Receptor Tyrosine Kinase Alterations in AML – Biology and Therapy

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Abstract Acute myeloid leukemia (AML) is the most common form of leukemia in adults, and despite some recent progress in understanding the biology of the disease, AML remains the leading cause of leukemia-related deaths in adults and children. AML is a complex and heterogeneous disease, often involving multiple genetic defects that promote leukemic transformation and drug resistance. The cooperativity model suggests that an initial genetic event leads to maturational arrest in a myeloid progenitor cell, and subsequent genetic events induce proliferation and block apoptosis. Together, these genetic abnormalities lead to clonal expansion and frank leukemia. The purpose of this chapter is to review the biology of receptor tyrosine kinases (RTKs) in AML, exploring how RTKs are being used as novel prognostic factors and potential therapeutic targets.

Introduction

Acute myeloid leukemia (AML) is the most common form of leukemia in adults, and despite some recent progress in understanding the biology of the disease, AML remains the leading cause of leukemia-related deaths in adults and children [132] AML is a complex and heterogeneous disease, often involving multiple genetic defects that promote leukemic transformation and drug resistance. The co-operativity model suggests that an initial genetic event leads to maturational arrest in a myeloid progenitor cell, and subsequent genetic events induce proliferation and block apoptosis. Together, these genetic abnormalities lead to clonal expansion and frank leukemia [64, 170, 127, 176].

Mutations in receptor tyrosine kinases (RTKs) or their downstream effectors are extremely common in AML, with estimated 40–60% of AML patients

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harboring a mutation abnormality in RTKs [144, 89, 45, 49]. In addition, another 15–25% of AML patients will have a mutation in one of the downstream effectors in RTK pathways [144, 168, 113, 69, 27]. More than 50 different RTKs have been identified, which are classified into 20 subfamilies based on their structural and functional characteristics (reviewed in Ref. [89]) [14, 38, 120]. A meticulous examination of all the different RTKs has yet to be performed, but such studies are underway. These studies will most likely discover that an even greater percentage of AML patients harbor mutations or abnormalities in RTK pathways. Although several RTKs have been implicated in malignancies, the vast majority of RTK mutations in AML, thus far, have been found in the RTK subclass III family (a.k.a. the PDGFR family) [120, 3, 124]. The subclass III RTK family consists of FLT3, KIT, PDGFRA, PDGFRB, and CSF1R, and the majority of this review will examine the biology, prognosis, and potential therapeutic targets of these RTKs, focusing on FLT3 and KIT mutations, which are by far the most common RTKs known to be affected in AML. The role of CSFR1 (a.k.a. C-FMS) and PDGFR in myeloid malignancies will also be briefly discussed. In addition, we will assess the growing interest in small molecule inhibitors against RTKs as potential therapeutic targets for AML.

Receptor Tyrosine Kinase Activation and Downstream Effectors

RTKs play a critical role in myeloid proliferation, differentiation, and apoptosis [115, 167, 126, 44, 133, 97, 102, 99, 93]. Structurally, subclass III RTKs consist of an extracellular ligand-binding (E) domain, transmembrane (TM) dimerization domain, juxtamembrane (JM) domain, and an intracellular tyrosine kinase domain (Fig. 1A). In their inactive state, RTKs exist primarily as monomers, and multiple autoinhibitory or intrinsic repressive forces prevent dimerization (a.k.a. receptor activation) [52, 123]. While in the monomeric state, the RTK displays a "closed" conformation, which prevents easy phosphorylation of specific tyrosine residues within the intercellular domains and limits inappropriate activation (Fig. 1A). Activation begins when a ligand binds to the extracellular domain (or domains), causing a conformational change in the receptor. The new conformation reverses the intrinsic repulsive forces of the receptor, promoting dimerization with either itself or other membrane-bound RTKs (Fig. 1B). Together, these changes lead to an "open" confirmation of the receptor, which facilitates the transfer of a phosphate from ATP to the tyrosine substrate within the intracellular kinase domain (Fig. 1B). This activated conformational change and/or phosphorylated tyrosine also promote the docking of adaptor proteins (e.g., SHC), which also become activated. The activated adapter proteins then interact with downstream effectors, efficiently transmitting the extracellular signal to the appropriate intracellular pathways (Fig. 2). After activation, RTKs are rapidly internalized and degraded, such that within 20 min the signal will start to dissipate [178, 163]. This rapid degradation and

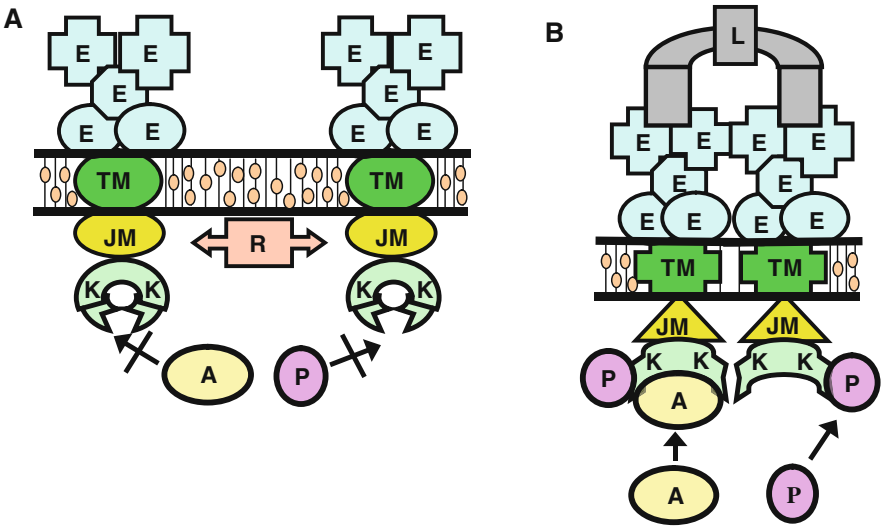

Fig. 1 Subclass III tyrosine kinase receptor structure and activation. (A) Inactive receptor. Subclass III RTKs consist of an extracellular ligand-binding domain (E), transmembrane dimerization domain (TM), juxtamembrane domain (JM), and an intracellular tyrosine kinase domain (K). In their inactive state, RTKs exist primarily as monomers, and multiple autoinhibitory or intrinsic repressive forces prevent dimerization (R). Note the "closed" conformation or the tyrosine kinase domain. (B) Activated receptor. RTKs become activated when a ligand (L) binds to their extracellular domain (E) causing a conformational change in the receptor. These changes lead to an "open" confirmation of the intracellular tyrosine kinase domain (K), which facilitates the transfer of phosphate (P) to the tyrosine kinase domain. This activated conformational change and/or phosphorylated tyrosine also promote the docking of adaptor proteins (A), which are then activated. The activated adapter proteins facilitate transmission of the extracellular signal to the appropriate intracellular pathways

downregulation of the receptor contributes to the tightly controlled signaling activity of RTKs.

Several different types of mutations (missense point mutations, deletions, insertions, and internal tandem duplications) in AML cells have been described in the different RTKs (mainly FLT3 and c-KIT, Fig. 3) [144, 89, 45, 49, 69, 101, 10]. Despite the different types of mutations, the RTK sequences tend to remain in frame, ensuring translation of the entire protein. These mutations have been found to be most frequent in JM domain or tyrosine kinase domain and will be described in more detail under the specific subclass RTKs; nevertheless, there are two general themes to these mutations. Those mutations involving the JM domain tend to be large insertions, which probably disrupt the intrinsic repulsive forces that naturally prevent dimerization. However, recently, small point mutations and deletions have been described in the JM domain of FLT3 [146]. Once these repulsive forces are disrupted, ligand-independent activation occurs. Whether the different types and sizes of

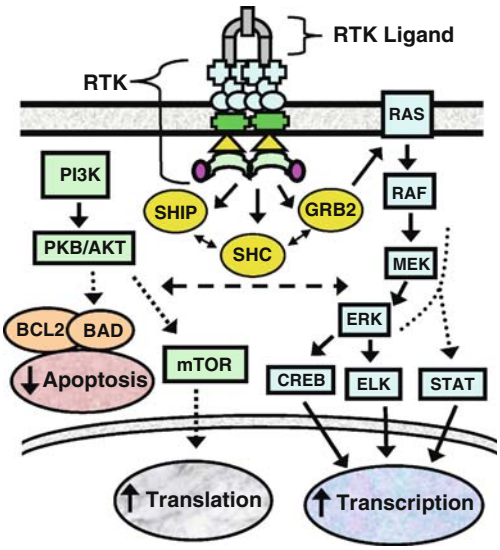

Fig. 2 Tyrosine kinase receptor pathway. Binding of a ligand (L) to tyrosine kinase receptor activates multiple downstream effectors, including the PI3K (phosphatidylinositol 3' kinase) and RAS pathways. *Solid* arrows are more direct interactions, while *broken* arrows represent associations that probably involve multiple steps between the proteins. The activated RTK interacts with multiple adapter proteins: SH2-containing sequence proteins (SHC), SH2-containing inositol phosphatase (SHIP), GRB2, and others, which connect the RTK to the PI3K and RAS pathways. RAS activation stimulates the MAPK kinase pathway: RAF, MAPK/ERK kinases (MEK), extracellular-signal-regulated kinase kinases (ERK), and 90-kDa ribosomal protein S6 kinase (RSK). These downstream effectors activate cyclic adenosine monophosphate-response element-binding protein (CREB), ELK, and signal transducer and activators of transcription (STATs), which lead to transcription of specific genes that promote proliferation. Activated PI3K stimulates protein kinase B (PKB/AKT) and other members of the PI3K pathway (e.g., rapamycin or mTOR), which promotes translation. In addition, activated PI3K induces phosphorylation of the pro-apoptotic BCL2 family protein (BAD), which blocks apoptosis by binding BCL2. Both pathways probably also interact with each other and other pathways/effectors such as BRCA1, p21WAF1, and p27KIP1

mutations in the JM domain have a unique biological and clinical significance is currently being investigated [145, 116]. The other domain of RTKs that is frequently mutated is that of the tyrosine kinase domain (or activation loop domain) [175, 1, 160, 42, 140, 67]. Missense point mutations in the tyrosine kinase domain (a.k.a. TKD mutations or FLT3/ALM) also constitutively activate the receptor. Similar to JM mutations, TKD mutations also activate downstream effectors, inappropriately stimulating pathways that are critical in the normal regulation of differentiation, proliferation, and apoptosis [175, 1].

In addition to RTK mutations, mutations in downstream effectors within RTK pathways may also play a critical role in leukemogenesis. For example, RAS, a component of many RTK pathways, is mutated in approximately

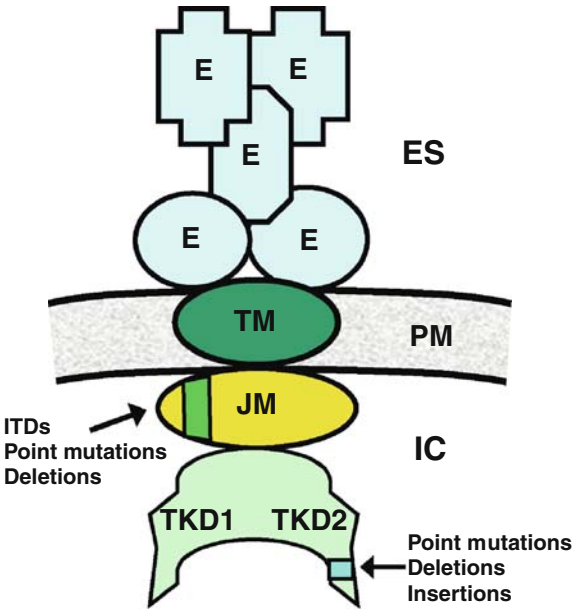

Fig. 3 Mutations in tyrosine kinase receptor. Several different types of mutations have been described in tyrosine kinase receptors. The vast majority of these mutations are either in the juxtamembrane (JM) domain or in the tyrosine kinase domain (TKD). Internal tandem duplications (*bright green*) are the most common type of mutations in JM domain (*star*), while point mutations (*bright blue*) are the most common type of mutations in the TKD. Other labels include the extracellular (E) and transmembrane (TM) domains, plasma membrane (PM), extracellular space (ES), and intracellular cytosol (IC)

20–25% of AML patients – usually either NRAS or KRAS [144, 89, 113, 35, 26, 104, 84, 17, 22, 119], and mutations in other members of the RTK pathways, such as PTNP11, have also been discovered in AML patients [27, 119, 138, 12]. Besides activating the RTK pathway, RTK mutations inappropriately activate other pathways such as JAK/STAT pathway [151, 54, 159, 13]. Recently, the JAK/STAT pathway has received attention due to novel small molecule inhibitors that make it a potential therapeutic target for several malignancies [95, 171, 174]. Whether it is the constitutive activation of the receptor by an RTK mutation [144, 89, 45, 49, 69, 101, 74, 2, 152, 130, 146, 175, 1, 19, 21, 9, 11, 24, 134, 129, 10, 160, 42, 140, 67, 143, 163, 117, 109], an autocrine/paracrine stimulation of the receptor by a ligand secreting tumor (e.g., VEGF) [161, 4, 48], or the activation of the downstream effectors [27, 119, 138, 12, 151, 54, 159, 13, 181, 96, 15, 76], inappropriate activation of RTK pathways directly contribute to pathogenesis of AML, progression of the disease, and its resistance to chemotherapy.

FLT3 Mutations

As FLT3 mutations have been implicated in the prognosis of AML, FLT3 mutations remain one of the most common genetic abnormalities in AML identified thus far [144, 89, 69, 101, 74, 2, 152, 130, 146, 175, 1, 145, 22, 157, 179, 59, 73, 37, 41]. As described above, FLT3 mutations occur within two specific regions within the FLT3 gene (juxtamembrane domain and tyrosine kinase domain). The most common type of a FLT3 mutation is that of internal tandem duplication (FLT3/ITD) in the JM domain, which occurs in 15–35% of AML patients [144, 89, 69, 74, 152, 130, 75, 90]. FLT3/ITDs are rare in infant AML, where only approximately 1% of children <1 year harbor a FLT3/ITD, but steadily increases with aging, such that 10–15% of pediatric and 20–35% of adult AML patients have FLT3/ITDs [144, 89, 69, 74, 152, 130, 75, 90]. FLT3/ITDs cause ligand-independent dimerization and autophosphorylation of the receptor, leading to constitutive activation of the FLT3 and many downstream effectors (SHC, RAS, ERK, AKT, and STAT5) [151, 54, 88, 71, 70, 94]. Besides FLT3/ITDs, smaller insertions, deletions, and missense point mutations have recently been described in the JM of AML patients [146]. These mutations are relatively rare, occurring in less than 5% of patients [146]. Although the clinical significance of these "non-ITD" mutations in the JM (a.k.a. FLT3-JM-PM) are currently unknown, Reindl et al. recently found that FLT3-JM-PMs promoted ligand-independent dimerization, autophosphorylation, and constitutive activation of the receptor; however, the activation seemed to be "weaker" than compared to classic FLT3/ITD transduced cells, as indicated by a lower level of phosphorylation of the receptor and less activation of downstream STAT5 in cells transduced with FLT3-JM-PM [116].

FLT3/ITDs are associated with rapid disease progression and resistance to conventional therapy [69, 74, 152, 130, 75]. Initial studies demonstrated a strong prognostic significance for the presence of FLT3/ITD, such that patients with this mutation had an extremely poor clinical outcome compared to the patients without FLT3/ITD [69, 75]. In more recent studies using contemporary chemotherapy, the prognostic significance of FLT3/ITD has been less dramatic, with an overall survival of approximately 30% for the FLT3/ITD population compared to 45% to that of patients without FLT3/ITD [74, 152]. However, these studies also identified a subclass of patients with FTL3/ITD, in which the mutant ITD to wild-type allele (ITD allelic ratio or ITD-AR) correlates with clinical outcome. ITD-ARs vary significantly from patient to patient, and this difference may have clinical implications [152, 18, 173]. For example, some AML patients have a predominantly mutant ITD product with little or no normal product (high ITD-AR), whereas others have an equal or higher distribution of normal product (low ITD-AR). The ITD-AR has been used to identify patients with FLT3/ITD at a higher risk of relapse and poor outcome in a number of clinical trials [152, 18, 173]. Although ITD-AR may become a critical tool in the risk identification of FLT3/ITD-positive patients, the exact

ITD-AR threshold "cut-off" that identifies high-risk patients has not been established. Thiede et al. used ITD-AR threshold of 0.78 to define relapse risk in FLT3/ITD-positive patients [152]. Patients with low allelic ratio (AR $\leq$ 0.78) had an overall survival of nearly 60% compared to overall survival of 0% in patients with ITD-AR of >0.78 ($p = 0.006$) [152]. They also suggested that ITD allelic ratio may be a continuous variable, as changing the ITD-AR cut-off altered the clinical outcome. Similar findings were shown in a pediatric AML population from European cooperative studies. Using the ITD-AR median, Zwaan et al. demonstrated that patients with high ITD-AR (ITD-AR > median of 0.69) had a poor outcome, whereas the outcome for those with low ITD-AR (ITD-AR $\leq$ 0.69) was no different than patients without FLT3/ITD [173]. More recent analysis of ITD-AR in a cohort of 630 children treated in CCG-2941/2961 has revealed that ITD-AR of 0.4 can identify the highest proportion of FLT3/ITD-positive patients at high risk of relapse (personal communication by Meshinchi, submitted for publication). Underlying mechanisms for the allelic ratio variation is under study. We and others have presented data in support of loss of heterozygosity (LOH) in chromosome 13q12 as a possible mechanism for high ITD-AR in some of the AML patients [75, 18]. Other studies, however, have failed to demonstrate LOH in patients with high ITD-AR [152], suggesting that there may be additional factors responsible for variation in the ITD-AR.

Recently, we demonstrated that size of duplicated region in FLT3/ITD-positive AML may have prognostic significance. In a study of adult 151 AML patients treated, those AML patients with larger ITDs had a significantly higher relapse compared to those with smaller ITDs [145]. Ongoing studies in other populations are underway, but this data would reinforce the hypothesis that different sizes and types of mutations in the JM may have unique biological and clinical significance, adding yet another layer of complexity to the use of FLT3/ITDs as a prognostic marker [145, 116, 182].

FLT3 mutations in activation loop of the tyrosine kinase (FLT3/ALM) occur in approximately 5–10% of AML patients [89, 175, 1, 140] making FLT3/ALM the second most common type of FLT3 mutation. Like FLT3/ITDs, FLT3/ALM constitutively activates the FLT3 receptor and downstream effectors [175, 140, 67, 151, 54, 13, 73, 88, 71]. However, it remains to be determined whether FLT3/ITDs and FLT3/ALMs have similar biological consequences. Recent evidence would suggest that mutations in JM and TKD cause biologically different responses. When Grundler et al. transduced mice marrow with FLT3/ITDs, the mice developed the classic myeloproliferative syndrome, which had previously been described in other FLT3/ITD transduction murine models [147, 53]. However, mice transduced with FLT3/ALM developed an oligoclonal lymphoid disorder [147]. These data argue against the hypothesis that the biological consequences of FLT3/ITDs and FLT3/ALMs are the same. In addition, global RNA expression studies have found distinct expression patterns between FLT3/ITDs and FLT3/ALMs, which readily differentiate the two types of mutations, providing additional evidence

that suggest biological differences between the two types of FLT3-activating mutations [65]. When one examines the clinical significance of FLT3/ALM, there appear to be clinical differences between patients with FLT3/ITDs and FLT3/AMLs. Unlike FLT3/ITDs, the frequency of FLT3/ALMs does not vary with age. In addition, available data suggest that patients with FLT3/ALM have a lower diagnostic white count, higher remission rates, lower relapse rate, and better overall survival than patients with FLT3/ITD [89, 152, 175, 78]. However, it must be noted that the frequency of FLT3/ALMs is considerably less than FLT3/ITDs, making it more difficult to obtain significant power to convincingly rule out a possible clinical significance for these mutations. Also, there have been fewer studies examining the clinical significance of FLT3/ALMs, since these mutations were first identified in 2001, which is approximately 5 years after FLT3/ITDs were discovered [101, 175, 1, 140].

Together, the data suggest that FLT3/ITDs, FLT3/ALM, and FLT3/JM/PM all promote constitutive activation of the receptor, but probably have biological differences that impact their prognostic significance. Therefore, in evaluating the prognostic relevance of FLT3-activating mutations, one must keep in mind not only the presence or absence of a mutation but also location, type, and allelic ratio of the mutation. The identification of a high-risk population in FLT3/ITD-positive patients is of great importance, as it may identify a significant number of patients who are destined for poor outcome and may benefit from alternative treatments such as early hematopoietic stem cell transplant. The ITD-AR or ITD size may provide additional tools to better risk-stratify AML patients. Besides understanding how these different FLT3 mutations respond to chemotherapy, it will be critical to determine how these different mutations impact the responsiveness to small molecule inhibitors. However, at this time, investigators are left with trying to better risk-stratify AML patients based on a variety of poorly understood surrogate FLT3 prognostic markers.

KIT Mutations

Activating mutations of KIT receptor gene have been reported in a variety of myeloid malignancies. Early studies implicated KIT mutations in the pathogenesis of mastocytosis [42, 87]; however, recent studies have found that these mutations may also be involved in the pathogenesis of AML, especially those with t(8;21) or inv(16) [170, 176, 89, 49, 27, 19, 24, 134, 129, 10, 85, 23]. Activating mutations in KIT receptor gene usually occur in either the JM domain, which regulates receptor dimerization, or the intracellular tyrosine kinase domain of the receptor gene. Mutations in both regions lead to constitutive activation of the KIT receptor [160, 42, 103, 68, 162].

KIT mutations have been reported in 3–15% of adult and pediatric AML [49, 24, 85] and in significantly higher proportion of those with t(8;21) or

inv(16), where KIT mutations were observed in nearly 40–50% of AML involving the core-binding factor (CBF) [49]. Data suggest that KIT-activating mutations may co-operate with t(8;21) translocation to contribute to myeloid leukemogenesis, where KIT mutation leads to proliferative advantage in cells which have undergone maturation arrest due to t(8;21) translocations [170]. Although presence of KIT mutations may not display prognostic significance in AML patients at large, their presence may have prognostic and possible therapeutic implications in AML patients involving CBF [49, 19, 21, 134]. Cairoli et al. evaluated 67 patients with t(8;21) or inv(16) and demonstrated that 46% of the AML patients harbored a missense, insertion, deletion, or internal tandem duplication mutations in either exon 8, 11, or 17 of the KIT receptor gene [19, 164]. Missense mutations in the tyrosine kinase domain (TKD) of the receptor (D816) was the most common mutation observed, where 20/67 patients (29%) had D816 missense mutations. Correlation of the D816 mutation in KIT with clinical outcome demonstrated that those patients with D816 mutations had a significantly higher relapse rate and worse survival. A similar study reported an adverse outcome for pediatric AML patients, in which these investigators identified mutations in the KIT TKD in 17% of pediatric AML patients with t(8;21) [134]. In contrast, Care et al. found more prognostic significance for KIT mutations in exon 8 in CBF leukemias harboring Inv16, in which 24% of patients with Inv16 had a mutation in exon 8 [24]. Besides the potential prognostic implication of these mutations, KIT mutations may have therapeutic implications, as there are data to suggest that primary leukemic cells that harbor some forms of KIT mutations may be susceptible to pro-apoptotic effects of Imatinib Mesylate [77], thus providing a therapeutic modality for some AML patients with CBF abnormalities at high risk of relapse.

Other RTKs, c-FMS, and PDGFR

Activating mutations in CSF1R (a.k.a. c-FMS) were initially described in myeloid cell lines, MDS, and AML, having an estimated frequency of 2–10% in myeloid malignancies [50, 125, 165, 118, 153]; however, a later study in AML patients did not identify any CSF1R mutations in their AML population [32]. The true prevalence of CSF1R mutations remains to be defined, but the importance of this receptor for the development of leukemia should not be underestimated. CSF1R resides on chromosome 5q33-35 and aberrant regulation of the receptor through methylation or loss of heterozygosity may play a critical role in leukemogenesis [141, 16]. If disruption of the CSF1R occurs at an "inappropriate" time during myeloid differentiation, studies have found that it may predispose cells to malignant transformation [36].

With respect to mutations in the PDGFR1α (chromosome 4q11-13) and PDGFR1β (5q31-32) genes, large-scale mutation analyses of the two genes have not been conducted; however, translocations involving PDGFR1β and

TEL (a.k.a. ETV6, 12p13) have been identified in chronic myelomonocytic leukemia, activating PDGR1β and promoting malignant transformation [122, 50, 25, 155]. Recently, translocations involving PDGFR1α and FIP1L1 (4q12) have also been found in hypereosinophilic syndrome and chronic eosinophilic leukemia [154, 28, 30, 166, 51], suggesting a possible role for PDGFR1α in malignant transformation.

In addition to the activating mutations at the receptor level, activating mutations of the secondary mediators of the RTKs (e.g., RAS, BRAF genes) have also been reported in AML [144, 113, 27, 138, 96, 29, 60, 100, 121, 135, 86, 40]. Some of these activating mutations, such as those involving RAS, are common, occurring in 10–20% of AML patients. While the clinical significance of these mutations remains uncertain, ongoing studies are investigating how these mutations may co-operate with other genetic abnormalities to promote leukemogenesis and affect prognosis [89, 113, 69].

Small Molecule Inhibitors as Therapeutic Options

Recently, there has been a surge in the development of small molecule inhibitors for myeloid leukemia [111]. Imatinib Mesylate (Gleevec) has proven to be a major advancement for the treatment of chronic myeloid leukemia in chronic phase (CML-CP). Imatinib induces a high percentage of complete cytogenetic remission for patients with CML-CP, and many of these patients have maintained this remission for 3–5 years [33, 58]. The results for Imatinib have been less impressive in more advanced forms of CML (e.g., accelerated or blast phase), in which patients will sometimes obtain responses, but almost universally relapse with resistant disease [5]. Multiple factors, including mutations in the bcr-abl gene, genomic amplification of the bcr-abl, and/or other genetic abnormalities, may account for some of the disparity in the responses between CML-CP and its more advanced counterparts [34]. To counteract the resistance secondary to bcr-abl mutations, newer small molecule inhibitors such as BMS-354825 (a.k.a. Dasatinib) and AMN 107 have been developed that display activity against cells harboring the mutated bcr-abl gene [57, 106]. Although early clinical trials seem promising for these drugs, long-term studies will be necessary to ensure that resistance against these newer small molecule inhibitors does not develop. Unlike CML-CP, AML is a much more heterogeneous disease, as indicated by the variety of different cytogenetic abnormalities and mutations within AML cells. Therefore, the development of a "universal" small molecule inhibitor for AML will be more challenging, if not impossible. Yet, the RTK pathways offer the "potential" for such drug development, and we will briefly describe some of the novel tyrosine kinase inhibitors (TKIs) that have recently been developed that target RTK pathways.

FLT3 Inhibitors

The FLT3 pathway is an obvious target for TKIs. FLT3 mutations are one of the most common mutations in AML. Because FLT3 mutations constitutively activate the receptor's pathway and contribute to leukemogenesis, small molecule inhibitors that block their activation may have therapeutic benefits for many AML patients. In addition, an increased expression of the wild-type (WT) receptor may also play a role in leukemogenesis for some AML patients [128], suggesting that FLT3 inhibitors may be effective in more than just AML patients with the FLT3 mutations. Initial in vitro studies using non-specific TKI (herbimycin A, AG1296, and AG1295) found that these drugs blocked constitutive activation of FLT3/ITDs and preferentially killed cells harboring FLT3/ITDs [7, 180, 83, 158]. However, all these compounds are highly toxic in humans, initiating searches for more selective and less toxic drugs for clinical use. Through molecular screening, numerous compounds have now been identified (MLN518, PKC412, SU5416, SU5614, SU11248, CEP-701, CEP-5214), and we will briefly describe the progress and limitations of these compounds.

MLN518 (a.k.a. CT53518 from Millennium) has been found to inhibit the activation of FLT3/ITDs and growth potential of cells harboring these mutations [92]. Like many TKIs, MLN518 is not specific for the FLT3/ITD receptor, also inhibiting WT forms of FLT3, PDGF, and KIT. Heinrich et al. published their results of a phase I study for high-risk AML patients in 2002, which found that two of six patients had $> 50\%$ reduction in bone blasts [66]. A phase II study evaluated the efficacy of MLN518 in 18 FLT3/ITD-positive AML patients with relapsed or refractory disease that were unfit for conventional chemotherapy. In this study, 6 of 18 patients demonstrated an objective response, as measured by a decrease in the peripheral blood blast by a mean of 92% (range 85–100%) [55]. However, no complete responses (CRs) were achieved.

PKC412 (from Novartis) was initially developed as a VEGF receptor inhibitor, but studies found that this benzoylstaurosporine blocked FLT3, including FLT3 mutated receptors [128, 31]. Armstrong et al. also found that MLL cells over-expressing WT FLT3 were preferentially killed by PKC412 [128,172]. A phase II trial examined the efficacy of PKC412 as a single agent for relapsed or refractory AML patients with poor performance status [6]. The PKC412 was given orally at 75 mg three times a day. Initial results found the drug to be well tolerated, with the most common side effect being nausea. However, no CRs were obtained in this heavily pretreated population. Additional follow-up of this study was recently presented at the American Society of Hematology (ASH) conference. A total of 20 FLT/ITD-positive AML patients with mutant FLT3 with either relapsed/refractory AML or high-grade myelodysplasia were treated with single agent PKC412. The peripheral blood blasts decreased by 50% in 6 of the 20 patients, with 2 responders obtaining a blast percentage of $<5\%$. Again, no CRs were observed in this high-risk population [150] but autophosphorylation of the mutant receptor was blocked in most of the responding patients, indicating an

in vivo target response using the dose in the study. Given these results, PKC412 has been combined with daunorubicin and cytarabine for induction of AML patients [148, 46]. In a phase I trial examining this combination approach, investigators recently reported at ASH that those AML patients harboring FLT3/ITDs had a CR rate of 91% (10/11) compared to 53% (17/32) for those without FLT3/ITDs ($p = 0.033$) [46]. Although not a randomized study, these data suggest that combination therapies adding TKI with chemotherapy may improve remission induction for those AML patients harboring FLT3/ITDs, although its efficacy for improving survival remains to be defined.

Sugen has several drugs under development as potential TKIs (SU5416, SU5614, and SU11248) [38, 149, 105]. Similar to most of the other TKIs, these agents also block the activities of other tyrosine kinase receptors (KIT, PDGFR, and VEGF) [38, 149, 105]. Giles et al. treated 55 patients with refractory or relapsed AML with SU5416 at a dose of 145 mg/m^2 [177]. AML patients without FLT3 mutations were also included in the trial. Grade 3/4 toxicities were few, but included headaches (14%), dyspnea (14%), infusion-related reactions (11%), and thrombotic episodes (7%). As a single agent, only three patients (5%) obtain a partial response. Another phase I trial examined SU11248 in 32 patients with advanced AML. Again, the drug was relatively well tolerated, with major dose-limiting toxicity being fatigue [38, 47]. Although approximately 50% of the AML patients had a >50% reduction in their blast count percentage, complete remission was not achieved [38, 47].

CEP-701 and CEP-5214 (from Cephalon) are two indolocarbazole compounds that inhibit autophosphorylation of the FLT3-WT and FLT3-mutant receptors [39, 81]. Unlike some of the other TKIs, these drugs have less activity against other RTKs such as KIT, FMS, and PDGF, and most clinical studies have focused on CEP-701 (Lestaurtinib). Initial studies with CEP-701 demonstrated the ability of this agent to kill FLT3-mutated primary AML cells from patients, and subsequent murine studies have found that CEP-701 extends the survival of mice injected with BaF3 cells transformed using FLT3/ITD [39]. A phase I/II trial evaluated CEP-701 as a single agent for patients with refractory, relapsed, or poor risk AML expressing FLT3-activating mutations. Fourteen heavily pretreated AML patients with FLT3 mutations were treated with CEP-701. CEP-701 toxicities were minimal, with nausea, fatigue, and neutropenia being most commonly described. Five patients obtained objective clinical responses, which correlate closely with a block in the constitutive phosphorylation of the mutated receptor. Clinical responses included significant reductions in bone marrow and peripheral blood blasts; however, no CRs were observed [80, 137]. These investigators recently opened a study to examine if the addition of CEP-701 to conventional chemotherapy may improve clinical outcomes. A total of 48 AML patients with FLT3 mutations in first relapse were randomized to either receiving standard chemotherapy or standard chemotherapy with CEP-701. Of the 24 patients who received CEP-701, 5 achieved complete CR and another 5 obtained a CR with incomplete count recovery. For those patients

receiving only standard chemotherapy, three achieved CR and an additional three obtained CR with incomplete count recovery. Accrual is continuing on this trial, and it is too early to know if the addition of CEP-701 will benefit these high-risk patients, but Levis et al. found that CEP-701 and chemotherapy killed a cell line harboring a FLT3/ITD in a synergistic fashion [136], suggesting that using CEP-701 in combination with chemotherapy may be beneficial.

Together, these results suggest that current TKIs probably are not extremely effective as single agents in heavily pretreated AML patients with FLT3 mutations. How are TKIs in previously untreated AML patients with FLT3 mutations remain to be determined? However, there is a push to use these agents in combination with more "standard" chemotherapy, believing that this offers the most potential for providing a therapeutic advantage with this class of drugs. Therefore, the efficacy of TKIs as a single agent in de novo AML patients may not ever be fully investigated. It will be critical to determine which AML patients with FLT3 mutations may have the highest likelihood of response with these novel drugs. As previously described, AML is a very heterogeneous disease event within the subgroup of patients with FLT3 mutations.

KIT and Other Small Molecule Inhibitors

There are few selective KIT inhibitors, and small molecules against KIT have not been extensively used for the treatment of AML patients. Many TKIs (e.g., SU5416, SU6668, Gleevec) have some activity against KIT [82, 56]. A recent study also found that both SU5416 and SU6668 inhibited KIT autophosphorylation and downstream effectors [56], and there has been at least one case report of an individual with refractory AML obtaining CR with SU5416 as monotherapy [139]. In addition, Gleevec has significant activity against WT-KIT and KIT harboring mutations in JM domain [82]. However, Gleevec has no activity against KIT with activating point mutations in the tyrosine kinase domain [82]; therefore, Gleevec's role in AML therapy may be limited to a highly selected group of AML patients, if at all. However, a newer TKI (BMS-354825 or Dasatinib) has been found to inhibit constitutive activation of KIT receptors with mutations in both the JMD and the TKD [106], suggesting that it may have a broader therapeutic application for AML with KIT mutations. To date, few large studies have actually targeted these drugs for AML patients with KIT mutations. This limited experience is probably due to lower prevalence of KIT mutations in AML as compared to FLT3 mutations, but given the recent discovery of the high rate of KIT mutations in AML involving CBF, additional studies using these drugs in AML patients with KIT mutations will certainly be developed [124].

In addition, small molecule inhibitors directed toward PDGF [91] or downstream effectors in the RTK pathway have been developed [43, 98, 156, 142, 108, 110, 169]. These drugs are currently in a variety of different stages of

investigation. Because RAS mutations occur frequently in many different types of tumors [144, 113, 96, 29, 60, 100, 121, 135, 86, 40], RAS has been a natural target of the RTK pathway for many years, and farnesyltransferase inhibitors have led the way in trying to block the effect of constitutive activation of RAS in AML [114, 112, 107]. In early clinical trials examining farnesyltransferase inhibitors, the response rates as a single agent have been encouraging, ranging from 20 to 35%, but the efficacy of these compounds have not correlated with RAS mutations [107]. Details about the biology and clinical implications of farnesyltransferase inhibitors are beyond the scope of this review and have recently been extensively discussed by the leaders in this field [61, 131, 62, 63]. Other inhibitors have targeted downstream effectors such as MEK or RAF [Morgan, 2001 #1171;Rahmani, 2005 #2428;Sridhar, 2005 #2423;Ouyang, 2006 #2425;Wallace, 2006 #2427; Baines, 2000 #1356]. Although some studies suggest efficacy of these agents, major responses have been limited in AML patients.

Future Directions

There are many challenges ahead in the treatment of AML. One of the major challenges will be to begin to interrogate our understanding of the RTK pathways into the development of novel therapeutic approaches for the treatment of AML. As a start, it would be critical to be able to better risk-stratify AML patients harboring abnormalities in RTK pathways. In order to do this successfully, additional investigations will need to determine the exact frequency of mutations in RTK pathway and the clinical significance of these mutations. In addition, it will also be important to understand how the different types of mutations affect the RTK pathways. These investigations will be difficult, given the large number of RTKs, downstream effectors, and heterogeneity within AML.

Researchers are examining how inhibitors of RTK pathways may be useful in the treatment of AML. As single agents, current compounds unfortunately have not induced complete responses in AML patients, but the potential of combining these agents with standard chemotherapy regimens may be more beneficial. In addition, combinations of small molecule inhibitors blocking inappropriate RTK activation at several points along the pathway may also be more effective than using them as a single agent. The major limitation to such approaches may be toxicity, given that the RTK pathway is critical in the regulation of the normal function of the hematopoietic system.

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