

FLT3 inhibitors: clinical potential in acute myeloid leukemia

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Abstract: Acute myeloid leukemia (AML) is an aggressive hematopoietic malignancy that is cured in as few as 15%–40% of cases. Tremendous improvements in AML prognostication arose from a comprehensive analysis of leukemia cell genomes. Among normal karyotype AML cases, mutations in the *FLT3* gene are the ones most commonly detected as having a deleterious prognostic impact. *FLT3* is a transmembrane tyrosine kinase receptor, and alterations of the *FLT3* gene such as internal tandem duplications (*FLT3*-ITD) deregulate *FLT3* downstream signaling pathways in favor of increased cell proliferation and survival. *FLT3* tyrosine kinase inhibitors (TKI) emerged as a new therapeutic option in *FLT3*-ITD AML, and clinical trials are ongoing with a variety of TKI either alone, combined with chemotherapy, or even as maintenance after allogeneic stem cell transplantation. However, a wide range of molecular resistance mechanisms are activated upon TKI therapy, thus limiting their clinical impact. Massive research efforts are now ongoing to develop more efficient *FLT3* TKI and/or new therapies targeting these resistance mechanisms to improve the prognosis of *FLT3*-ITD AML patients in the future.

Keywords: AML, *FLT3*, *FLT3*-ITD, tyrosine kinase, target therapy, resistance mechanisms

Introduction

Acute myeloid leukemia (AML) is an aggressive hematopoietic cancer arising from bone marrow immature myeloid progenitor cells transformed by recurrent genetic alterations that cooperate to induce cell proliferation and survival. These driver genetic events may include gene fusion (such as *PML-RARA*) or alterations of genes involved in epigenetic (eg, *DNMT3A*), metabolism (eg, *IDH1/2*), transcription (eg, *NPM1*), and signaling (eg, *FLT3*, fms-like tyrosine kinase 3) regulation.^{1,2} Among these genes, *FLT3* is the one most frequently altered in AML: up to 30% of cases harbor an internal tandem duplication (*FLT3*-ITD), or a tyrosine kinase domain (TKD) amino acid substitution (*FLT3*-TKD) is seen in 5%–10%.^{3,4} Currently, only a minority of patients with AML are cured by highly intensive therapies that encompass high-dose cytotoxic chemotherapy and hematopoietic stem cell transplantation.⁵ The detection of a *FLT3*-ITD mutation is a hallmark of adverse prognosis in AML as less than 15% of these patients experience long-term survival despite intensive therapies.⁶ In contrast, the detection of a *FLT3*-TKD mutation does not have the same negative impact on survival.⁷ Hence, finding new therapeutic options for the *FLT3*-ITD AML patient represents a major goal for physicians and scientists since years. In this review, we will focus on a novel therapeutic modality, the *FLT3* tyrosine kinase inhibitors (TKI), and discuss the mechanisms involved in the frequent clinical resistance to these molecules.

FLT3 mutations in AML

FLT3 structure and function

FLT3 belongs to a family of 58 protein tyrosine kinase receptors (TKR) characterized by an extracellular domain for ligand binding, a transmembrane helix, and a C-terminal intracytoplasmic part supporting the tyrosine kinase.⁸ FLT3 is a Class III TKR characterized by the presence of five immunoglobulin-like motifs within their extracellular part that are exclusively expressed in hematopoietic cells and induce the activation of intracellular signaling pathways such as PI3K/AKT or ERK/MAPK upon ligand binding (Figure 1).⁹ Mice knockout experiments revealed the critical role of FLT3 in normal hematopoiesis, as FLT3^{-/-} hematopoietic progenitors fail to compensate for hematopoietic deficiency in lethally irradiated mice.^{10,11} Moreover, Boyer et al¹² demonstrated that in mice, FLT3 is expressed on very immature hematopoietic progenitor cells lacking self-renewing potential but retaining capacity of differentiating into all hematopoietic lineages including megakaryocytes and erythrocytes, highlighting the critical role of FLT3 in the early stages of hematopoiesis.

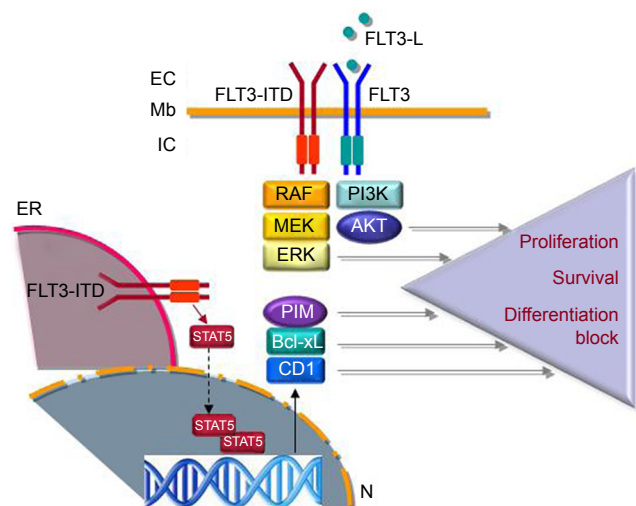


Figure 1 Schematic view of FLT3 and FLT3-ITD signaling.

Notes: FLT3 and FLT3-ITD receptors are expressed at the cell Mb. Their EC part is composed of five immunoglobulin-like domains that bind the FLT3-L. Their IC portion contains the TKD that supports the enzymatic activity of these receptors. FLT3 receptors are activated upon FLT3-L binding and, while remaining sensitive to FLT3-L, FLT3-ITD receptors are capable of activating downstream signaling pathways after FLT3-L-independent homodimerization. From the cell Mb receptors, several signaling pathways may be activated including the RAF/MEK/ERK and PI3K/AKT (the most studied). In contrast to FLT3 receptors, FLT3-ITD proteins may be found in the ER due to alteration of their glycosylation. Aberrant signaling from the ER activates STAT5 transcription factors that translocate into the nucleus (N) and subsequently activate a transcriptional program leading to the accumulation of oncogenic proteins such as Pim kinases, Bcl-xL, or CD1. Signaling pathway activation from both Mb and ER contribute to cell proliferation and survival as well as to a differentiation block that contributes to AML propagation.

Abbreviations: Mb, membrane; EC, extracellular; FLT3-L, FLT3 ligand; IC, intracellular; TKD, tyrosine kinase domain; ER, endoplasmic reticulum; CD1, cyclin D1; AML, acute myeloid leukemia; Pim, proviral integration site.

FLT3 in AML: clinical perspectives

Early reports demonstrated an overexpression of FLT3 mRNA in AML and acute B-cell (but not T-cell) leukemia¹³ as well as an overexpression of FLT3 ligand (FLT3-L).¹⁴ However, a breakthrough in our understanding of AML pathophysiology came from the discovery of mutations within the FLT3 gene located on chromosome 13q12. Nakao et al¹⁵ identified FLT3 FLT3-ITD, detected in up to 30% of diagnosis AML cases, and then Yamamoto et al¹⁶ found point mutations within FLT3-TKD, leading to activating amino acid substitutions, which are detected in 5%–10% of AML samples.

From genome-wide sequencing studies, we learned that FLT3 is the most frequently mutated gene in AML, generally detected in samples with normal karyotype, and these mutations frequently co-occur with alterations of other genes such as DNMT3A or NPM1.^{3,4} The presence of a FLT3-ITD mutation (but not of FLT3-TKD mutations) adversely impacts on prognosis, with a less than 20% long-term overall survival.^{3,6} In their recent, very large-scale genomic study on AML, Papaemmanuil et al³ observed a FLT3-ITD alteration in as much as 22% of a cohort of 1,540 AML patients. Interestingly, these mutations frequently co-occurred with NPM1 and DNMT3A mutations (39%) and chromatin or RNA splicing gene mutations (15%), and were also found to be associated with t(15;17) and t(6;9) translocations (35% and 80%, respectively). In this large study, FLT3-TKD mutations were found in less than 5% of the cohort, mostly co-occurring with MLL-PTD (1%) and NPM1 (3%) abnormalities. This study unambiguously confirmed the adverse impact of FLT3-ITD mutations, particularly when co-occurring with both DNMT3A and NPM1 mutations, and observed an adverse impact of the gene–gene interaction between MLL-PTD and FLT3-TKD mutations.³

While FLT3-ITD patients experience the same complete remission rate after induction therapy compared with other AML patients, they frequently relapse even after receiving allogeneic stem cell transplantation. The prognosis of relapsing patients is particularly poor as virtually none of the currently used therapies – including TKI that will be discussed later – induces long-term remissions.¹⁷ New therapeutic approaches based on a better understanding of FLT3-ITD AML biology are, therefore, warranted to improve patient outcome.

FLT3 in AML: biological perspectives

Recent evidence suggests that AML arises from a clonal hematopoietic compartment referred to as preleukemic stem

cells, which is expanded due to early genetic events such as *DNMT3A* mutations and thereafter transformed due to the occurrence of secondary driver mutations in other genes such as *NPM1* or *FLT3*.¹⁸ From the comparison between AML genomes, we also learned that FLT3 mutations occur as secondary events during AML clonal evolution.² While these data suggest that FLT3 mutations are secondary, late events during leukemogenesis, Chu et al¹⁹ demonstrated that FLT3-ITD drives a myeloproliferative disorder in mice due to proliferation and subsequent depletion of a quiescent long-term hematopoietic stem cell population, which was reverted by the FLT3 inhibitor sorafenib. A model to reconcile these findings may involve a preleukemic hematopoietic population amplified by initiating genetic lesions including *DNMT3A*, *ASXL1*, or *TET2* mutations, which will be transformed into AML due to mutations – such as FLT3-ITD – acquired later in the differentiation process.²⁰ In the absence of a survival advantage during the early stages of hematopoiesis, the occurrence of a *FLT3-ITD* mutation may in contrast deplete the hematopoietic stem cell compartment, preventing the development of a FLT3-ITD-driven leukemic clone.¹⁹

FLT3-ITD mutation produces receptors with a constitutive tyrosine kinase activity due to homodimerization or heterodimerization with the normal FLT3 receptors.²¹ While FLT3 receptors induce signal transduction from the cell membrane, FLT3-ITD receptors are mostly located in the endoplasmic reticulum (ER) due to glycosylation defects leading to a distinct signaling pattern including the phosphorylation and activation of the STAT5 transcription factor.²² Interestingly, Chu et al¹⁹ have shown that FLT3 receptors are not detected at the cell surface of hematopoietic stem cell, but they found FLT3 mRNA and proteins and evidence for FLT3-driven signal transduction in this cellular compartment, suggesting that intracellular ER-dependent FLT3-ITD signaling may be critical in disease propagation.

The constitutive activation of STAT5 by FLT3-ITD promotes AML cell proliferation and survival through the transactivation of target genes including *BCL2L1*, *PIMI*, *CDKN1A*, and *CCND1*.²³ Surprisingly, among STAT5 targets is the suppressor of cytokine signaling 1 gene encoding a negative regulator of cytokine-induced signaling, which promoted FLT3-ITD-induced myeloproliferative disease through escape from interferon growth inhibitory effects during cellular transformation.²⁴ Recently, Chatterjee et al²⁵ demonstrated that STAT5 nuclear translocation was due to a FAK/Rac1-Tiam1/PAK1 axis in FLT3-ITD and also in KIT D816V-positive cells and that targeting FAK, Tiam1,

or PAK1 significantly reduced leukemia propagation in part through STAT5 inhibition.

FLT3-ITD also regulates myeloid cell differentiation, which is made evident by the observation of differentiation syndromes in AML patients treated by FLT3 inhibitors.²⁶ In fact, FLT3-ITD blocks the activity of the C/EBP α transcription factor through direct phosphorylation of serine 21 and indirectly through CDK1 activation, inhibiting myeloid differentiation.^{27,28} In addition, other FLT3-ITD target genes are involved in differentiation including *RGS2* and *GADD45*.^{29,30}

Interestingly, metabolic pathways – ie, glycolysis and mitochondrial tricarboxylic acid cycle – recently emerged as new targets for cancer therapy.³¹ In FLT3-ITD AML cells, Alvarez-Calderon et al³² demonstrated that targeting mitochondrial metabolism using oligomycin A, an inhibitor of the mitochondrial ATP synthase, enhanced the antileukemic activity of the FLT3 TKI quizartinib against primary AML samples in vitro and in vivo in humanized patient-derived xenograft mouse models, suggesting that FLT3-ITD could induce an addiction of AML cells to mitochondrial metabolism.

Finally, recent data implicated a deregulation of oxidative metabolism in FLT3-ITD-induced leukemogenesis. FLT3-ITD receptors increase the expression of reactive oxygen species (ROS) due to increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase isoform 4 expression through STAT5 and also p22(phox), a small membrane-bound subunit of the NADPH oxidase complex.^{33,34} Besides DNA damage and genomic instability, ROS production may affect the function of signaling effectors as illustrated by Godfrey et al,³⁵ showing that ROS overproduction due to FLT3-ITD inactivates the protein tyrosine phosphatase DEP-1 – a negative regulator of FLT3-ITD signaling – through oxidation of its catalytic cysteine.

FLT3 tyrosine kinase inhibitors

The characterization of frequent FLT3 mutations in AML patients and their correlation to adverse clinical outcome in the case of FLT3-ITD prompted the search for FLT3 TKI. These TKI are heterocyclic compounds blocking FLT3 activity through competition with ATP at the TKD of FLT3.³⁶ While generally demonstrating in vitro activity against multiple tyrosine kinases, their efficacy in AML patients is markedly enhanced in FLT3-ITD cases and correlates with inhibition of FLT3 phosphorylation and downstream signaling relays.³⁶ Comprehensive reviews on the use of FLT3 TKI in clinical trials have been recently published.^{17,36}

Schematically, three categories of studies currently encompass FLT3 inhibitors in AML. First, studies using

FLT3 TKI as monotherapy were reported mostly in a refractory/relapse context of FLT3-ITD AML. Some of them are already reported and show that, while of significant clinical activity, FLT3 TKI, such as quizartinib or sorafenib, fail to induce long-lasting remissions in this context, while some patients were successfully bridged to allogeneic transplant after achieving TKI-induced remission.^{37,38} Second, several clinical trials were recently designed to combine a TKI to Vidaza, a hypomethylating agent with activity in AML, due to a possible synergistic activity of these compounds in FLT3-ITD AML.¹⁷ Third, important results recently came from clinical trials combining a TKI with conventional chemotherapy in AML. In a Phase II randomized trial (SORAML), 276 AML patients were treated by conventional chemotherapy plus sorafenib (400 mg twice daily, n=134) or plus placebo (n=133), resulting in a 40% versus 22% 3-years event-free survival in favor of the sorafenib group. In this study, a trend to improved survival was detected in patients treated by sorafenib when a FLT3-ITD was present, although the results were not statistically significant possibly due to the limited size of the cohort (n=46).³⁹ In a recent abstract, Stone et al⁴⁰ reported the results of a Phase III study (RATIFY) that combined midostaurine or placebo to a standard chemotherapy in 717 FLT3-ITD AML patients. They showed that the addition of midostaurine extended the median overall survival from 25 to 75 months, which represents the first demonstration of a significant activity of a TKI in FLT3-ITD⁺ AML in randomized trials. These results prompted the designation of midostaurine as a breakthrough therapy in *FLT3*-mutated AML by the US Food and Drug Administration in 2016. Finally, as posttransplant relapses have been successfully managed by the FLT3 TKI sorafenib,^{41,42} the idea emerged to administer a FLT3 TKI in patients achieving a complete remission after allogeneic transplant. In fact, several recent studies report promising results using this maintenance approach, albeit follow-up intervals remain short.⁴³⁻⁴⁵ In a recent retrospective study, sorafenib posttransplant maintenance was associated with an improved 2-year progression-free survival and a reduced risk of relapse.⁴⁶ However, these preliminary results warrant confirmation in prospective clinical trials, and several questions need to be addressed in this perspective such as the timing of TKI initiation and the duration of therapy.¹⁷

Resistance to therapy in FLT3-ITD AML

The presence of a *FLT3-ITD* mutation has a strong negative prognosis impact in AML due to an increased risk of relapse after chemotherapy. The current treatment guidelines in

FLT3-ITD AML have been recently reviewed by König and Levis¹⁷ and support the use of allogeneic stem cell transplantation whenever possible in first remission to decrease the risk of relapse. For refractory/relapse patients, no guidelines are available and their survival is generally very short regardless of the therapeutic intervention used.¹⁷

Resistance to cytotoxic chemotherapy

Genomic pairwise comparisons between diagnosis and relapse AML samples revealed the expansion of a founding clone or subclone, which may acquire new oncogenic mutations at relapse.⁴⁷ In FLT3-ITD AML, relapse-specific mutations were found at low frequency in several genes including *FLT3* (*FLT3-TKD* mutations), *WT1*, *ASXL1*, *MLL3*, and *FAT4*, which may provide some insights on the mechanisms of resistance to chemotherapy.⁴⁸ Interestingly, *MLL3* and *FAT4* were tumor suppressors in the FLT3-ITD context, as knockdown of their expression by RNA interference stimulated AML propagation including in mouse models, suggesting that inactivating mutations in these genes may have favored AML relapse after chemotherapy.⁴⁸

Interestingly, FLT3-ITD AML samples collected at the time of relapse appear to be more sensitive to FLT3 TKI in vitro compared to diagnosis samples, suggesting that chemotherapy is selective for an AML clone addicted to FLT3-ITD signaling.⁴⁹ This clonal selection might be directly favored by chemotherapy, which markedly enhance the production of FLT3-L by the bone marrow microenvironment and activate FLT3-ITD-dependent signaling pathways in AML cells.⁵⁰

Oncogene addiction to FLT3-ITD

A significant number of reports have shown that FLT3 TKI frequently induce clinical responses in FLT3-ITD AML, including some complete responses, when used as a monotherapy.¹⁷ While some transient responses in blast count are reported in the absence of *FLT3-ITD* mutation, probability due to the activity of TKI against other kinases, complete responses are seen only in FLT3-ITD patients, attesting for an oncogenic addiction to FLT3-ITD in their AML cells.³⁶ However, responses are generally lost within month, even when the TKI is given continuously.³⁸ The addiction of AML cells to FLT3-ITD suggests that this deregulated TKR may represent a very attractive target for therapy, and in fact, important efforts were undertaken to understand the molecular basis of resistance to TKI in FLT3-ITD AML to develop new efficient therapeutic strategies in the future. The current state of the art of these resistance mechanisms is depicted in Figure 2.

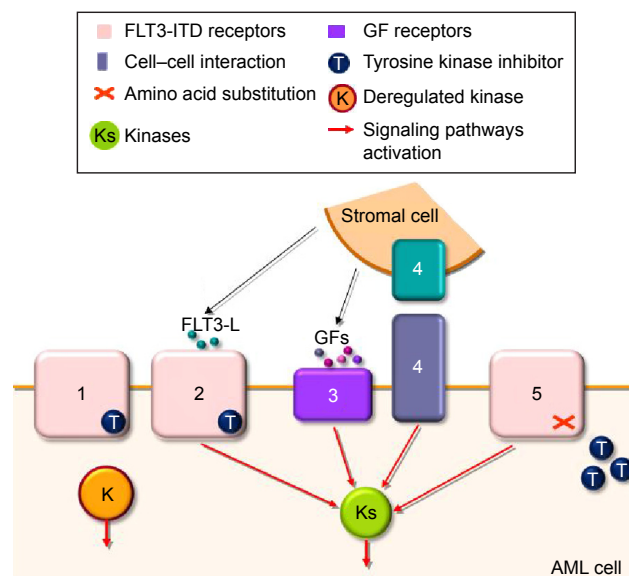


Figure 2 Overview of the resistance mechanisms to FLT3 TKI in AML.

Notes: 1) FLT3-ITD receptors are efficiently targeted by TKI (T) but bypass activation of oncogenic tyrosine kinases (SYK or AXL) or serine/threonine kinases (Pim) reduce the activity of TKI and enhance AML cell proliferation and survival. 2) FLT3-L overproduction from bone marrow stromal cells, for example, as observed in AML patients treated with cytotoxic chemotherapy, stimulates FLT3-ITD receptors and reduces the efficacy of TKI. 3 and 4) Stromal cells from the bone marrow microenvironment may favor FLT3-ITD AML cell survival upon TKI therapy through the secretion of soluble cytoprotective factors that activate signaling pathways such as ERK (3) or through cell-to-cell interactions, involving, for example, integrins (4). 5) Amino acid substitutions within the FLT3-TKD (generally at position D835) decrease the affinity of TKI for their target receptors. The mechanisms hereby referenced from 1 to 5 result in the activation of intracellular signaling pathways such as ERK or STAT5 even in the presence of TKI, which explains why AML cells escape from their cytotoxic effects.

Abbreviations: TKI, tyrosine kinase inhibitors; AML, acute myeloid leukemia; FLT3-L, FLT3 ligand; Pim, proviral integration site.

FLT3 allele burden

FLT3-ITD allele burden is defined by the ratio between the mutated and the wild-type allele. A high (>0.5) FLT3-ITD burden is associated with an adverse prognosis after conventional therapy, even if allogeneic stem cell transplant may benefit this category of AML patients.^{51,52} In contrast, the antileukemic activity of six different FLT3 TKI appeared superior in vitro against samples with a high FLT3-ITD allele burden.⁴⁹ Interestingly in this report, FLT3 inhibition did not correlate to TKI-induced cytotoxicity in all cases, suggesting that some samples may not have been addicted to FLT3-ITD-driven signaling.⁴⁹ The follow-up of FLT3 allele burden in the course of conventional or TKI therapy will be facilitated by new sensitive sequencing techniques, and the impact of minimal residual disease monitoring will probably be widely evaluated in FLT3-ITD AML in the future.^{53,54}

Overexpression of oncogenic kinases

The activation of bypass signaling pathways in response to target inhibition of an oncogenic signaling pathway is

a well-recognized mechanism of resistance to therapy in cancer, as illustrated, for example, by the activation of CRAF in *BRAF* V600-mutated cells treated with vemurafenib or by the activation of the MET tyrosine kinase in *EGFR*-mutated cells treated by gefitinib.⁵⁵ In FLT3-ITD AML, the cytosolic tyrosine kinase SYK is overactivated and directly activates FLT3-ITD by direct binding and phosphorylation. Moreover, SYK appears critical for myeloid disease propagation in mice, with potential implications in resistance to TKI.⁵⁶ AXL is a receptor tyrosine kinase overexpressed in many cancer types including in AML. Upon incubation with TKI, FLT3-ITD cells – and particularly cells that were resistant to TKI – demonstrated an increased expression and phosphorylation of AXL, and AXL inhibition by small molecules and by RNA interference facilitated the activity of FLT3 TKI in vitro.⁵⁷

The impact expression of the PIM serine/threonine kinase family members have been extensively studied in cancer and AML.^{58,59} PIM kinases are FLT3-ITD targets dependent on – at least for PIM1 – STAT5 activity that controls several oncogenic relays including transcription, protein translation, cell cycle, apoptosis, and cell migration.⁵⁸ Early reports showed that PIM kinases overexpression may contribute to FLT3-ITD-induced cell transformation and resistance to FLT3 TKI in vitro.^{60–62} Several groups reported a significant antileukemic activity of PIM inhibitors in preclinical models of AML,^{63–65} and currently a clinical trial is ongoing with the LGH447 pan-PIM kinase inhibitor in AML regardless of their FLT3 mutational status (NCT02078609). We recently observed that PIM kinases overexpression decreases the efficacy of FLT3 TKI due to a direct action of PIM kinases on FLT3-ITD receptors. In AML cells resistant to TKI, PIM kinase inhibition by RNA interference or by the pan-PIM kinase inhibitor LGB321 (similar to LGH447) restored the cytotoxic activity of FLT3 TKI against these cells, providing the molecular basis to understand the synergy between FLT3 and PIM kinase inhibition in FLT3-ITD⁺ AML.⁶⁶ In fact, the SGI-1776 compound is a dual PIM/FLT3 inhibitor that reached a clinical trial development stage. Unfortunately, unanticipated cardiac toxicity – possibly due to an off-target activity against the hERG potassium channel – resulted in withdrawal of SGI-1776 from clinical use.⁶⁷ Future development of dual PIM/FLT3 inhibitors might be of great interest after TKI failure in FLT3-ITD AML patients.

FLT3-L

While the expression of FLT3 receptors is restricted to hematopoietic cells, FLT3-L is expressed by a wide variety of cells, particularly by bone marrow stroma and T lymphocytes

after chemotherapy.⁵⁰ In fact, increased levels of FLT3-L significantly impaired the ability of FLT3 TKI to inhibit FLT3-ITD activity and induce cytotoxicity in FLT3-ITD AML.⁵⁰ As FLT3-L levels gradually increased after repeated cycles of chemotherapy, these results suggested to combine chemotherapy with a TKI frontline, an approach that seems fruitful in the clinics as attested by the recent results of the RATIFY clinical trial as discussed earlier.⁴⁰ In addition, evidence suggests that, in contrast to cytotoxic chemotherapy, treatment with hypomethylating agents such as 5-azacytidine may prevent the release of FLT3-L. On the basis of these observations, several clinical trials are currently ongoing combining Vidaza to different TKI molecules in FLT3-ITD AML.¹⁷ An interesting approach to bypass FLT3-L-induced resistance to therapy is the development of anti-FLT3 antibodies. These antibodies bind FLT3 regardless the concentration of FLT3-L and induce cytotoxicity mainly through antibody-dependent cell-mediated cytotoxicity.⁶⁸ FLYSYN is such a therapeutic antibody that will be used in clinical trials to target minimal residual disease in AML in complete remission (NCT02789254).

Microenvironment

The bone marrow microenvironment has long been recognized as a feeder for hematopoietic cancer cell growth and has been involved in resistance to FLT3 TKI in FLT3-ITD AML.⁶⁹ Soluble molecule secretion as well as cellular interactions between AML and stromal cells was reported as cytoprotective factors toward AML cells including against TKI, particularly through a sustained activity of signaling pathways such as ERK.⁷⁰ A recent report also suggests an important role for integrin $\alpha v \beta 3$ in activating β -catenin signaling in FLT3-ITD AML cells, promoting resistance to FLT3 TKI.⁷¹ Clinical use of TKI revealed a differential activity of these molecules between the blood and the bone marrow compartment. First-generation FLT3 TKI frequently produced a peripheral blast cell clearance, while the bone marrow blast cells were generally persistent.¹⁷ Second-generation TKI more frequently induced complete bone marrow blast cell clearance, but occurring later compared to the blood compartment (several weeks compared to few days). In fact, bone marrow blast cell clearance seems to be due to progressive myeloid differentiation rather than apoptosis with the implication of C/EBP α downstream of FLT3-ITD inhibition.²⁶ These data suggest that leukemia-permissive interactions with the bone marrow microenvironment are important mediators of FLT3 TKI resistance for which therapeutic strategies must be refined by combining TKI

to agents that target these interactions, such as the CXCR4 inhibitor plerixafor.

FLT3 mutation

FLT3 TKIs are small molecules that bind to the TKD through establishing hydrogen bond interactions. The TKI/kinase complex is thereby stabilized, which prevents ATP from reaching its binding site.⁷² Mutations occurring within the binding site of TKI may reduce their affinity for these molecules, hence reducing their antileukemic potential. This therapeutic resistance mechanism by modification of the target is commonly found in bacteria with acquired resistance to antibiotics and has been widely studied in the face of resistance to TKI in chronic myelogenous leukemia (CML).⁵⁵ While occurring in virtually all cases of TKI resistance in CML, with a particular mention of the T315I “gatekeeper” mutation preventing the binding of most TKI to ABL TKD, the detection of mutations within FLT3-TKD only recently emerged as an important mechanism of resistance to FLT3 TKI.³⁶

These *FLT3-TKD* mutations may be detected at diagnosis in the absence of *FLT3-ITD* mutation in 5%–10% of AML cases, and the nature as well as the clinical impact of these mutations was recently reviewed by Leung et al.³⁶ The same mutations may occur on FLT3-ITD alleles and induce amino acid substitutions within the kinase domain generally at position D835.³⁸ The F691L amino acid substitution, which is found less frequently than D835 mutations, is a “gatekeeper” mutation similar to the T315I mutation found in CML and also induces a global resistance to FLT3 TKI.⁷² Other TKD mutations were reported at low frequency in FLT3-ITD relapse samples, and overall, Alvarado et al⁷³ reported the occurrence of *TKD* mutations in 22% of relapse samples of FLT3-ITD AML patients treated by quizartinib, sorafenib, lestaurtinib, or KW-2449 in clinical trials. Smith et al⁷⁴ reported *FLT3-TKD* mutations in all samples from patients relapsing from quizartinib therapy and demonstrated that these mutations were not detectable in diagnosis samples, suggesting a strong selective pressure against FLT3 during the course of TKI therapy that supported the notion of oncogene addiction to FLT3-ITD of AML cells, pinpointing FLT3-ITD as an important therapeutic target in AML.

However, the mechanisms by which these additional mutations confer resistance to TKI are not fully understood. *TKD* mutations may stabilize the FLT3 kinase domain in an open ATP-binding configuration that enhance FLT3 activity and reduce TKI binding.³⁶ In fact, Type 2 TKI such as quizartinib or sorafenib bind the TKD in an inactive conformation state, while Type 1 TKI (midostaurine, crenolanib)

may target both active and inactive conformations of tyrosine kinases.⁷⁵ Interestingly, Smith et al⁷⁴ reported that crenolanib, a Type I TKI with a strong selectivity for FLT3, was powerful to induce cytotoxicity in FLT3-ITD cells harboring *TKD* resistance mutations.

How to target these mutations arising after TKI therapy is a central question that should be addressed to improve long-term disease control.³⁶ First, new, more potent TKI may be discovered, as discussed earlier in the case of crenolanib. In fact, several other TKI, such as AUZ454 or ponatinib, are currently being developed to challenge these *FLT3-TKD* mutations, with ponatinib being routinely used in *BCR-ABL* T3151-mutated leukemia.⁷⁶ Second, multikinase inhibitor or dual therapy with a FLT3 TKI and another inhibitor may be considered to circumvent signaling pathways activated in *TKD*-mutated AML cells. Following this concept, several approaches have been developed including combination of FLT3 inhibitors with mTOR, NFκB, MEK, or PI3K inhibitors and also new compounds with dual activity against FLT3 and PIM, JAK, or aurora kinases.³⁶ Virtually any pathway involved in TKI resistance may be considered as a potential target for dual targeting, and a rigorous preclinical evaluation of these new therapeutic options is required to select the best candidates for further clinical development in the future.

Other mechanisms

Other resistance mechanisms may involve changes in the regulation of target genes and/or in activation of signaling pathways downstream of FLT3-ITD.³⁸ Interestingly, microarray analysis on primary samples from FLT3-ITD AML patients who became refractory to sorafenib showed an upregulation of the *TESC* gene, which encodes a calcium-interacting protein with impact on intracellular pH regulation and resistance to sorafenib.⁷⁷ Agnostic approaches such as those developed in this study may represent powerful approaches to identify new strategies to target TKI resistance mechanisms in FLT3-ITD AML cells. Interestingly, Lam et al⁷⁸ recently identified (by using a high-content chemical screen) homoharringtonine, a plant alkaloid with cytotoxic activity in AML achieved through protein translation inhibition, as having a synergistic activity with sorafenib in vitro, in mouse models, and in a clinical trial conducted in patients with refractory/relapsed FLT3-ITD AML, some of whom were resistant to FLT3 TKI.

Perspectives

AML is currently cured in only a minority of patients, and this is even more so when an *FLT3-ITD* mutation is detected

in leukemic blasts. Tremendous efforts led to the development of highly active and clinically pertinent FLT3 TKI, as accumulated evidences pinpointed that AML cells may be addicted to FLT3-ITD-dependent signaling. However, relapses are commonly observed after TKI therapy due to different resistance mechanisms that encompass FLT3 TKD amino acid substitutions, bypass activation of oncogenic kinases, and leukemia-permissive interactions with the bone marrow microenvironment. In contrast to CML in which resistance mechanisms appear to be uniform, the pattern of resistance mechanisms in FLT3-ITD AML is far from being fully understood and represent an active field of research. However, recent results from a Phase III clinical trial in which FLT3-ITD AML patients were treated with chemotherapy and midostaurine or placebo strongly suggested a survival benefit to FLT3 inhibition. Future strategies will include more potent and/or specific FLT3 inhibitors and integration into a global strategy in which TKI may be given in combination with chemotherapy and/or as a consolidation strategy, particularly after allogeneic stem cell transplantation or even as a maintenance therapy, possibly combined to hypomethylating agents or other molecules for which a synergy may be found with FLT3 TKI.

Disclosure

The authors report no conflicts of interest in this work.

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