Midostaurin added to standard therapy in \textit{FLT3}-positive acute myeloid leukaemia treatment

Andrzej Szczepaniak, Zuzanna Rzetelska

Department of Haematology and Bone Marrow Transplantation, Medical University of Poznan, Poznań, Poland

Abstract

Acute myeloid leukaemia (AML) is a complex disease with a dynamic course associated with a series of acquired and cumulative genetic changes. In recent years, significant advances have been made in the understanding of its pathogenesis. Moreover, diagnostic and therapeutic options have expanded. The current classifications consider cytogenetic and molecular disorders, including the presence of, among others, mutations within FMS-like tyrosine kinase 3 (FLT3) transmembrane tyrosine kinase, regulating the proliferation and differentiation of hematopoietic cells at an early development stage. FLT3 mutation is detected in approximately 30\% of newly diagnosed AML cases and concerns mutations: internal tandem duplication (ITD) or tyrosine kinase domain (TKD) gene. The high ratio of FLT3-ITD mutation is associated with an unfavourable prognosis. It is recommended to include patients in clinical trials due to insufficient standard therapy effects. The new AML treatment strategies include first- and second-generation tyrosine kinase inhibitors. Midostaurin, a non-specific kinase inhibitor, was approved in 2017 for treatment of patients with newly diagnosed FLT3-positive AML in combination with standard chemotherapy. The paper presents the experience of the Department of Haematology and Bone Marrow Transplantation in Poznan in the use of FLT3 tyrosine kinase inhibitors based on a case report of two patients with newly diagnosed AML.

Key words: acute myeloid leukaemia, mutation \textit{FLT3}, tyrosine kinase inhibitors, midostaurin

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Introduction

Acute myeloid leukaemia (AML) is a complex, dynamic disease, characterized by multiple acquired somatic mutations, the coexistence of competing clones, and evolution over time. Genetic alterations include amplifications, deletions, gene rearrangements, and point mutations. Tremendous progress has been made recently in understanding disease pathogenesis, and diagnostic as well as therapeutic options. Both the current World Health Organization (WHO) 2016 classification and the European LeukaemiaNet (ELN) risk stratification [1, 2].

FMS-like tyrosine kinase (\textit{FLT3}) is a transmembrane tyrosine kinase activated by binding the ligand to the receptor and expressed on hematopoietic and progenitor cells. It plays an important role in the early stages of myeloid and lymphoid lines development. The attaching extracellular ligand stimulates cell survival, proliferation and differentiation through PI3K-, RAS-, and STAT5-related signalling pathways. The \textit{FLT3} mutation is detected in approximately 30\% of newly diagnosed AML. Internal tandem duplication (ITD) mutation constitutes about 25\% cases, resulting in the...
duplication of 3 to 100 amino acids attached to the cell membrane, and 7–10% of cases include point mutations of the tyrosine kinase domain (TKD) in codons D835 and I836 or deletion in the codon I836. The mutation causes ligand-independent kinase activation, resulting in the proliferation and survival of leukemic cells. For the FLT3-ITD gene, it is also important to calculate the wild-to-mutant FLT3-ITD allelic ratio (AR), as its prognostic significance has been proven in patients with AML. The presence of the FLT3-ITD mutation is associated with hyperleukocytosis at diagnosis, worse prognosis, higher relapse rate and shorter survival [3]. The results of treatment of AML FLT3 with standard chemotherapy are suboptimal. The understanding of mutations in the genes encoding the FLT3 tyrosine kinase led to the development of targeted inhibitors. The first generation includes tandutinib, sunitinib, lestaurtinib, sorafenib and midostaurin, and the second generation quizartinib, crenolanib and gilteritinib. These compounds differ in respect of the selectivity of FLT3 kinase inhibition and toxicity profile. Midostaurin is a non-specific kinase inhibitor originally developed for the treatment of solid tumours. In the clinical trials using midostaurin as monotherapy in patients with refractory/relapsed AML (R/R AML), as in the case of other inhibitors, a weak anti-leukemic effect was found; however, relatively better tolerance was the advantage [4]. The breakthrough studies were RATIFY and AMLSG 16-10, which showed the effectiveness of midostaurin in combination with intensive chemotherapy in terms of overall survival (OS) [5, 6]. These studies became the basis for drug registration in 2017 in the treatment of newly diagnosed AML with the FLT3 mutation and the development of currently applicable therapeutic recommendations [7, 8]. The presented publication presents the experience of the Department of Haematology and Bone Marrow Transplantation in Poznań in the use of FLT3 tyrosine kinase inhibitors, based on two case reports of patients with newly diagnosed AML.

**Case reports**

**Case 1**

A 36-year-old female patient was referred to the haematology department in July 2019 with suspected AML. In the medical history, the patient reported weakness and pharyngitis treated ineffectively with empiric antibiotic therapy. So far, she has not been chronically ill. Upon admission, the patient was in good general condition first grade of performance status according to Eastern Cooperative Oncology Group (ECOG) [1]. Laboratory tests confirmed hyperleukocytosis [white blood cells (WBC) 176.3 G/L], anaemia [haemoglobin (Hb) 4.4 mmol/L], thrombocytopenia [platelets (PLT) 95 G/L]. In addition, biochemical tests showed increased activity of lactate dehydrogenase [(LDH) 1117 U/L], as well as C-reactive protein [(CRP) 113.7 mg/L] and D-dimers (27,171 ng/mL) levels. The cytology and flow cytometry (FCM) evaluation confirmed 95% infiltration by blast cells with leukaemia-associated immunophenotype (LAIP): CD13+, CD33+, CD117+, HLA DR(dim), CD38+, CD31+, CD11c+, CD64+, CD4+, CD123+, MPO(−). The cytogenetic test revealed a normal karyotype (46, XX), and the fluorescent in situ hybridization (FISH) method excluded the presence of PML-RARA and CBFB-MYH11-gene fusions. In molecular tests with the polymerase chain reaction (PCR) method, the presence of the FLT3-ITD (AR 0.66) and NPM1 mutations were confirmed and CEBPA, RUNX1-RUNX1T1, and BCR-ABL mutations were excluded. Due to the hyperleukocytosis and leukemic cells phenotype (monoblastic), cerebrospinal fluid (CSF) analysis was also performed, showing involvement of the central nervous system (CNS) by AML cells with the baseline phenotype (12%). The diagnosis of AML with NPM1 mutation according to WHO 2016 classification was established. The patient was classified as an intermediate-risk group according to ELN 2017 due to the presence of FLT3-ITD (high ratio) and NPM1 mutations with normal karyotype. Abdominal screening ultrasound revealed hepatomegaly (16 cm), fluid in the pouch of Douglas (24 mm) and a trace fluid in both pleural cavities. The patient was qualified for intensive induction therapy according to DA-60 protocol (daunorubicin 60 mg/m² on days 1–3 and cytarabine 200 mg/ m² on days 1–7 during a 24-hour infusion) along with intrathecal drugs administration (cytarabine, dexamethasone, methotrexate). Due to at this time of reimbursement for midostaurin in Poland, an application for the drug was submitted to a pharmaceutical company. According to complex administrative procedures, it was not possible to add midostaurin during the induction phase of chemotherapy. After induction treatment, a complete response (CR) was achieved with minimal residual disease (MRD) of 1.8%. In consolidation therapy, high doses of cytosine arabinoside (HD-Ara-C) 3 g/m² on days 1, 3, 5) were used in combination with midostaurin at a dose of 50 mg every 12 hours, administered orally on days 8–21. The
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The cytogenetic examination revealed a normal karyotype (46,XY), and the FISH method excluded the presence of PML-RARA and CBFB-MYH11 gene fusions. In molecular tests using PCR, the presence of the FLT3-ITD mutation (AR 0.68) was confirmed and FLT3-TKD D835, NPM1, CEBPA, RUNX1-RUNX1T1, and BCR-ABL mutations were excluded. Moreover, in the study with the next-generation sequencing (NGS) method, the presence of the RUNX1, TET2, and DNMT3A mutations was demonstrated. Based on this, the diagnosis of AML with the RUNX1 mutation was established (provisional entity according to WHO 2016 classification), morphologically without signs of maturation. The patient was classified as an unfavourable prognostic group according to ELN 2017. In the echocardiography performed before the treatment, asymmetric septal hypertrophy and left ventricular relaxation disorders with normal contractility were described. The patient was qualified for intensive induction treatment according to DA-90 protocol (daunorubicin 90 mg/m² on days 1–3 and cytarabine 100 mg/m² in 24-hour infusion on days 1–7). Due to at this time of reimbursement for midostaurin in Poland, an application for the drug was submitted to a pharmaceutical company. Due to complex administrative procedures, it was not possible to add midostaurin during the induction phase of chemotherapy. Early haematological evaluation on day 14 showed aplastic bone marrow with no increased blasts in FCM. During the neutropenia, fever was present which was resolved with empiric antibiotic therapy. The evaluation after induction treatment showed a CR with an MRD of 0.3%. Due to the high cytogenetic and molecular risk, the patient was qualified for allo-SCT and the procedure of selecting an unrelated donor was initiated under the protocol used in the transplant centre. The patient then received post-regeneration treatment: 2 cycles with HD-Ara-C at the dose of 2 g/m² on days 1, 3, and 5 in combination with midostaurin at a dose of 50 mg every 12 hours administered orally on days 8–21. In FMC evaluation after the second consolidation cycle, MRD was negative (0.004%), and there was no FLT3-ITD mutation in the PCR test. However, in the assessment immediately before the allo-SCT procedure, an increase in MRD of up to 0.7% was observed and a reappearance of the FLT3-ITD mutation. After conditioning according to the FluBu2 regimen (fludarabine, busulfan), a stem cell transplant from a matched unrelated donor was performed in September 2020. In the prevention of graft-versus-host disease (GVHD), anti-thymocyte globulin (ATG), cyclosporin and methotrexate were used. The post-regeneration evaluation showed...
CR with MRD and the mixed donor chimerism of 93%. Immunosuppressive treatment was limited and active MRD monitoring was implemented. Symptoms of GvHD were not observed.

Discussion

The first evidence of an unfavourable prognosis associated with the FLT3-ITD mutation comes from retrospective studies that compared the presence of the mutation at diagnosis and in possible relapse. A significantly higher frequency of mutations present in resistant patients indicated the persistent subclone as the cause of relapse [9]. Three-quarters of patients with FLT3-ITD mutation at diagnosis also show its presence in the relapse, with an increasing AR ratio. In the case of the FLT3-ITD mutation, the relapse rate and shorter OS depend mainly on AR value. The prognosis is also influenced by the coexistence of other mutations, including NPM1 mutation, found in approximately 30% of patients. Detection of NPM1 mutation in the absence or with an FLT3-ITD mutation with a low AR (< 0.5) is associated with a favourable prognosis. The presence of FLT3-ITD mutation with a high AR (≥ 0.5) together with the NPM1 mutation indicates an intermediate prognosis, while the wild-type of NPM1 gene results in an unfavourable prognosis [1, 10]. The coexistence of other high-risk mutations (e.g. TP53, KMT2A) also significantly worsens the prognosis. The prognostic significance of the TKD mutation is less well documented.

According to the current standard, FLT3 mutation status should be assessed in every AML patient within 48–72 hours [1]. Although routine testing for the presence of FLT3 mutation has been recommended by ELN since 2010, the diagnostics methods differ significantly between centres [11]. The challenge for diagnosticians is the technique of determinations and the lack of appropriate standardization. So far, the use of AR determinations mainly in clinical trials, less frequently in daily clinical practice, may be a challenge both in the determination and interpretation of the obtained results concerning insufficiently validated reference values. It seems necessary to develop international testing standards [12]. The presence of FLT3 mutation may evolve in the course of the disease, therefore it is suggested that genetic tests should be repeated at various stages of treatment, especially at the time of disease recurrence. To date, the exact value of the AR cut-off point, above which the unfavourable prognosis and the risk of relapse increase, is ambiguous. The value above which a shorter OS was observed significantly differs depending on the studies (AR 0.51 and 0.78) [13, 14]. In the case of low and intermediate AR values, the prognostic significance is unconfirmed [14]. Absent at diagnosis FLT3 gene mutation may reveal itself in relapse and worsen the prognosis (this applies more often to FLT3-ITD than FLT3-TKD mutation, 8% vs. 2%, respectively). According to the currently applicable standards, FLT3 mutation cannot be used for MRD monitoring due to the lack of sufficient standardization of determinations [1]. However, the analysis of FLT3-ITD mutation status monitoring with NGS in patients treated in AMLSG 16-10 study, presented at the 62nd American Society of Haematology (ASH) Annual Meeting, seems to be a promising diagnostic tool, although still unavailable in routine practice [15].

The treatment of newly diagnosed AML with the presence of FLT3 mutation is one of the major challenges in the daily practice of haematologists. The results of treatment with the first FLT3 inhibitors in monotherapy were disappointing as their effects were limited to an only transient decrease in blasts percentage. The effects of combining with standard chemotherapy remain the subject of clinical trials. In the SORAML trial with the use of sorafenib in combination with standard chemotherapy in patients with the newly diagnosed disease, an extended event-free survival (EFS) was achieved, but without a significant effect on OS [16]. The combination of sunitinib with conventional chemotherapy during induction and consolidation allowed to obtain CR in 50% and 38% of patients with FLT3-ITD and FLT3-TKD mutations, respectively [17]. Only the introduction to the treatment of newly diagnosed AML patients midostaurin with standard chemotherapy (in the group of 360 patients up to 60 years of age), for the first time in many years resulted in a statistically significant EFS [median 8.2 vs. 3 months, hazard ratio (HR) 0.78; p = 0.002] and OS (median 74.7 vs. 25.6 months; HR 0.78; p = 0.009) prolongation as compared to chemotherapy alone. The 4-year survival ratio in the study groups was 51.4% to 44.3% in favour of the group receiving the study drug. The CR rate was higher in patients taking midostaurin, but without statistical significance. In a posthoc analysis of all reported CRs within 30 days of the end of the intervention, the ratio was 68% versus 59% (p = 0.04) [18]. Limited access to midostaurin in Poland, lack of reimbursment at the time when our patients were treated, resulted in a delay in treatment initiation and insufficient
experience in haematology centres. This may translate into the effects of the treatment and the depth of the responses obtained. In both of the presented cases, midostaurin was administered in combination with high doses of cytarabine, as the drug was unavailable at the induction chemotherapy phase. However, despite this, the use of combination therapy allowed for remission (case 1) and negative MRD (case 2).

There is no single standard of care in the case of relapse and primary refractory disease, and the treatment of this group of patients remains a huge challenge. Recruitment to clinical trials should be a priority. The efficacy of midostaurin in the group of patients with R/R AML has not been confirmed [4]. The use of lestaurtinib in refractory patients did not show any improvement in response rates or increased OS [12]. In phase I clinical trial with R/R AML patients receiving sunitinib, only a short-term partial response was achieved [19]. In the QuANTUM-R study, the survival of patients in the quizartinib group was similar to that of patients receiving salvage chemotherapy, while the toxicity of the treatment turned out to be unacceptable [20]. The hopes were raised by the results of the ADMIRAL study, in which the use of gilteritinib compared to placebo resulted in OS extension (9.3 vs. 5.6 months, respectively) and a higher CR and CRi rate (35% vs. 15.3%, respectively) [21]. Also, the percentage of patients who qualified for allo-SCT was higher in the gilteritinib group. This study became the basis for the registration of the drug was unavailable at the induction chemotherapy phase. However, despite this, the use of combination therapy allowed for remission (case 1) and negative MRD (case 2).

Based on the above results, it seems justified to implement maintenance treatment with an FLT3 kinase inhibitor in the second presented case to reduce recurrence risk. This therapy, however, is still not registered or reimbursed for this indication.

In conclusion, targeted therapy with midostaurin in combination with chemotherapy for AML with FLT3 mutation has become the current standard of care, however, the incidence of disease relapses remains a serious problem. Therefore, it is necessary to conduct further studies on the use of tyrosine kinase inhibitors and combination therapy.

References


