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Quizartinib for the treatment of FLT3/ITD acute myeloid leukemia

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Abstract

FLT3/ITD acute myeloid leukemia is a poor prognosis disease driven by a constitutively activated receptor tyrosine kinase, making it an obvious target for drug development. The development of clinically effective FLT3 inhibitors has been slow, in part because many are multi-targeted inhibitors that are not selective or specific for FLT3. Quizartinib is the first small molecule FLT3 tyrosine kinase inhibitor expressly developed as a FLT3 inhibitor. It is potent, selective and has ideal pharmacokinetics in comparison to other compounds previously tested. This article summarizes its advantages and limitations, and details the insights into the biology of the disease that have been uncovered through the laboratory and clinical use of quizartinib.

Keywords

c-KIT; FLT3; kinase; leukemia; quizartinib

Acute myeloid leukemia (AML) is a hematopoietic malignancy with an aggressive clinical course that occurs across all age groups, although it is more common in older adults. To a large degree, the molecular subtype determines the clinical characteristics and prognosis [1]. For example, AML with a *PML—RARα* fusion typically presents with a low peripheral white blood cell (WBC) count and disseminated intravascular coagulation, but has an excellent prognosis when created with a specifically tailored therapy [2] AML with a double mutation of the *C/EBPα* gene likewise has a favorable prognosis when treated using conventional induction and consolidation chemotherapy [3]. By contrast, AML wirh an internal tandem duplication of the *FL13* gene (*FLT3/ITD* mutation) typically presents with leukocytosis, and a bone marrow packed with undifferentiated or monocytic blasts. FLT3/ITD AML, which accounts for 20—25% of adult AML cases, will often go into remission with conventional chemotherapy, but typically relapses and overall has a poor prognosis [4].

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FLT3/ITD mutations, which consist of tandem duplications of variable length inserted into the juxtamembrane domain or the first part of the kinase domain, result in constitutive activation of the tyrosine kinase function of the FLT3 receptor [5], although the mutant receptor is still dependent on the presence of its cognate ligand, FLT3 ligand (FL), for complete activation [6]. Since their discovery in 1996 [7], the role that these mutations play in the pathogenesis of this important AML subtype has been the focus of intense scrutiny. The wild-type FLT3 receptor, when acrivated by FL, undergoes dimerization and autophosphorylation, followed by activation of the RAS/MAPK and PI3K pathways, along with transient activation of STAT5 [8,9]. *FLT3/ITD* mutations lead persistent activation of these signaling pathways, with constitutive activation of STAT5 mediated by an immature form of the receptor mislocalized to the endoplasmic reticulum [10,11].

A constitutively activated kinase occurring prominently in a cancer is a tempting target for drug development, and FLT3 has been no exception. However, progress in this regards has been slow for a number of reasons [12]. While inhibition of the mutant receptor in cell lines or AML blasts in suspension culture with a FLT3 tyrosine kinase inhibitor (TKI) results in apoptosis, blasts within the marrow are more resistant to this therapy, in part because of local production of FL and other cytokines [13]. In order to induce apoptosis in a FLT3/ITD blast with a FLT3 inhibitor, the degree of inhibition must be relatively profound and sustained [14,15]. This is problematic when using nonselective multi-targeted inhibitors. Indeed, the first generation of drugs studied as FLT3 inhibitors were really ‘repurposed’ TKIs. Two examples are lestaurtinib and midostaurin, which are both pan-kinase inhibitors with particular activity against FLT3 [14,16–17]. When used at concentrations necessary to achieve complete FLT3 inhibition, these drugs inhibited numerous other kinases, leading to significant off-target effects. Even the second-generation FLT3 inhibitors, such as sorafenib and crenolanib, were originally developed to target other receptors, such as VEGF receptors and PDGF receptors [I8–21].

Quizartinib (formerly known as AC220) is the first drug to have been identified and developed exclusively as a FLT3 inhibitor [22]. It has proven to be more potent and selective than any previous FLT3 TKI, and its development thus far has resulted in important new findings about this disease. Perhaps more importantly, the preliminary clinical activity induced by this drug has fueled a new enthusiasm for the field as well as a clear indication that FLT3 inhibition will ultimately be clinically useful for this difficult to treat subset of AML. This review will focus on the discovery, clinical development and correlative laboratory data pertaining to quizartinib.

Identification & characterization of quizartinib

At the time quizartinib was introduced into the literature in 2009, there were several other compounds under investigation for this purpose, including lestaurtinib, midostaurin, tandutinib, sunitinib and sorafenib [23–28]. All of these compounds, however, were originally isolated and developed with other kinase targets in mind, and as such, none of them were particularly selective for FLT3. Given the profound degree of FLT3 inhibition necessary to affect blasts within the marrow, and the fact that inhibition needs to be sustained to induce apoptosis [15], it was not surprising that these early repurposed

compounds were not generally effective as single agents [12,28]. Their predictable off-target effects prevented them from being tolerable at FLT3 inhibitory concentrations *in vivo.* Furthermore, a number of them had suboptimal pharmacokinetic properties, such as a short *in vivo* half-life and/or high plasma protein binding, which impeded efficacy [15].

A bis-aryl urea derivative was initially identified as a potent and selective FLT3 inhibitor using a unique phage display screening method [29]. While this lead compound was both potent and selective for FLT3, optimization was carried out to improve its water solubility and pharmacokinetic profile through removal of a carboxamide group, along with addition of a solubilizing morpholinoethoxy group [30]. The result was AC220, subsequently designated quizartinib [22], the first compound to be identified and developed specifically as an orally administered FLT3 inhibitor for potential therapeutic use in FLT3/ITD AML.

In keeping with the manner in which it was isolated, quizartinib is much more selective for FLT3, and, in particular, the FLT3/ITD mutant receptor, than other compounds commonly used as FLT3 inhibitors. As an illustration of the significant difference in selectivity between quizartinib and other FLT3 inhibitors, Box 1 lists kinases with binding constants that fall within a fivefold range of the K_d of quizartinib, compared with sorafenib and lestaurtinib, for inhibition of the FLT3/ITD receptor in the phage display assay [22]. Toxicity, presumably from off-target effects, was a major limitation for both sorafenib and lestaurtinib in clinical studies [31,32], and quizartinib would be predicted to have a noticeable advantage in this regard.

Quizartinib was systematically studied as a FLT3 inhibitor in cell lines, primary blast samples, and murine xenograft models [22,33]. In assays using MV4–11 or Molm 14 cells (both of which express FLT3/ITD mutant receptors) as well as blasts isolated from FLT3/ITD AML patients, quizartinib inhibited phosphorylated FLT3 and induced apoptosis with an IC_{50} of 1—2 nM. More importantly, however, quizartinib retained its relative potency against FLT3 in human plasma. Most small molecule kinase inhibitors are very hydrophobic and highly protein bound — a low nanomolar IC_{50} in culture medium (typically containing only 10% serum) does not translate into a similar potency in plasma [17]. In contrast with other inhibitors (Table 1), quizartinib is at least 25-times more potent against FLT3 in plasma.

Clinical results with quizartinib

Quizartinib was first tested in human patients in a conventional Phase I 3 × 3 dose-escalation trial with relapsed and refractory AML patients irrespective of FLT3 mutation status [34]. The primary end points were safety, tolerability and the determination of pharmacokinetic parameters. Patients were initially treated intermittently starting at 12 mg orally per day, 2 weeks on and 2 weeks off, and then the trial was modified to allow continuous dosing. Of the 76 patients enrolled, 17 (22%) were FLT3/ITD positive and 37 (49%) were negative, while in 22 (29%) the FLT3 mutation status was unknown. The maximum tolerated dose was 200 mg per day of continuous dosing, with asymptomatic prolongation of the duration of cardiac repolarization, referred to as the QT interval (calculated by the Fridericia method [39]) as the dose-limiting toxicity. Other grade 3/4 adverse events, possibly attributable to

quizartinib, were anemia, thrombocytopenia and fatigue, all toxicities associated with the underlying disease. The drug proved to have a longer-than-expected half-life of greater than 1.5 days, and an active metabolite (AC886) of similar properties as the parent drug was detected. Responses occurred at very early dose levels (18 mg/day of intermittent dosing). There were responses in 23 of 76 patients, consisting of two complete remissions (CR), three CRs with incomplete platelet recovery (CRp), five CRs with incomplete hematologic recovery (CRi) and 13 partial responses. The median duration of response was 13.3 weeks and the median survival was 14 weeks.

In this trial, quizartinib was exceptionally well tolerated, and the remarkably high response rate in a group of patients with an extraordinarily poor prognosis was obviously very encouraging. Moreover, the toxicities of QT prolongation, anemia and thrombocytopenia were relatively modest, given the tenuous nature of the patients being treated. The maximum tolerated dose of 200 mg/day continuously was chosen as the Phase II dose. However, this trial serves as an illustration that the traditional 3 × 3 dose-escalation trial design, which was developed primarily for the testing of cytotoxic chemotherapy, is probably not an ideal way to find an optimal dose for a targeted drug, such as quizartinib. Quizartinib was specifically designed with the goal of profoundly and selectively inhibiting FLT3 in sustained fashion *in vivo*, and clearly it met that goal. Correlative assays indicated that FLT3 was completely inhibited in patients at the lowest dose levels, and yet the Phase II dose was several fold higher [34]. In retrospect, a better approach might have been to use a dose one or two levels above the dose necessary to achieve target inhibition (to ensure inhibition in the large majority of patients, accounting for individual variability). This would have been 40 or 60 me per day, instead of the 200-me dose.

Five clinical studies of quizartinib have opened subsequent to the Phase I trial, four of which have been presented in abstract form. The largest of these was a trial of single agent quizartinib for relapsed/refractory AML patients. During an exploratory phase in which 62 patients were enrolled, the dose was decreased to 135 mg/day for men and 90 mg/day for women because of a high incidence of reversible, asymptomatic QT prolongation [40]. Using this modified dosing regimen (still higher than needed to achieve *in vivo* inhibition), two larger cohorts of patients were enrolled [41,42]. The first cohort consisted of 133 patients, 92 *FLT3/ITD* positive and 41 *FLT3/ITD* negative, age 60 years and over who were either refractory to primary therapy or in first relapse. For patients who were *FLT3/ITD* positive, the composite remission rate was 54% (0 CR; 3% CRp; and 51% CRi), with a median duration of response of 12.7 weeks and median overall survival of 25.3 weeks. Patients who were refractory to their last AML therapy achieved a 39% composite complete remission rate with quizartinib. For *FLT3/ITD*-negative patients the composite complete remission rate was 32% (2% CR; 2% CRp; and 27% CRi). The second cohort of the Phase II study enrolled primarily younger patients, 99 *FLT3/ITD* positive and 38 *FLT3/ITD* negative, who were refractory to or had relapsed after a second line of therapy. In this cohort, the response rate was similar to the cohort of older patients, with *FLT3/ITD* positive patients achieving a composite complete remission rate of 44% (4% CR; 0 CRp; and 40% CRi), and *FLT3/ITD* negative patients responding at 34% (3% CR; 3% CRp; and 29% CRi). This response rate was similar to that seen in the older patient cohort, but a CRi in a younger patient often allows an allogeneic transplant to be attempted. In the younger cohort, 34% of

patients discontinued quizartinib to undergo a transplant. While the responses in the *FLT3/ITD*-negative patients in some cases may have been due to inhibition of wild-type *FLT3* or c-KIT, most likely occurred in patients that were actually *FLT3/ITD* positive, but in whom the assay yielded a false-negative result. The sensitivity of the PCR-based assay for *FLT3/ITD* mutations is such that it can detect the presence of the mutation when the percentage of blasts in the marrow is 5—10% or greater. When FLT3/ITD AML patients are in early relapse, the marrow sample assayed may contain only a few percentage blasts, leading to a negative mutation test despite the patient actually being positive [43].

QT prolongation remained a relatively common problem in both cohorts (25—26%). Furthermore, the most common response was CRi, consisting of elimination of blasts but without count recovery, and with persistent platelet and red cell transfusion dependence. Off-target inhibition of c-KIT almost certainly contributed to this myelosuppression [21]. In a follow-up trial, relapsed/refractory FLT3/ITD AML patients were randomized to receive either 30 or 60 mg/day, continuously [44]. Preliminary results from this trial (presented in abstract form) indicate that either dose resulted in approximately the same response rate, and responding patients could be successfully bridged to transplant.

Given the overall high response rates induced by single agent quizartinib in this very difficult population, it was logical to incorporate it into existing treatment paradigms. To this end, three separate clinical trials of quizartinib have been launched. The first is a trial of the quizartinib combined with conventional chemotherapy in newly diagnosed patients and the second is a combination with salvage chemotherapy for pediatric AML patients. Allogeneic transplant has become a standard part of the therapy for FLT3/ITD AML, and so the third trial is of quizartinib administered as maintenance therapy following allogeneic transplant. The differentiation induced by FLT3 inhibition in general and by quizartinib specifically have spurred investigators to combine these drugs with hypomethylating agents such as 5-azacitidine [45], and several such trials (with quizartinib or other inhibitors) are due to begin accrual in 2014. Finally, efforts are underway to explore combinations of quizartinib with inhibitors of other signal transduction targets, such as MAPK, AKT and PIM. PIM is of particular interest, given that the PIM kinases are convergence points for a number of growth factor signaling pathways, and that both PIM1 and PIM2 activity are influenced by FLT3/ITD signaling [46,47]. Given that resistance to FLT3 inhibition can occur through activation of parallel growth factor signaling pathways (which converge on the PIM kinases) [48], targeting one or both of the two major isoforms of PIM may be a way of circumventing resistance.

Nature of the response to quizartinib

During the large Phase II single agent trial of quizartinib, in which relatively uniform groups of patients were treated with a consistent dose, it became clear that most of the responses were different that the type of response that would be expected from cytotoxic chemotherapy. There were a few complete remissions in which all blasts rapidly disappeared and normal hematopoiesis was completed restored, but these occurred predominantly in patients relapsing after an allogeneic transplant. This phenomenon, which may in part be

mediated by activation or potentiation of an allogeneic effect, has been observed with other FLT3 inhibitors [49]. However, these represented only a minority of the responses.

The typical patient enrolling on the Phase II trial had circulating blasts in the peripheral blood and a hypercellular bone marrow almost completely effaced by blasts. Initiation of quizartinib almost invariably cleared the peripheral blood of circulating blasts within a few days. On day 14 of treatment, the marrow typically remained hypercellular, but by this point the cells consisted of differentiating myeloid elements, all still harboring the *FLT3/ITD* mutation [50]. By day 29 of treatment, when most responses were achieved, mature neutrophils were in abundance and blasts had been reduced to less than 5% or were even undetectable. There was often a surge of neutrophils into the peripheral blood at this point, occasionally accompanied by manifestations of a differentiation syndrome resembling that seen in APL treated with retinoic acid. With ongoing quizartinib therapy, the marrow eventually became hypocellular, with the neutrophil count falling back down to relatively low levels. Throughout treatment, most patients remained transfusion dependent, and some developed hair depigmentation indicative of c-KIT inhibition [51]. At this point, the leukemia was essentially reduced to a minimal residual disease (MRD) state, still often detectable by PCR but with blasts maintained at less than 5% in the marrow. Younger patients were eligible for allogeneic transplant anytime past the first month of treatment by virtue of the fact that marrow blasts had been reduced to an acceptable level. While relapsed/refractory FLT3/ITD AML carries an exceptionally dismal prognosis, the ability to bridge these patients to transplant clearly resulted in a number of patients achieving long-term survival. The clinical benefits conferred by this drug were apparent to most practitioners enrolling patients on these studies.

These responses was labeled CRi, in an attempt to conform to the previously defined International Working Group criteria for responses to therapy in AML [52]. However, labeling them in this manner is somewhat misleading, because a CRi achieved via a DNA-damaging drug, such as cytarabine or daunorubicin, has a very different physiology as compared with a CRi obtained during quizartinib treatment. In the former, the failure to recover normal hematopoietic activity is usually attributed to a toxic effect on stem and progenitor cells, possibly accompanied by persistent MRD. A CRi from quizartinib occurs from a reasonably well-understood series of cellular effects that were predicted by preclinical studies. FLT3/ITD AML blasts in suspension culture undergo apoptosis in response to effective sustained FLT3 inhibition [33,53]. However, in the presence of marrow-derived cytokine stimulation, FLT3/ITD blasts have been shown to undergo terminal myeloid differentiation [54,55]. In FLT3/ITD blasts on bone marrow stroma *in vitro*, FLT3 inhibition leads to cell cycle arrest and gradual terminal myeloid differentiation rather than apoptosis, exactly what was observed clinically in patients treated with quizartinib [50], and what has been observed in some patients treated with sorafenib [56,57]. The failure to undergo apoptosis is due to the persistence of the MAPK pathway activation mediated by stromal-derived cytokines [13]. Thus, when a relapsed FLT3/ITD AML patient with circulating peripheral blasts and a packed bone marrow is treated with quizartinib, peripheral blasts undergo apoptosis relatively quickly, while bone marrow blasts undergo terminal myeloid differentiation over a few weeks. The differentiated cells leave the marrow, resulting in the mild surge of peripheral blood neutrophils and occasionally in a

differentiation syndrome. The marrow generally remains hypocellular from this point on, likely due to a combination of persistent MRD and ongoing c-KIT inhibition (which is myelosuppressive) [21]. The marrow is rarely cleared of the leukemic clone, as evidenced by the persistence of circulating neutrophils with the FLT3/ITD mutation, and by the eventual emergence of resistant clones. The failure to eradicate the leukemia initiating cells is consistent with recent genome sequencing studies suggesting that FLT3/ITD mutations occur as secondary or cooperating events in leukemogenesis [58].

The clearest evidence that all of the above described responses to quizartinib occur via inhibition of FLT3 is the emergence of leukemia cells expressing resistance-conferring point mutations in the *FLT3/ITD* allele [59]. Resistance mutations have been observed sporadically in patients treated with FLT3 inhibitors [60], but their relative scarcity was likely a reflection of the lack of sustained FLT3 inhibition provided by these other inhibitors. Point mutations within the codon for the D835 residue within the activation loop represented the most common type of mutation conferring resistance to quizartinib, but mutations at position F691 were also found. This has resulted in efforts to identify compounds with activity against these *FLT3* variants [21,61].

Conclusion

A number of lines of evidence accumulated over the past decade, including whole-genome sequencing studies, suggest that FLT3/ITD AML is a disease that evolves from diagnosis to relapse [43,58,62]. At diagnosis, the disease is polyclonal, with only a subset of cells truly dependent on the mutant FLT3 signaling for survival. At relapse, the disease is monoclonal or oligoclonal, and the blasts are usually addicted to the FLT3/ITD receptor activity, such that selective FLT3 inhibition will result in cell cycle arrest and/or apoptosis [33]. The quizartinib trials completed thus far have enrolled relapsed/refractory patients, patients whose leukemia cells are predominantly FLT3-addicted. The nature of the clinical responses observed have been entirely consistent with what might be predicted from the preclinical data. Pertinent to this is the fact that blasts from newly diagnosed FLT3/ITD AML patients do not routinely respond to quizartinib *in vitro.* How then should a drug such as quizartinib be incorporated into current treatment regimens? The current standard of care for a patient with FLT3/ITD AML who is eligible for intensive therapy is an induction course composed of infusional cytarabine combined with an anthracycline, with or without the addition of etoposide. Although still somewhat controversial, the preponderance of clinical data indicates that consolidation with an allogeneic transplant offers the patient the best chance at avoiding a relapse and achieving long-term survival [43,63]. The key, therefore, is to achieve a remission and maintain that remission long enough to proceed to a transplant. Newly diagnosed FLT3/ITD AML will go into remission will standard chemotherapy at a similar rate as other AML subytpes, although patients with a high mutant allelic burden at presentation will often be refractory, and even when remission is achieved, FTL3/ITD AML relapses quickly. Quizartinib could be used during induction to increase the remission rate, and to maintain that remission up to transplant. As quizartinib and other FLT3 inhibitors induce a cell cycle arrest, it may be preferable to administer the drug in sequence, after or towards the end of the cytarabine infusion [64]. Quizartinib could then be administered afterwards as maintenance therapy, in a manner similar to the BCR–ABL inhibitors in

Philadelphia chromosome-positive acute lymphoblastic leukemia. To establish the efficacy of this approach, it would be necessary to conduct a Phase III trial in which newly diagnosed FLT3/ITD AML patients receiving induction therapy and eventual transplant are randomized to receive quizartinib versus placebo. Indeed, two of the clinical trials described above are directed just towards this paradigm.

In summary, quizartinib is a rationally designed, orally administered, selective, potent inhibitor of FLT3. It has an ideal pharmacokinetic profile, with a long *in vivo* half-life and relatively low protein binding. Its off-target effects are limited to QT prolongation and inhibition of c-KIT. It produces a high response rate in relapsed/refractory FLT3/ITD AML and these responses are entirely consistent with what was predicted from pre-clinical studies. It represents a major advance in this field, and will hopefully be approved by regulatory agencies for the treatment of FLT3/ITD AML in the near future.

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Box 1

Relative selectivity of quizartinib: off-target kinases inhibited within fivefold of the K_d for the FLT3/ITD receptor

Quizartinib

- FLT3 (wild-type), KIT, PDGFRB, PDGFRA, RET and CSF1R

Sorafenib

- DDR1, HIPK4, ZAK, DDR2, RET, CSF1R, FLT1, PDGFRB, ERK8, VEGFR2, PDGFRA, TIE1, FLT4, YSK4, CDKL2, MKNK2, MUSK, CDK7, LOK, AURKC, MKNK1, p38-B, RAF1, EPHB6, CDK11, CDK11, TNNI3K and CDK8

Lestaurtinib

- PHKG1, YSK4, LATS2, SNARK, MKNK2, PLK4, PHKG2, PKN2, JAK3, MST2, CHEK1, TAK1, CLK4, MST1, AAK1, DRAK1, MAPSK15, PDPK1, MEK1, MLK1, MINK, ARK5, JAK2, MEK4, MEK2, AURKC, MAP4K2, MAP3K2, ERK8, ULK3, DRAK2, PKN1, TNIK, ERN1, LOK, MAP3K3, IRAKI,TTK, TNK2, GRK7 and GAK

Lists of different kinases that are inhibited by quizartinib, sorafenib and lestaurtinib within a fivefold range of the inhibition of the FLT3/ITD receptor are demonstrated. Data taken from [22].

EXECUTIVE SUMMARY

Identification & characterization of quizartinib

- Quizartinib is a bis-aryl urea derivative isolated using a phage display assay system.
- Quizartinib was pecifically optimized pharmacodynamically and pharmacokinetically to inhibit the FLT3/ITD mutant receptor.
- The only other receptors inhibited by quizartinib are in the type III receptor tyrosine kinase family–c-KIT, CSF1R and PDGF receptor.

Clinical results with quizartinib

- Quizartinib induces a high response rate in relapsed/refractory FLT3/ITD AML.
- Side effects of quizartinib include QT prolongation and myelosuppression from c-KIT inhibition.
- Long-term survival is achieved in younger patients by using quizartinib to bridge them to allogeneic transplant.
- Registration and chemotherapy combination trials of quizartinib are about to begin accrual.

The nature of the response to quizartinib

- Quizartinib clears peripheral blasts through induction of apoptosis.
- Quizartinib induces cell cycle arrest and terminal myeloid differentiation in marrow blasts.
- Differentiation syndrome, similar to retinoic acid syndrome, can be seen with quizartinib therapy.
- Responses induced by quizartinib are most commonly a complete remission with incomplete hematologic recovery, consisting of a minimal residual disease state.
- Resistance-conferring point mutations can emerge after response to quizartinb.

Table 1

Pharmacodynamic and pharmacokinetic parameters of quizartinib.

Drug	IC_{50} (medium); nM	IC_{50} (plasma); nM	*In vivo* half-life; h
Lestaurtinib	2	700	8
Midostaurin	6	1000	2
Sorafenib	3	265	24
Quizartinib	1	18	36+

Shown are values for inhibition of FLT3 autophosphorylation by immunoblot using Molm14 cells in either culture medium (RPM11640/10% fetal calf serum) or 100% human plasma.

Data taken from [22,34–38].