S. Amadori

Department of Hematology,
University Tor Vergata,
Rome, Italy

New agents for the treatment of acute myeloid leukemia: midostaurin

FLT3, a membrane-bound receptor tyrosine kinase (TK) expressed by immature hematopoietic cells, plays an important role in an early stage of hematopoiesis.[1] FLT3 is among the most commonly mutated genes in acute myeloid leukemia (AML). Approximately 30% of AML patients have mutations of FLT3 that result in constitutive phosphorylation and activation of FLT3, and subsequent activation of downstream signal molecules important for cellular proliferation, differentiation, and survival.[2] These mutations typically result in either an internal tandem duplication (ITD) of between 3 and 33 or more amino acids in the juxtamembrane region of the FLT3 protein (25% of patients), or a less common (5-10%) point mutation in the activation loop of the thyrosine kinase domaine (TKD).

The importance of FLT3 mutations in the pathogenesis of AML has been well established and, in most studies, these have been shown to confer an adverse prognosis. The outcome appears more unfavorable in the normal cytogenetic subgroup,[3] especially if associated with the loss of the normal allele.[4]

Recently, attention has been focused on developing small molecule inhibitors that target FLT3. Midostaurin (N-benzoylstaurosporine), formerly known as PKC412, is a potent inhibitor of both mutated and wild type FLT3, as well as of other molecular targets thought to be important for the pathogenesis of AML (VEGFR-1, PDGFR, c-kit, H- and K-ras, FGFR-1, and the multidrug resistant gene, MDR).[5-7] *In vitro*, midostaurin induces apoptosis in Ba/F3 cells that have been transformed to IL-3-independent growth by FLT3-ITD.[8] Furthermore, midostaurin is effective in FLT3-ITD-induced disease in a murine bone marrow transplantation model.[8]

Midostaurin has been investigated in phase 1 and phase 2 studies of various malignancies, and has shown a favorable toxicity profile with significant biologic activity. In a phase 1 study in 20 patients with relapsed or refractory FLT3-mutated AML, single-agent midostaurin at a dose of 75 mg 3 times daily by mouth was generally well tolerated.[9] Toxicities included grade 1-2 nausea/vomiting, diarrhea, fatigue and 2 patients developed fatal pulmonary events of unclear etiology. In an extension of the phase 1 study, there were two additional episodes of severe pulmonary toxicity both occurring in patients on antifungal azoles known to be potent CYP3A4 inhibitors. Because of this, patients were subsequently not allowed to receive antifungal azoles and no

further severe pulmonary events with unknown etiology were seen. Fourteen of the 20 patients (70%) achieved more than 50% reduction in peripheral blast counts, and 7 of them experienced a greater than 2-log reduction for at least 4 weeks. For reasons that are not fully understood, the bone marrow response was less evident with only 6/20 patients showing a greater than 50% reduction in marrow blast counts. FLT3 autophosphorylation in blast cells of responding patients was inhibited by >90% by day 3, thus demonstrating *in vivo* target inhibition.

Altogether, these results indicate that single-agent midostaurin has modest clinical activity in patients with advanced AML whose blasts have been shown to harbor an activating mutation of FLT3, although biological responses are common. While a decrease in peripheral and bone marrow blast cells are seen, there are few, if any, patients who achieve complete remission (CR). In part because of the lack of significant clinical activity as single agent, and because of *in vitro* data showing sequence-specific additive or synergistic interactions between FLT3 inhibitors and chemotherapy, combination trials are underway.[10]

In a phase Ib trial, Stone et al. treated newly diagnosed patients <60 years of age with midostaurin plus daunorubicin and infusional cytarabine (3+7 regimen) induction followed by high-dose cytarabine consolidation and maintenance midostaurin.[11] Results with the original dose schedules of midostaurin of 100 mg bid given either from day 8 continuously (arm 1) or day 1 continuously (arm 2), or on an amended schedule of day 8-21 (arm 1) or days 1-7 and 15-21 (arm 2) demonstrated poor tolerability due to nausea and vomiting. However, tolerability improved substantially once the dose of midostaurin was reduced to 50 mg bid. Of the 40 patients treated with the definitive schedule, 38 [26 with wild type FLT3 (FLT3wt) and 12 with mutated FLT3 (FLT3mut)] were evaluable for efficacy and 37 for safety. Overall, CR occurred in 27/38 (71%). The CR rate in $FLT3^{wt}$ patients was 18/26 (69%), with 9/13 (69%) patients achieving remission in each arm. CR occurred in 11/12 (92%) $FLT3^{mut}$ patients. The CR rate in $FLT3^{mut}$ patients was 7/7 (100%) in Arm 1, and 4/5 (80%) in arm 2. 4/11 CR patients relapsed after 7, 7, 10 and 15 months; 7 patients remained in first CR for 3-15 months. No drug-related deaths were reported, and tolerability was slightly better in patients receiving the sequential schedule compared to those receiving simultaneous midostaurin and chemotherapy. An international phase 3 trial of conventional chemotherapy plus or minus sequential midostaurin in newly diagnosed patients 18-60 years of age with $FLT3^{mut}$ AML is now in progress.

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A.K. Burnett

Department of Haematology, School of Medicine, Cardiff University, UK

Lestaurtinib as a FLT3 inhibitor

Overexpression of the FLT-3 receptor is common in Acute Myeloid Leukaemia (AML) and mutations represent one of the commonest mutations which occur in approximately 30% of adult cases, although less frequent in older patients. The most frequently detected mutation is an Internal Tandem Duplication (ITD) in the juxtamembrane position of the receptor (24%), and a point mutation in the activation loop usually at positive 385 (7%). The mutations are in frame and constitutively activate via STAT5. Mutations are unevenly distributed in FAB and cytogenetic groups. They have highest frequency in Acute Promyelocytic Leukaemia (35-40%), and are associated with a normal karyotypic or trisomy 8. They are less frequent in poor risk karyotypes or in core binding leukaemias. The association with normal karytoype has the additional interest of being associated with mutations of the nucleophosmin 1 mutation in approximately two-thirds of cases. Whether these mutations are themselves leukaemogenic is thought to be unlikely.

The major clinical interest is that the presence of an ITD predicts a significantly increased risk of relapse, but does not reduce the prospect of initial response. The frequent association with the APL subtype, which is highly curable, does not significantly reduce survival. Its impact in that context is difficult to distinguish from the presence of a high white count. Point (TK) mutations do not have an adverse prognosis, and indeed may indicate a favourable feature. The mutation characteristics patients with proliferative disorders ie with high WBC's and hypercellular marrows. Several agents with pre-clinical *in vivo* and *in vitro* activity have been developed. None of the agents who are furthest down the clinical development path are specific for FLT3 mutations. Lestaurtinib (CEP-701) is a small molecule kinase inhibitor which has shown impressive preclinical activity in *in vivo* models, against cell lines bearing the mutation and against primary cells where it is active against both mutated and non-mutated cases, although at a lower IC against mutants.

In phase I/II studies which recruited mutated AML cases with relapsed disease a maximum tolerated dose was not reached at doses capable of inhibition. Clinical activity was seen as clearance of blasts in approximately half of patients and reduction in bone marrow blasts but no complete remissions. It was suggested that greater benefit would be seen in combination with standard chemotherapy, and some pre-clinical studies suggested