A Novel FLT3 Y842D Mutation Carries a Potentially Highly Sensitivity to Midostaurin? Case Report and Literature Review

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Short report

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Abstract

**Background:** Y842D mutation in the activation loop of FLT3 caused a strong resistance to Sorafenib in vitro, whether this kind of mutation would exert clinical significance on acute myeloid leukemia (AML) patients remain to be clarified.

**Case presentation:** Here, we described a novel type of activating mutation (Y842D) within the kinase domain of FLT3 in a pregnancy with de novo acute myeloid leukemia. Following induction failure with standard dose of idarubicin and cytarabine (IA), the patient received a re-induction combined with midostaurin, a promising agent targeting mutant-FLT3, and IA regimen. Fortunately, morphological remission was achieved. During the period of midostaurin treatment, the patient exhibited a symptom which was quite similar to the differentiation syndrome which disappeared following the treatment with methylprednisolone.

**Conclusions:** Clinically, we showed for the first time that Y842D, a novel activating mutation in the activation loop of FLT3, tend to be a sensitive mutation form to midostaurin in acute myeloid leukemia patients.

Introduction

FMS-like tyrosine kinase 3 (FLT3) is a type 3 receptor tyrosine kinase. Certain alteration of this protein can constitutively active receptor tyrosine kinase causing uncontrolled proliferation, reduced apoptosis, and malignant transformation of myeloid cells\(^1\). Thus, mutant FLT3 is well known as a promising target for molecular therapy in acute myeloid leukemia (AML)\(^\[1\].2\]. FLT3 mutation occurred in 30% of de novo AML, with internal tandem duplication (FLT3-ITD) in juxtamembrane domain observed in 75% of mutant patients and point mutation within the activation loop of tyrosine kinase domain (FLT3-TKD) in the rest of 25% patients\(^3\). Based on previous clinical research, even hematopoietic stem cell transplantation (HSCT) cannot reverse the adverse effect of FLT3-ITD on AML patients\(^4\). Given the rare probability of FLT3-TKD mutation in AML, it is reasonable that no large sample randomized controlled clinical trial was reported. However, the current results shown that the negative impact of TKD mutation seems comparable to that of ITD with regard to disease free survival time (DFS) in patients with AML\(^5\).

D835 was the most common mutation site in FLT3-TKD, other types of mutation such as 836 and 840, could also be found in AML patients\(^6\). Y842 was a key residue in regulating exon 20 of FLT3, which formed a hydrogen bond with Asp811, the latter in turn was linked to Arg834 through an ion pair which leads to closed (inactive) conformation of the FLT3 activation loop\(^7\). When 842 tyrosine residue was substituted by cysteine (FLT3-Y842C), the FLT3 activation loop switched from closed conformation to the open (active) conformation and led to constitutive activation of FLT3 and signal transducer and activator of transcription 5 (STAT-5) tyrosine phosphorylation, thus contributed to malignant transformation of myeloid cells\(^8\). Consequently FLT3-Y842C was a potentially sensitive mutation type to sorafenib, a multitargeted kinase inhibitor which inhibited activation of FLT3 signaling and suppressed proliferation.
of leukemia cells in preclinical models[8]. However, in vitro analyses showed replacement of 842 tyrosine residue with Aspartic acid (Y842D) possessed different biological characteristics comparing with Y842C, as Y842D mutant cells were much more resistant to sorafenib[9].

Here, we described the characterization of a novel type of activating mutation (Y842D) within the kinase domain of FLT3. To the best of our knowledge, this was the first reported de novo novel case with mutant Y842D and who achieved complete remission (CR) after re-induction with midostaurin and IA regimen (idarubicin and cytarabine). Our result showed that Y842D in FLT3-ITD tend to be a sensitive mutation form to chemotherapy regimen containing midostaurin.

Case Presentation

A 29-year-old, gravida 1 parity 0 woman at 18 weeks of pregnancy admitted to our hospital for intermittent fever for a week on August 30th, 2020. Routine blood test (RBT) showed white blood cells (WBC) count of 203.39×10^9/L (normal range: 4.0–10×10^9/L), hemoglobin (Hb) count of 78g/L (normal range: 120-175g/L), and platelets (PLT) count of 96×10^9/L (normal range: 100–350×10^9/L). Notably, a month ago, on July 21st, 2020, the patient has undergone RBT during her pregnancy examination which revealed WBC of 7.63×10^9/L, Hb of 121g/L, and PLT of 193×10^9/L. In just 40 days, the patient's white blood cells increased by 25.66 times (Fig. 1). Meanwhile, blood cell smear indicated 95% blast cells in peripheral blood, bone marrow smear showed extremely active hyperplasia with 91% blast cells, the proliferation of granulocytes, macrophages, megakaryocytes and erythroblasts were significantly inhibited. Blast cells were strongly positive for esterase staining which could be inhibited by sodium fluoride. Flow cytometry revealed that myeloid blasts exhibited monocytic differentiation tendency (CD34-, CD117-, CD33+, CD64+, CD4 dim, CD13-, HLA-DR+). Karyotypic analysis of bone marrow cells expressed the translocation of t (8;22) (p11.2; q13) (Fig. 2). Gene mutation detection showed that FLT3 point mutation T2524G which led to replacement of alanine by aspartic acid at site 842 within the receptor tyrosine kinase domain (FT3-TKD, Y842D), allelic ratios was 36.4%. Meanwhile, another mutant gene, KMT2D, was detected with allelic ratios 46.6%. Comprehensively, the patient was diagnosed as AML-M5 and classified into unfavorable category according to French–American–British (FAB) classification systems and 2019 version of National Comprehensive Cancer Network (NCCN)[10].

Hydroxyurea was used to dealing with hyperleukocytosis and artificial abortion was conducted prior to induction chemotherapy. On September 14, first induction therapy was carried out using cytarabine (100 mg/m^2/d, d1-7) and idarubicin (10 mg/m^2/d, d1-3). On October 12, 8.8% primitive monocytes and 30% immature monocytes still resided in bone marrow according to bone marrow smear analysis. Meanwhile flow cytometry revealed 35.2% myeloid blasts showing monocytic differentiation tendency. To obtain better clinical efficacy, FLT3-ITD inhibitor midostaurin (50mg, q12h, d8-21) combined with IA regimen was adopted in the re-induction therapy on October 14. On October 30, 10 days after midostaurin treatment, the patient suffered from fever, body temperature gradually raising from 38.1 to 40.2 degrees Celsius. At the same time, skin rash accompanied by pruritus spread from both lower extremities rapidly to the whole
body, including face, and continued to progress 3 to 4 days (Fig. 3). Blood biochemistry test revealed glutamic-pyruvic transaminase and glutamic oxalacetic transaminase reached 89U/L and 160U/L, respectively. Following treatment with methylprednisolone for a week, on November 6 patient gradually recovered from skin rash. November 11, no primitive monocytes and immature monocytes were found in bone marrow, meanwhile minimal residual disease (MRD) revealed myeloid blast cells account for less than 0.1% of non-erythroid cells of the bone marrow, namely morphological remission was achieved. Considering the negative effect of FLT3-TKD on clinical prognosis, another cycle of consolidation therapy with cytarabine (1.5g/m2, q12h, d1, 3, 5) and midostaurin was conducted, then the patient was subjected to hematopoietic stem cell transplantation (HSCT). Her whole disease course and clinical outcomes were summarized in Fig. 4.

Discussion

The past 30 years have witnessed the thriving development of molecular related fundamental researches on hematopoietic cells, which in turn greatly enriched therapeutic strategies for acute myeloid leukemia and led the priority treatment of leukemia entered the era of molecularly guided precision therapy\textsuperscript{[11–13]}. Take the treatment of acute promyelocytic leukemia (APL) as an example, application of all-trans retinoic acid and arsenous cured about 90% of APL patients\textsuperscript{[14]}. Undoubtedly, therapies based on genetics and epigenetics changes played an increasing role in the treatment of non-M3 AML. In combination with standard 3 + 7 regimen, molecular targeted agents were supposed to improve remission rate of induction therapy effectively and provide more opportunities for subsequent HSCT\textsuperscript{[14]}.

Mutant FLT3 contained two kinds of mutation forms, internal tandem duplication (ITD) in juxtamembrane domain and point mutation within the activation loop of tyrosine kinase domain (TKD)\textsuperscript{[2]}. FLT3 mutation occurred in 30% of de novo AML patients and accounted for the most predominant mutation type in AML\textsuperscript{[1]}. Notably, FLT3-ITD was considered as an independent unfavorable risk factor in AML, for patients with FLT3-ITD always be resistant to chemotherapy and present a higher relapse rate and more early death cases. Though the clinical significance of FLT3-TKD on AML remains to be confirmed due to relative rare incidence, meta-analysis showed that FLT3-TKD exerted similar unfavorable effect on AML as FLT3-ITD\textsuperscript{[5]}.

Generally, patients with mutant FLT3 frequently accompanied with hyperleukocytosis\textsuperscript{[15]}. Nevertheless, the effect of each mutation form, particularly Y842D, on clinical phenotype remains to be clarified. Back to the case we reported, in just 40 days, patient’s white blood cell count raised from 7.63×10\textsuperscript{9}/L to 203.39×10\textsuperscript{9}/L. The doubling time was as short as 8.45 days, which was very similar to the clinical characterization of patients with FLT3-ITD. It was generally known that consecutive activation of FLT3 and downstream signaling pathways resulted in uncontrolled proliferation of hematopoietic cells. According to Kindler T\textsuperscript{[8]} research, point mutation at Y842 altered the conformation of FLT3 receptor tyrosine kinase domain and drove the formation of activation loop. Mediated by downstream signaling pathway, including STAT5, myeloid cells with Y842C acquired the ability to proliferate indefinitely. Murine
32D cells transfected with human FLT3-Y842C showed activated FLT3 and STAT5 signaling pathway which can be inhibited by FLT3 inhibitor (N-benzoyl staurosporine, PKC412). The above results demonstrated that mutation of FLT3-Y842 played a critical role in the development of AML. Comprehensively, it was reasonable to deduced that hyperleukocytosis of the patient might attribute to continuous activation of FLT3 caused by Y842D.

Another important biological characteristic of FLT3-Y842D mutant AML cells was that they may be resistant to certain FLT3 inhibitors. Through cultivating cells with different gradient concentrations of FLT3 inhibitors, researchers successfully screened out FLT3 inhibitors-resistant FTL3 mutant cells. Base on this screening experiment, von Bubnoff N and his colleagues found that, at the same oncentration, myeloid cells with FLT3-Y842D was resistant to sorafenib instead of midostaurin $[^9]$. This may be the reason why sorafenib was only used in the treatment of FLT3-ITD mutant AML patients$[^{16}]$. In a phase III, randomized control clinical trial, researchers compared induction therapy effect of daunorubicin (60mg/m2, d3-5), cytarabine (20mg/m2, d1-7) with or without midostaurin in 717 AML patients with mutant FLT3, including 162 patients with FLT3-TKD. Statistically, the median overall survival time in midostaurin group was 74.7 months (95%CI:31.5-not achieved), significantly better than 25.6 months (95%CI:18.6–42.9) in placebo group. Meanwhile, the incidence of adverse events between the above two groups revealed no significant difference$[^{14}]$. That was also a critical reason for clinical application of midostaurin in AML patients with FLT3 mutation and why we chose midostaurin, cytarabine, and idarubicin as re-induction regimen in this case. 23 days after re-induction, morphological remission was achieved, which might indicate that patient with FLT3-Y842D potentially be sensitive to midostaurin.

The third characteristic of this case was that the patient presented with differentiation syndrome on the 10th day of midostaurin treatment. Red nodular rash firstly appeared in symmetrical parts of both lower limbs, accompanied by pruritus and fever. This type of rash often occured in patients treated with selective FLT3 inhibitors, such as cuzatinib, gliptinib$[^{17},^{18}]$. Pathologically, FLT3 inhibitors induced differentiation of primordial cells into neutrophils, this rash was exactly caused by the infiltration of neutrophils in deep skin and subcutaneous tissues$[^{18}]$. Take AML patients who received cuzatinib monotherapy as an example, though the proliferative degree of nucleated cells did not alter significantly 29 days after therapy, the proportions of cells with different degrees of differentiation changed a lot. The proportion of primordial cells decreased from 77–6%, the proportion of myelocyte, metamyelocyte, and neutrophils raised from 9–57%. Since the mutation frequency of FLT3-ITD did not changed, it was sufficiently to conclude that the mature neutrophils derived from differentiation of primordial cells$[^{17}]$.Mc Mahon cm and his colleague evaluated the validity of gliptinib in 21 AML patients with FLT3 mutation, 10 patients suffered from differentiation syndrome during treatment. The overall survival time and morphological remission rate of these 10 patients was much more worse than that of the other 11 ones$[^{19}]$. Similar with the prevention of retinoic acid-induced differentiation syndrome, it was confirmed that, combination of glucocorticoid with FLT3 inhibitor not only upregulated the expression of proapoptotic protein Bim, but also induced leukemia cell apoptosis by promoting the degradation of antiapoptotic protein Mcl-1$[^{20}]$. Therefore, FLT3 inhibitor combined with glucocorticoid in the prevention of
FLT3 inhibitor induced differentiation syndrome needed further study. In addition, patient in this report did not concurrent with rearrangement of NPM1 or CBF genes, which were also regarded as important unfavorable prognostic factors in AML\textsuperscript{[21]}. To sum up, it was sensible to treat this patient with allogeneic-HSCT as soon as possible, and maintenance therapy after transplantation with midostaurin would further improve the clinical outcome.

In conclusion, this case showed that FLT3-Y842D mutation was a new activating mutation form of FLT3 in AML. Patients with this mutation tend to be sensitive to midostaurin. In the process of midostaurin treatment, we should be alert to the existence of differentiation syndrome, once observed, glucocorticoid should be used as soon as possible. It is notable that allogeneic hematopoietic stem cell transplantation should be conducted since patients achieved complete remission as soon as possible.

**Declarations**

**ETHICS STATEMENT**

Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

**Availability of data and materials**

The raw data supporting our findings can be requested from the corresponding author.

**Ethics approval and consent to participate**

Not applicable.

**AUTHOR CONTRIBUTIONS**

MJ composed the manuscript and performed literature review. SJ did the acquisition and analysis of laboratory data for the work. JY and WQ did the acquisition and analysis of laboratory data for the work. YL critically revised and interpreted the data. PY took care of the patient from the clinical point of view. MJ and SJ wrote the manuscript while YP fully revised and improved it. All authors contributed to the article and approved the submitted version.

**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**References**


At the time of diagnosis, white blood cell count of the patient was about 26 times more than that of 40 days ago.
Figure 2

The karyotype of the patient at the time of diagnosis.
Figure 3

Diffuse red subcutaneous nodules can be seen on the abdomen.

Figure 4
Timeline of clinical events. IA regimen: cytarabine and idarubicin; NR, No Remission; DS: Differentiation syndrome.