway is aberrantly activated in more than 70% of cases [42,43]. mTOR forms two complexes, mTOR complex 1 (mTORC1) and mTORC2. The mTORC1 complex, defined by the binding of regulatory associated protein of mTOR (RAPTOR), is regulated mainly by nutrients and growth factors upstream of PI3K/AKT. mTORC2 was identified more recently and differs from mTORC1 through complex formation with the rapamycin-insensitive companion of mTOR (RICTOR). This complex is insensitive to nutrients [44–46].

Rapamycin, originally developed as an antifungal agent in the 1970s, is the most selective targeted drug available. So far, the only identified direct target of rapamycin is mTORC1, but to fully appreciate the potential antileukemic potential of rapamycin, one aspect of mTOR signaling needs to be elucidated. Maximal activation of AKT requires the phosphorylation of two amino acid residues: Thr308 and Ser473. Although Thr308 is phosphorylated by phosphoinositide-dependent kinase 1 (PDK1), Ser473 is phosphorylated by the mTORC2 complex [47]. It was recently demonstrated that rapamycin blocked the full activation of AKT, possibly through the inhibition of the mTORC2 complex formation via substrate depletion of mTOR [48•]. This second mechanism of inhibitory activity of rapamycin was confirmed in human AML samples [49].

Next to its established role as a potent immunosuppressant, rapamycin also acts as a cytostatic, inducing apoptosis and cell cycle arrest in G1. In vitro studies have revealed potent growth arrest in several cancer cells, such as neuroblastoma, glioblastoma, and leukemia [50]. Rapamycin suppressed tumor growth of human and murine tumor models in vivo and showed antileukemic effects in IM-resistant CML in vitro and in vivo [51]. Several studies demonstrated that a combination of rapamycin with either conventional chemotherapy [52] or other targeted drugs [53] had a synergistic effect in leukemia models. In 32D cells expressing a c-Kit ITD derived from childhood AML, a combination of IM with rapamycin induced cell cycle arrest, apoptosis, and sensitization for radiation-induced damage [14].

Conclusions

c-Kit mutations are frequently observed in adult and childhood AML. Yet unidentified *c-Kit* mutations and clinically relevant epigenetic modifications may augment the relevance of targeting c-Kit in AML.

The role of *c-Kit* mutations as prognostic factors needs to be elucidated in a larger patient population, but it seems that these mutations (at least in children) play a pivotal role as markers of poor prognosis.

Although AML is not a myeloproliferative disorder like CML, but rather the consequence of clonal evolution, c-Kit seems to play a central role in the pathogenesis of leukemia, similar to observations made in GIST. There is evidence that targeting molecular abnormalities such as c-Kit can be more effective and less toxic than standard chemotherapy approaches. Because of the complexity of cancer mechanisms, however, along with the observed development of resistance in similar disease entities and the synergy acting on receptor and downstream molecules, the most powerful therapy is likely to consist of a combination of several targeted drugs, together (at least for the time being) with conventional chemotherapy.

Furthermore, it is likely that targeted drugs will force us to modify our current concepts of clinical trials. Adaptive and more flexible trial designs are needed for therapeutic trials in molecularly defined subgroups of patients.

Disclosure

No potential conflicts of interest relevant to this article were reported.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Kinzler KW, Vogelstein B: **Cancer-susceptibility genes. Gatekeepers and caretakers.** *Nature* 1997, **386:**761,763.
2. Weinstein IB: **Cancer. Addiction to oncogenes—the Achilles heal of cancer.** *Science* 2002, **297:**63–64.
3. Kelly LM, Gilliland DG: **Genetics of myeloid leukemias.** *Annu Rev Genomics Hum Genet* 2002, **3:**179–198.
4. Gari M, Goodeve A, Wilson G, et al.: **c-kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia.** *Br J Haematol* 1999, **105:**894–900.
5. Beghini A, Peterlongo P, Ripamonti CB, et al.: **C-kit mutations in core binding factor leukemias.** *Blood* 2000, **95:**726–727.
6. Wang YY, Zhou GB, Yin T, et al.: **AML1-ETO and C-KIT mutation/overexpression in t(8;21) leukemia: implication in stepwise leukemogenesis and response to Gleevec.** *Proc Natl Acad Sci U S A* 2005, **102:**1104–1109.
7. Tyner JW, Walters DK, Willis SG, et al.: **RNAi screening of the tyrosine kinome identifies therapeutic targets in acute myeloid leukemia.** *Blood* 2008, **111:**2238–2245.
8. Ikeda H, Kanakura Y, Tamaki T, et al.: **Expression and functional role of the proto-oncogene c-kit in acute myeloblastic leukemia cells.** *Blood* 1991, **78:**2962–2968.
9. • Boissel N, Leroy H, Brethon B, et al.: **Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML).** *Leukemia* 2006, **20:**965–970.

c-Kit mutations are identified as poor prognostic markers in a large (n = 103) pediatric and adult patient population.

10. Goemans BF, Zwaan C, Miller M, et al.: **Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia.** *Leukemia* 2005, **19:**1536–1542.
11. Corbacioglu S, Kilic M, Westhoff MA, et al.: **Newly identified c-kit receptor tyrosine kinase ITD in childhood AML induces ligand independent growth and is responsive to a synergistic effect of imatinib and rapamycin.** *Blood* 2006, **108:**3504–3513.
12. Paschka P, Marcucci G, Ruppert AS, et al.: **Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study.** *J Clin Oncol* 2006, **24:**3904–3911.
13. •• Li L, Piloto O, Nguyen HB, et al.: **Knock-in of an internal tandem duplication mutation into murine FLT3 confers myeloproliferative disease in a mouse model.** *Blood* 2008, **111:**3849–3858.

This elegant knock-in mouse model demonstrates the unique potential of a single constitutively activated RTK.

14. Schnittger S, Kohl TM, Haferlach T, et al.: **KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival.** *Blood* 2006, **107:**1791–1799.
15. Shimada A, Taki T, Tabuchi K, et al.: **KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group.** *Blood* 2006, **107:**1806–1809.
16. • Advani AS, Rodriguez C, Jin T, et al.: **Increased C-kit intensity is a poor prognostic factor for progression-free and overall survival in patients with newly diagnosed AML.** *Leuk Res* 2008, **32:**913–918.

In patients with newly diagnosed AML in a multivariate analysis, the mean fluorescent index (MFI) as a measure of c-Kit expression correlated strongly with decreased progression-free survival and overall survival.

17. • Mahadevan D, Cooke L, Riley C, et al.: **A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors.** *Oncogene* 2007, **26:**3909–3919.

This report identifies a potentially novel mechanism for the development of resistance in primarily IM-sensitive GIST through a kinase switch to the RTK AXL.

18. Kindler T, Breitenbuecher F, Marx A, et al.: **Efficacy and safety of imatinib in adult patients with c-kit-positive acute myeloid leukemia.** *Blood* 2004, **103:**3644–3654.
19. Cortes J, Giles F, O'Brien S, et al.: **Results of imatinib mesylate therapy in patients with refractory or recurrent acute myeloid leukemia, high-risk myelodysplastic syndrome, and myeloproliferative disorders.** *Cancer* 2003, **97:**2760–2766.
20. Schittenhelm M, Aichele O, Krober SM, et al.: **Complete remission of third recurrence of acute myeloid leukemia after treatment with imatinib (STI-571).** *Leuk Lymphoma* 2003, **44:**1251–1253.
21. Cairoli R, Beghini A, Morello E, et al.: **Imatinib mesylate in the treatment of Core Binding Factor leukemias with KIT mutations. A report of three cases.** *Leuk Res* 2005, **29:**397–400.
22. 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.
23. 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.

24. Schittenhelm MM, Shiraga S, Schroeder A, et al.: **Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies.** *Cancer Res* 2006, **66:**473–481.
25. Kolb EA, Gorlick R, Houghton PJ, et al.: **Initial testing of dasatinib by the pediatric preclinical testing program.** *Pediatr Blood Cancer* 2008, **50:**1198–1206.
26. Hu S, Niu H, Minkin P, et al.: **Comparison of antitumor effects of multitargeted tyrosine kinase inhibitors in acute myelogenous leukemia.** *Mol Cancer Ther* 2008, **7:**1110–1120.
27. Zhang W, Konopleva M, Shi YX, et al.: **Mutant FLT3: a direct target of sorafenib in acute myelogenous leukemia.** *J Natl Cancer Inst* 2008, **100:**184–198.
28. Safaian NN, Czibere A, Bruns I, et al.: **Sorafenib (Nexavar®) induces molecular remission and regression of extramedullary disease in a patient with FLT3-ITD(+) acute myeloid leukemia.** *Leuk Res* 2009, **33:**349–350.
29. Fiedler W, Serve H, Dohner H, et al.: **A phase 1 study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease.** *Blood* 2005, **105:**986–993.
30. Dulucq S, Bouchet S, Turcq B, et al.: **Multidrug resistance gene (MDR1) polymorphisms are associated with major molecular responses to standard-dose imatinib in chronic myeloid leukemia.** *Blood* 2008, **112:**2024–2027.
31. Burger H, van Tol H, Boersma AW, et al.: **Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump.** *Blood* 2004, **104:**2940–2942.
32. Steinbach D, Legrand O: **ABC transporters and drug resistance in leukemia: Was P-gp nothing but the first head of the Hydra?** *Leukemia* 2007, **21:**1172–1176.
33. Steinbach D, Lengemann J, Voigt A, et al.: **Response to chemotherapy and expression of the genes encoding the multidrug resistance-associated proteins MRP2, MRP3, MRP4, MRP5, and SMRP in childhood acute myeloid leukemia.** *Clin Cancer Res* 2003, **9:**1083–1086.
34. Chapuy B, Koch R, Radunski U, et al.: **Intracellular ABC transporter A3 confers multidrug resistance in leukemia cells by lysosomal drug sequestration.** *Leukemia* 2008, **22:**1576–1586.
35. Heidel F, Cortes J, Rucker FG, et al.: **Results of a multicenter phase II trial for older patients with c-Kit-positive acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (HR-MDS) using low-dose Ara-C and imatinib.** *Cancer* 2007, **109:**907–914.
36. Gleixner KV, Mayerhofer M, Aichberger KJ, et al.: **PKC412 inhibits in vitro growth of neoplastic human mast cells expressing the D816V-mutated variant of KIT: comparison with AMN107, imatinib, and cladribine (2CdA) and evaluation of cooperative drug effects.** *Blood* 2006, **107:**752–759.
37. Walker AR, Komrokji RS, Ifthikharuddin J, et al.: **Phase I study of cladribine, cytarabine (Ara-C), granulocyte colony stimulating factor (G-CSF) (CLAG Regimen) and simultaneous escalating doses of imatinib mesylate (Gleevec) in relapsed/refractory AML.** *Leuk Res* 2008, **32:**1830–1836.
38. Bradeen HA, Eide CA, O'Hare T, et al.: **Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations.** *Blood* 2006, **108:**2332–2338.
39. Harousseau JL, Lancet JE, Reiffers J, et al.: **A phase 2 study of the oral farnesyltransferase inhibitor tipifarnib in patients with refractory or relapsed acute myeloid leukemia.** *Blood* 2007, **109:**5151–5156.
40. Widemann BC, Salzer WL, Arceci RJ, et al.: **Phase I trial and pharmacokinetic study of the farnesyltransferase inhibitor tipifarnib in children with refractory solid tumors or neurofibromatosis type I and plexiform neurofibromas.** *J Clin Oncol* 2006, **24:**507–516.
41. Nishioka C, Ikezoe T, Yang J, et al.: **Inhibition of MEK/ERK signaling synergistically potentiates histone deacetylase inhibitor-induced growth arrest, apoptosis and acetylation of histone H3 on p21waf1 promoter in acute myelogenous leukemia cell.** *Leukemia* 2008, **22:**1449–1452.
42. Grandage VL, Gale RE, Linch DC, Khwaja A: **PI3-kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF-kappaB, Mapkinase and p53 pathways.** *Leukemia* 2005, **19:**586–594.
43. Min YH, Eom JI, Cheong JW, et al.: **Constitutive phosphorylation of Akt/PKB protein in acute myeloid leukemia: its significance as a prognostic variable.** *Leukemia* 2003, **17:**995–997.
44. Wullschleger S, Loewith R, Hall MN: **TOR signaling in growth and metabolism.** *Cell* 2006, **124:**471–484.
45. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM: **Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex.** *Science* 2005, **307:**1098–1101.
46. Guertin DA, Sabatini DM: **Defining the role of mTOR in cancer.** *Cancer Cell* 2007, **12:**9–22.
47. Sarbassov DD, Ali SM, Kim DH, et al.: **Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton.** *Curr Biol* 2004, **14:**1296–1302.
48.• Sarbassov DD, Ali SM, Sengupta S, et al.: **Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB.** *Mol Cell* 2006, **22:**159–168.

The first in vitro evidence that rapamycin has a dual inhibitory capacity through an unforeseen mechanism of action suggests that rapamycin can be used to inhibit Akt/PKB as well as the mTORC1 complex.

49. Zeng Z, Sarbassov dos D, Samudio IJ, et al.: **Rapamycin derivatives reduce mTORC2 signaling and inhibit AKT activation in AML.** *Blood* 2007, **109:**3509–3512.
50. Huang S, Bjornsti MA, Houghton PJ: **Rapamycins: mechanism of action and cellular resistance.** *Cancer Biol Ther* 2003, **2:**222–232.
51. Sillaber C, Mayerhofer M, Bohm A, et al.: **Evaluation of antileukaemic effects of rapamycin in patients with imatinib-resistant chronic myeloid leukaemia.** *Eur J Clin Invest* 2008, **38:**43–52.
52. Calabro A, Tai J, Allen SL, Budman DR: **In-vitro synergism of m-TOR inhibitors, statins, and classical chemotherapy: potential implications in acute leukemia.** *Anticancer Drugs* 2008, **19:**705–712.
53. 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 U S A* 2004, **101:**3130–3135.