VIA MEDICA

CASE REPORT

Hematology in Clinical Practice
2022, vol. 13, no. 1, 23–30
DOI: 10.5603/HCP.2022.0004

ISSN 2720–1015
e-ISSN 2720–2690

Efficacy of midostaurin combined with intensive chemotherapy followed by allogeneic hematopoietic stem cell transplantation in a patient with *NPM1* and *FLT3*-TKD mutated acute myeloid leukaemia with clinical high-risk features

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Abstract

Many genetic disorders occur in patients suffering from acute myeloid leukaemia (AML). The most common mutations found in such patients are in the nucleophosmin (NPM) gene and the FLT3 tyrosine kinase receptor gene. NPM1 mutation is observed in 30–35% of adult AML patients (50––60% of AML with normal karyotype), showing a favourable prognosis. The presence of point FLT3 comutations occur in the tyrosine kinase domain (TKD) are associated with even more favourable prognosis. However FLT3 comutations i.e. internal tandem duplication (ITD) in particular with high allelic ratio (ITD^{high}) worsen the course of leukemia and treatment outcomes. Deploying FLT3 tyrosine kinase inhibitors thus offers a prospect for improving treatment and prolonging the survival of patients with AML, burdened with the FLT3 gene mutation. Midostaurin and gilteritinib are type I FLT3 inhibitors which are used to treat patients with AML FLT3-TKD due to their mechanism of action.

This paper presents the case of a 30-year-old AML patient diagnosed with NPM1 and FLT3-TKD mutations, bone marrow reticuline fibrosis and extramedullary sites of AML. Treatment was individualized and induction chemotherapy was combined with midostaurin. After first-line treatment with midostaurin, complete remission was achieved, as confirmed by histopathological examination of the bone marrow. Subsequently, two cycles of consolidation chemotherapy were given, and allogeneic haematopoietic stem cells were transplanted from an unrelated donor after myeloablative conditioning. The patient has remained in complete leukaemia remission, 3 years after diagnosis.

Key words: acute myeloid leukaemia, *FLT3*-TKD, *NPM1*, midostaurin

Hematology in Clinical Practice 2022; 13, 1: 23–30

Introduction

Current knowledge suggests that the most important and independent prognostic factors in patients with acute myeloid leukaemia (AML) are cytogenetic and molecular aberrations in leukaemia cells. The dynamic development of genetics and the improvement of diagnostic methods in recent

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years have contributed to a better understanding of the biology of this disease, the discovery of new genetic disorders, and the introduction of targeted drugs into clinical practice [1–4]. Targeted therapy consists of the inhibition of specific signalling pathways involved in the processes of oncogenesis using compounds that selectively modify the properties of cancer cells or their surrounding environment [5]. The selective action of drugs against leukaemia cells optimizes the treatment, which translates into better outcomes and survival [1, 3].

The largest group of patients with AML include individuals with intermediate cytogenetic risk (about 60–70%) and with normal karyotype (about 40–45%), in whom molecular assessment of genetic alteration is important in diagnosis, prognosis and choosing the right therapy [6–8]. Apart from cytogenetic analyses, at the time of diagnosis in AML patients, tests for mutations in the following genes are advised and recommended: *NPM1*, *FLT3*, *CEBPA*, *RUNX1*, *TP53*, and *ASXL1* [6].

The *FLT3* (fms-like tyrosine kinase 3) gene encodes a receptor tyrosine kinase found in haematopoietic progenitor cells (HPCs). Under physiological conditions, regulatory proteins with tyrosine kinase activity are critical regulators of signal transduction pathways that control cellular functions including, but not limited to, growth, differentiation, cell cycle, and transcription. Mutations within the *FLT3* gene are responsible for the constant activation of the tyrosine kinase receptor, uncontrolled proliferation of leukemic cells, inhibition of apoptosis and activation of signalling pathways [9, 10]. Internal tandem duplication (ITD) and point mutations in the tyrosine kinase domain (TKD) are the most common *FLT3* gene alterations in AML patients [11–13].

The *FLT3*-ITD mutation is found in approximately 20–30% of AML patients, in a cytogenetic group with both good and intermediate as well as poor prognosis [8, 14]. The presence of the *FLT3*-ITD mutation is an independent, unfavourable prognostic factor that can be used to stratify patients, especially in the group with intermediate cytogenetic prognosis, including patients with normal karyotype [10, 13]. *FLT3*-ITD is associated in these patients with a shorter duration of complete remission (CR), a shorter disease-free survival (DFS), and shorter overall survival (OS) [14].

Point mutations in the *FLT3* tyrosine kinase domain are detected in approximately 8% of AML patients [12, 14]. Most often they are in the activation loop in the codon area 835–836 [15]. One of the meta-analyses confirmed the negative impact of this defect on DFS, while in another study the prognosis of *FLT3*-TKD-positive patients was better than that of *FLT3*-ITD-positive patients [16, 17].

The targeted therapies used to treat AML patients with the *FLT3* mutation were first approved by the American Food and Drug Administration (FDA), and then by the European Medicines Agency (EMA). Midostaurin was approved for use in the first-line treatment of AML in combination with intensive chemotherapy, whereas gilteritinib was approved for use in the second-and third-line treatments of refractory/relapsed (R/R) AML as a monotherapy [18, 19].

Due to their mechanism of action, type I FLT3 inhibitors, such as midostaurin, gilteritinib, crenolanib, sunitinib, and lestaurtinib, have proven to be effective in the treatment of patients with *FLT3*-TKD-positive AML [10].

Mutations in the nucleophosmin 1 *(NPM1)* gene or exon 12 *NPM* occur in more than half of normal karyotype patients with AML and are the most common single somatic mutations in a subgroup with a favourable prognosis [20–22]. The *NPM1* gene encodes the sequence of nuclear-shuttling factor, which modulates the p53 signalling pathway [23, 24]. An important clinical element is the ability to monitor minimal residual disease (MRD) with quantitative real-time polymerase chain reaction (RT-PCR) in AML with *NPM1* gene mutation [25, 26].

The paper presents the case of a 30-year-old man with AML *NPM1*^{mut} *FLT3*-TKD bone marrow reticuline fibrosis, as well as features of multilineage dysplasia (MLD) and a high likelihood of bone involvement at diagnosis. Due to the availability of midostaurin in the Novartis Managed Access Program (MAP), the patient was treated with DA (daunorubicin, cytosine arabinoside) induction chemotherapy regimen "3 + 7" combined with midostaurin. After first-line treatment with midostaurin, CR was obtained. Due to dry tap obtained on bone marrow aspiration, the response to treatment was assessed based on histopathological examination of the trephine biopsy. Then the patient was treated with two cycles of consolidation chemotherapy, which included high-doses of cytosine arabinoside combined with midostaurin. Due to unfavourable prognosis factors, including the presence of increased bone marrow reticuline fibrosis, extramedullary locations: splenomegaly, peripheral lymphadenopathy, and probability of bone involvement, the patient was qualified for allogeneic haematopoietic stem cell transplantation (allo-HSCT) from an unrelated donor. Currently,

2,5 years after transplantation, the patient is still in complete remission.

Case report

In March 2019, a thirty-year-old male patient was transferred from the Hospital Emergency Department (HED) of the Teaching Hospital in Warsaw to the Department of Haematology, Institute of Haematology and Transfusion Medicine (IHiT, *Instytut Hematologii i Transfuzjologii*) due to suspicion of acute haematologic malignancy. A patient's medical history includes generalized bone and joint pain, the most severe in the hip joints, weight loss (approx. 2–3 kg in 2 weeks), night sweats, and occasional headaches. A history of hepatitis B was discovered during the physical examination, as well as systemic peripheral lymphadenopathy and splenomegaly (4 cm below the left costal margin). Complete blood count (CBC) revealed increased white blood cell (WBC) count — 18.57 G/L, normocytic anaemia [haemoglobin level — 9.7 g/dL, mean cell volume (MCV) — 83.1 fL), and thrombocytopenia with platelets (PLT) count of 41 G/L. The peripheral blood smear showed a left shift with the presence of 9% blasts, 12% promyelocytes, 13% myelocytes, 6% metamyelocytes, 11% bands, 14% segments, 3% basophils, 1% monocytes, 31% lymphocytes, as well as anisocytosis, microcytosis and macrocytosis of red blood cells (RBC). Additional tests showed an increased activity of lactate dehydrogenase (LDH) accounting for 2709 U/L. The bone marrow cytology showed moderate hypocellularity with 16% blasts and 10% promyelocytes. Flow cytometric analysis of bone marrow aspirate indicated 18% of myeloblasts with features of dysplasia. The presence of PML-RARA aberration was ruled out based on molecular tests. However, the obtained results did not allow to establish the diagnosis, therefore the results of the trephine biopsy were awaited. The results of the remaining molecular tests were also obtained: however, the obtained results did not allow to establish the diagnosis, therefore the results of the trephine biopsy were awaited. The results of the remaining molecular tests were obtained: in DNA Fragment Length Analysis (FLA, Gene scan) the presence of the mutant *FLT3*-ITD allele was not detected, while in restriction fragment length polymorphism (RFLP) using polymerase chain reaction (PCR-RFLP) the D835/I836 point mutation was found in the *FLT3* kinase domain. Direct sequencing confirmed the existence of pathogenic variation W288fs*12 in exon 12 of the *NPM1* gene (heterozygous) — type A.

During this time hip pain worsened as did bone pain, which required the use of opioid analgesics. No abnormalities were detected in the hip joints radiograph (X-ray). The ultrasound (US) examination of the soft tissues of the right inguinal area revealed enlarged inguinal lymph nodes. Low dose computed tomography (CT) of the skeleton showed irregular trabeculae of woven bone, especially the area of both hip bones, the sacrum, and the lumbar vertebral bodies. Tissue changes that may correspond to marrow infiltrate were visible in both femoral bone medullary cavities. At the same time, a further increase in leukocytosis to 42 G/L in CBC and an increase in the percentage of blasts up to 30% in the peripheral blood smear was observed, confirmed by immunophenotyping, showing 26% of myeloblasts. Therefore, the diagnostic criteria for AML were met, namely the presence of more than 20% blasts in the cytological blood test. Midostaurin has been requested as part of Novartis' MAP Early Access Program. Remission-inducing chemotherapy was administered according to the DA scheme "3 + 7" (DA, daunorubicin at a dose of 60 mg/m^2/day on days 1–3, cytosine arabinoside at a dose of 200 mg/m^2/day on days 1–7), in combination with subsequent therapy with midostaurin at a dose of 2 × 50 mg/day on days 8–21. The treatment was without severe complications. Midostaurin was well tolerated. At the same time, due to the presence of hepatitis B virus (HBV), and HBV DNA in the blood, appropriate treatment was commenced, initially with lamivudine and subsequently with entecavir. Bilateral pneumonia developed during pancytopenia after treatment with midostaurin. Broad-spectrum antibiotics, caspofungin, and sulfamethoxazole/trimethoprim in therapeutic doses were used, resulting in the resolution of inflammatory changes. At a later stage of the treatment, the result of the cytogenetic bone marrow examination with the binding method was obtained with a normal male karyotype as well as histopathological examination of the bone marrow, showing the infiltration of 95% of poorly differentiated blasts: CD15+ MPO+/– CD33–/+ CD163+/ /CD68PGM1+, CD117+ CD71– CD61– TdT– CD30– MCT– CEAmono– CD25– CD1a– CD20– CD3– CD34–, and significantly increased stromal reticulin fibres (MF-3), which constituted the picture corresponding to the diagnosis of AML with the *NPM1* mutation (Figure 1). The differentiation included acute panmyelosis with myelofibrosis (APMF), the transformation from myelofibrosis to AML, or AML with myelodysplasia-related changes (AML-MRC) with myelodysplasia. Therefore,

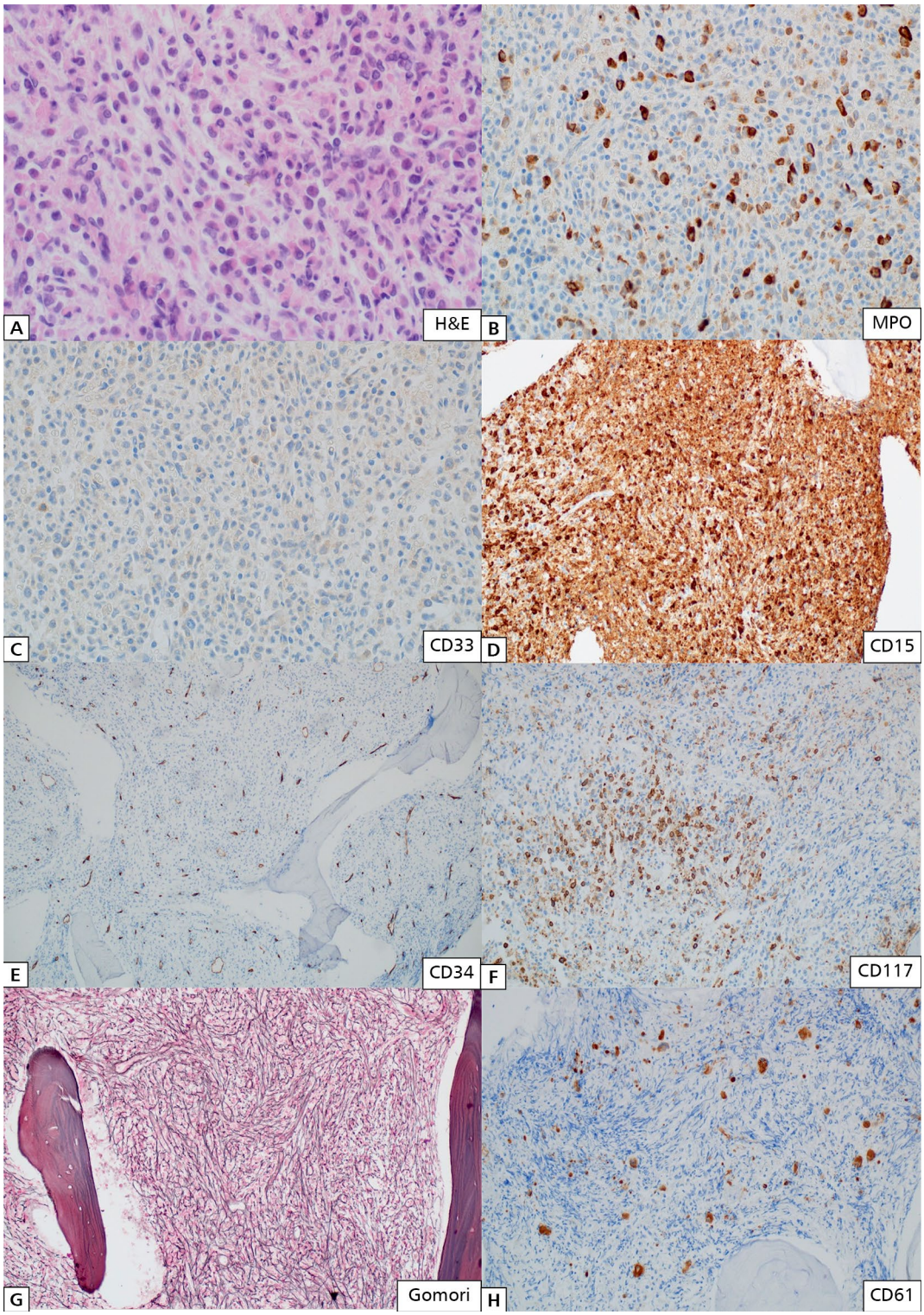

Figure 1A–H. Microscopic images of the bone marrow biopsy in a patient with acute myeloid leukaemia (AML) and coexisted *NPM1* and *FLT3*-TKD mutations. Hypercellular bone marrow with the proliferation of poorly differentiated myeloid lineage cells, with mild maturation [haematoxylin–eosin (HE) staining]. Phenotype of proliferative cells: MPO (expressed in portion of cells), CD33 (weak expression), CD15 (positive), CD34 (negative), CD117 (positive in about 30% of cells), CD61 (positive in dysplastic megakaryocytes); stromal reticulin fibrosis (Gomori, MF = 3); FLT3 (fms-like tyrosine kinase 3) — FMS-3-like tyrosine kinase; MF — myelofibrosis; NPM1 — nucleophosmin 1; TKD — tyrosine kinase domain

in the presence of increased bone marrow fibrosis and splenomegaly, additional molecular diagnostics were performed. Based on reverse transcriptase--PCR (RT-PCR) analysis the presence of the *BCR*/ /*ABL1* p190, p210, and p230 fusion gene was excluded, amplification-refractory mutation system-PCR (ARMS-PCR) test excluded the presence of *JAK2 V617F* mutation, and the presence of pathogenic variants in exon 12 of the *ASXL1* gene, exon 10 of the *MPL* gene, and in exon 9 of the *CARL* gene was excluded by Sanger sequencing. Finally, the diagnosis of AML with the *NPM1* mutation was made as a distinct entity in the AML group with recurrent genetic abnormalities according to the 2017 WHO classification, with a favourable cytogenetic/ /molecular risk according to the 2017 European LeukemiaNet (ELN) classification. To assess the effects of cytostatic treatment, a bone marrow aspiration biopsy was performed, but the tissue sample was too small to do cytological tests; therefore, a trephine biopsy was performed. The histopathological examination revealed extremely hypoplastic marrow with a haematopoietic structure occupying about 15–20% of medullary cavities, single blasts expressing CD34 and CD117, reticulin stromal fibrosis (MF2), and visible stromal degenerative changes. CR was confirmed with no obvious AML marrow infiltration. Then 2 cycles of consolidating chemotherapy were administered using high-dose cytosine arabinoside at a dose of 2 g/m^2 every 12 hours on days 1, 3, 5 and midostaurin at a dose of 2 × 50 mg/day on days 8–21. Midostaurin was well tolerated, and no significant side effects were observed. In October 2019, the patient underwent allo-HSCT from an unrelated donor with human leukocyte antigen (HLA) compatibility (10/10 matching). After administration of a myeloablative conditioning regimen with fludarabine 30 mg/m^2/day for 5 days, busulfan 3.2 mg/kg/day for 4 days, and thymoglobulin at a total dose of 4.5 mg/kg, CD34 cells collected from peripheral blood were transfused (total dose 5.3 × × 10^6/kg). Cyclosporin A and methotrexate were used as standard prophylaxis for graft-versus-host disease (GvHD). The haematopoietic system was successfully implanted and regenerated. Around day +60 post-transplant a cutaneous form of acute GvHD, grade II/III according to the Glucksberg classification [27] was detected. According to commonly accepted guidelines regarding the treatment of acute GvHD, glucocorticosteroids were used with good results. The patient is still in CR, with 100% donor chimerism monitored by STR-PCR (short-tandem repeat PCR), with no GvHD symptoms, and does not require immunosuppressive.

Discussion

Acute myeloid leukaemia with *NPM1* mutation was recognized by World Health Organization (WHO) classification of hematopoietic neoplasms as distinct entity in 2017. Usually it developes *de novo* in inviduals with normal karyotype, similar to the presented case. The aberrant cytoplasmic dislocation of mutant *NPM1* plays an important role in leukemogenesis [28, 29]. *NPM1* mutation is frequent in middle age adults and tends to decrease in patients > 70 years, quite rarely occurs in children (6,5%) [30, 31]. Simple techniques, such as immunohistochemistry (IHC) and molecular assays, allow the detection of *NPM1* mutations [32]. Molecular techniques were used to detect *NPM1* mutation in the presented case. Clinical manifestation of *NPM1* AML includes the involvement of extramedullary organs such as gums, lymph nodes and skin, as well as anaemia, thrombocytopenia, and leukocytosis. *NPM1* gene mutation is associated with a favourable prognosis, prolonged event-free survival (EFS) i OS [33]. However prognosis of *NPM1* AML, in addition of *FLT3* mutation status, depends on other comutated genes. *NMP1* AML without *FLT3*-ITD or with *FLT3*-ITDlow (ratio < 0.5) are classified as favourable-risk group while *NPM1* AML with *FLT3*-ITD^{high} (ratio > 0.5) is classified as intermediate-risk group [34]. *NPM1*/ /*FLT3*-TKD, *NPM1*/N-RAS and *NPM1*/RAD21 genotypes are regarded as favourable risk [35]. Whereas *NPM1*/DNMT3A/*FLT3*-ITD and *NPM1*^{wt} genotypes are associated with poor prognosis [35, 36]. *NMP1* leukaemia cells most often exhibit either myelomonocytic or monocytic morphology; in 1/4 of cases, multilinear dysplasia (MLD) is observed. Blast cells do not express CD34 with positive expression of CD117, CD123, CD33^{high}, CD13^{low} [37]. Due to specific features, including MLD or bone marrow fibrosis, similarly to the present case, the AML *NPM1*^{mut} subtype may be a major diagnostic challenge [38]. Therefore, differential diagnosis of AML with *NPM1* mutation and fibrosis includes APMF, a transformation from myelofibrosis to AML and AML-MRC. Acute panmyelosis with myelofibrosis is characterized by an acute trilinear proliferation with an increased blasts percentage (≥ 20%) and fibrosis. Bone marrow is hypercellular, with features of hyperplasia of three lines precursors as well as dysplasia (mainly affecting megakaryocytes) and fibrosis. The disease develops *de novo* and has a sudden onset. Patients with APMF usually have severe general symptoms, bone pain, pancytope-

nia, and slight or no splenomegaly [39, 40]. The prognosis is poor. Myeloblasts show CD34, CD13, CD33, and CD117 expression; the myeloperoxidase reaction is negative. In genetic testing the karyotype is complex [41]. In the described case, a proliferation of only one haematopoietic cell line and a normal karyotype were observed, which contradicts the APMF diagnosis. According to the 2017 WHO criteria, if acute hyperplasia affects only one haematopoietic cell line (e.g. myeloblasts) and is accompanied by myelofibrosis, the disease is classified as a specific AML subtype with myelofibrosis [37]. The features that excluded the transformation of primary myelofibrosis (PMF) into AML were no history of PMF, other than PMF morphology of megakaryocytes and no genetic abnormalities typical for PMF (*JAK-2* V617F, *MPL*, and *CARL* mutations) [37]. Due to the presence of dysplastic changes in the megakaryocytic and granulocytic lines, the diagnosis of AML-MRC was considered in the differential diagnosis [38]. The features ruling out this diagnosis included no history of myelodysplastic syndrome (MDS) and the absence of karyotyping changes typical for MDS, such as complex karyotype and defects in chromosomes 5 and 7 [37]. Finally, AML with *NPM1* mutation was diagnosed as a distinct entity in the AML group with recurrent genetic abnormalities according to the WHO 2017.

The AML-MRC subtype, in which the features of myelodysplasia are present, or its occurrence is preceded by MDS, or there are genetic myelodysplasia-related abnormalities, is characterized by an unfavourable prognosis compared to AML without MRC features [6]. Other studies report lower complete remission rates after intensive chemotherapy, with median overall survival of approximately 9––12 months [42–44]. Different observations were made in the case of AML *NPM1*^{mut} with the presence of multilineage dysplasia. Falini et al. [45] assessed the impact of MLD on survival in AML *NPM1*^{mut} patients. There were no significant differences in OS and EFS in patients with AML N*PM1*^{mut} with or without MLD [45].

The impact of reticulin fibrosis on prognosis in AML *NPM1*^{mut} patients was also assessed. In Naous et al. [46] study 78.6% of 14 AML *NPM1*^{mut} patients showed the presence of grade ≥ 2 reticulin fibrosis, and 45.5% of patients had *FLT3* gene mutation. Increased marrow reticulin fibrosis was demonstrated in all patients with *FLT3* and *NPM1* mutations. Patients with reticulin fibrosis had an unfavourable prognosis. Therefore, it was concluded that increased marrow reticulin fibrosis is a negative prognostic factor [46].

FLT3 mutations often coexist with *NPM1* mutations [24, 47]. The coexistence of the *FLT3*--TKD and *NPM1* mutation is associated with a better prognosis than in the case of *FLT3*-ITD and NMP*1* mutations, and, above all, isolated *NPM1* mutation [48]. Boddu et al. study indicated that the coexistence of the *FLT3*-TKD and *NPM1* mutations is associated with more favourable AML relapse-free survival compared to patients with an isolated *NPM1* mutation [49]. Moreover, Pappaemmanuil et al. [8] found a significant OS prolongation in AML patients with *FLT3*-TKD and *NPM1* mutations compared to the general population of AML patients.

The pathogenetic role of *NPM1* mutation in AML development has not been fully understood, and targeted therapies have not been discovered. However recent studies have identified therapies e.g. venetoclax-based, that may be particulary effective in *NPM1* AML [50]. *FLT3* mutations may promote resistance to venetoclax by increasing expression of the BCL-2 family, including BCL-XL and MCL-1 [51]. Therefore it is justified to use venetoclax in combination with FLT3 inhibitors in *NPM1*/*FLT3* comutated AML. Early phase clinical trials combining venetoclax with gilteritinib or quizartinib are ongoing. New agents in *NPM1* AML including XPO1 and MLL-menin inhibitors, alone or in combination with FLT3 inhibitors or venetoclax and drugs targeting the interaction between *NPM1* and its ligand, are under exploration [2, 29, 52–55]. Contrary to this, in the case of the *FLT3* mutation, its pathogenetic role is recognized, and numerous FLT3 kinase inhibitors are also assessed in clinical trials (www.clinicaltrials.gov). In addition, for several years, the combination of midostaurin with intensive chemotherapy has been the standard of care in AML patients as well as the possibility of using gilteritynib as monotherapy in patients with R/R AML.

The RATIFY study (CALGB10603) has shown that the combined treatment of midostaurin with intensive chemotherapy significantly improves outcomes and prolongs EFS in AML patients with *FLT3*-TKD mutation [18]. Moreover, it was found that the coexistence of *NPM1* and *FLT3*-TKD mutations is associated with a significant OS prolongation in these patients compared to patients without *NPM1* mutation [56].

Patients in the first remission of AML are qualified for allo-HSCT considering cytogenetic and molecular risk groups according to ELN 2017, while in the case of R/R AML, allo-HSCT should be considered in each patient [6]. The favourable risk group according to ELN 2017 is not an indication

for allo-HSCT in the first remission unless there are additional risk factors, mainly positive MRD [57]. In the intermediate-risk group, the indication for allo-HSCT is a positive MRD in CR1, while in patients with negative MRD allo-HSCT is an option with a low risk of treatment-related mortality (TRM). In the case of high-risk leukaemia, allo-HSCT should be considered in all patients as post-remission therapy [57].

Patients with *FLT3*-TKD and *NPM1* mutations are qualified for a favourable prognosis group and appropriate therapeutic methods are implemented. In Perry et al. [58] study *NPM1* mutation coexisted with *FLT3*-TKD mutation in 9.5% of patients, and the treatment outcomes in this group of patients were beneficial. Interestingly, it was observed that the *NPM1* mutation "lost" its favourable effect in patients without *FLT3*-TKD mutation [58].

In the present case, the patient was qualified for allo-HSCT, despite the favourable genetic pattern with *NPM1* and *FLT3*-TKD mutations, due to the presence of additional risk factors, including extramedullary infiltrates and increased bone marrow reticulin fibrosis. As a patient remains in complete remission, it seems that allo-HSCT was the right choice of treatment.

The outcomes in patients with acute leukaemia are not satisfactory so far, and new drugs with the potential to improve the treatment effectiveness are still being explored in clinical trials. It seems particularly important to search for further genetic abnormalities and associations with their coexistence. Understanding the complex structure and hierarchy of genetic and molecular alterations may support further development of targeted therapies in AML, and enable the combination of chemotherapy or hypomethylating drugs not only with a single targeted drug but with their combinations - doublets, triplets or in the sequential use.

References

1. Winer ES, Stone RM. Novel therapy in Acute myeloid leukemia (AML): moving toward targeted approaches. Ther Adv Hematol. 2019; 10: 2040620719860645, doi: 10.1177/2040620719860645, indexed in Pubmed: 31321011.
2. Kayser S, Levis MJ. Advances in targeted therapy for acute myeloid leukaemia. Br J Haematol. 2018; 180(4): 484–500, doi: 10.1111/bjh.15032, indexed in Pubmed: 29193012.
3. Bohl SR, Bullinger L, Rücker FG. New targeted agents in acute myeloid leukemia: new hope on the rise. Int J Mol Sci. 2019; 20(8), doi: 10.3390/ijms20081983, indexed in Pubmed: 31018543.
4. Rowe JM. Will new agents impact survival in AML? Best Pract Res Clin Haematol. 2019; 32(4): 101094, doi: 10.1016/j.beha.2019.101094, indexed in Pubmed: 31779986.
5. Lee YT, Tan YiJ, Oon CE. Molecular targeted therapy: treating cancer with specificity. Eur J Pharmacol. 2018; 834: 188–196, doi: 10.1016/j.ejphar.2018.07.034, indexed in Pubmed: 30031797.
6. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017; 129(4): 424–447, doi: 10.1182/blood-2016-08-733196, indexed in Pubmed: 27895058.
7. Ley TJ, Miller C, Ding Li, et al. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013; 368(22): 2059–2074, doi: 10.1056/NEJMoa1301689, indexed in Pubmed: 23634996.
8. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016; 374(23): 2209–2221, doi: 10.1056/NEJMoa1516192, indexed in Pubmed: 27276561.
9. Lagunas-Rangel FA, Chávez-Valencia V. FLT3-ITD and its current role in acute myeloid leukaemia. Med Oncol. 2017; 34(6): 114, doi: 10.1007/s12032-017-0970-x, indexed in Pubmed: 28470536.
10. Daver N, Schlenk RF, Russell NH, et al. Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia. 2019; 33(2): 299–312, doi: 10.1038/s41375-018-0357-9, indexed in Pubmed: 30651634.
11. Iwai T, Yokota S, Nakao M, et al. Internal tandem duplication of the FLT3 gene and clinical evaluation in childhood acute myeloid leukemia. Leukemia. 1999; 13(1): 38–43, doi: 10.1038/sj.leu.2401241.
12. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002; 99(12): 4326–4335, doi: 10.1182/blood.v99.12.4326, indexed in Pubmed: 12036858.
13. Kiyoi H, Naoe T, Nakano Y, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood. 1999; 93(9): 3074–3080, indexed in Pubmed: 10216104.
14. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012; 366(12): 1079–1089, doi: 10.1056/NEJMoa1112304, indexed in Pubmed: 22417203.
15. Yamamoto Y, Kiyoi H, Nakano Y, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood. 2001; 97(8): 2434–2439, doi: 10.1182/blood.v97.8.2434, indexed in Pubmed: 11290608.
16. Yanada M, Matsuo K, Suzuki T, et al. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations for acute myeloid leukemia: a meta-analysis. Leukemia. 2005; 19(8): 1345–1349, doi: 10.1038/sj.leu.2403838, indexed in Pubmed: 15959528.
17. Mead AJ, Linch DC, Hills RK, et al. FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. Blood. 2007; 110(4): 1262–1270, doi: 10.1182/blood-2006-04-015826, indexed in Pubmed: 17456725.
18. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. N Engl J Med. 2017; 377(5): 454–464, doi: 10.1056/NEJMoa1614359, indexed in Pubmed: 28644114.
19. Gorcea CM, Burthem J, Tholouli E. ASP2215 in the treatment of relapsed/refractory acute myeloid leukemia with FLT3 mutation: background and design of the ADMIRAL trial. Future Oncol. 2018; 14(20): 1995–2004, doi: 10.2217/fon-2017-0582, indexed in Pubmed: 29498296.
20. Boissel N, Renneville A, Biggio V, et al. Prevalence, clinical profile, and prognosis of NPM mutations in AML with normal karyotype. Blood. 2005; 106(10): 3618–3620, doi: 10.1182/blood-2005-05-2174, indexed in Pubmed: 16046528.
21. Schnittger S, Schoch C, Kern W, et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood. 2005; 106(12): 3733–3739, doi: 10.1182/blood-2005-06-2248, indexed in Pubmed: 16076867.
22. Suzuki T, Kiyoi H, Ozeki K, et al. Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. Blood. 2005; 106(8): 2854–2861, doi: 10.1182/blood-2005-04-1733, indexed in Pubmed: 15994285.
23. Falini B, Mecucci C, Tiacci E, et al. GIMEMA Acute Leukemia Working Party. Cytoplasmic nucleophosmin in acute myelogenous

leukemia with a normal karyotype. N Engl J Med. 2005; 352(3): 254–266, doi: 10.1056/NEJMoa041974. , indexed in Pubmed: 15659725.
24. Falini B, Nicoletti I, Martelli MF, et al. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. Blood. 2007; 109(3): 874–885, doi: 10.1182/blood-2006-07-012252, indexed in Pubmed: 17008539.
25. Ivey A, Hills R, Simpson M, et al. UK National Cancer Research Institute AML Working Group. Assessment of minimal residual disease in standard-risk AML. N Engl J Med. 2016; 374(5): 422–433, doi: 10.1056/nejmoa1507471.
26. Krönke J, Schlenk RF, Jensen KO, et al. Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. J Clin Oncol. 2011; 29(19): 2709–2716, doi: 10.1200/JCO.2011.35.0371, indexed in Pubmed: 21555683.
27. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974; 18(4): 295–304, doi: 10.1097/00007890-197410000-00001, indexed in Pubmed: 4153799.
28. Falini B, Bolli N, Liso A, et al. Altered nucleophosmin transport in acute myeloid leukaemia with mutated NPM1: molecular basis and clinical implications. Leukemia. 2009; 23(10): 1731–1743, doi: 10.1038/leu.2009.124, indexed in Pubmed: 19516275.
29. Brunetti L, Gundry M, Sorcini D, et al. Mutant NPM1 maintains the leukemic state through HOX expression. Cancer Cell. 2018; 34(3): 499–512.e9, doi: 10.1016/j.ccell.2018.08.005.
30. Cazzaniga G, Dell'Oro MG, Mecucci C, et al. Nucleophosmin mutations in childhood acute myelogenous leukemia with normal karyotype. Blood. 2005; 106(4): 1419–1422, doi: 10.1182/blood-2005-03-0899, indexed in Pubmed: 15870172.
31. Nagel G, Weber D, Fromm E, et al. German-Austrian AML Study Group (AMLSG). Epidemiological, genetic, and clinical characterization by age of newly diagnosed acute myeloid leukemia based on an academic population-based registry study (AMLSG BiO). Ann Hematol. 2017; 96(12): 1993–2003, doi: 10.1007/s00277-017-3150-3, indexed in Pubmed: 29090343.
32. Falini B, Martelli MP, Pileri SA, et al. Molecular and alternative methods for diagnosis of acute myeloid leukemia with mutated NPM1: flexibility may help. Haematologica. 2010; 95(4): 529–534, doi: 10.3324/haematol.2009.017822, indexed in Pubmed: 20378574.
33. Thiede C, Koch S, Creutzig E, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006; 107(10): 4011–4020, doi: 10.1182/blood-2005-08-3167, indexed in Pubmed: 16455956.
34. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017; 129(4): 424–447, doi: 10.1182/blood-2016-08-733196.
35. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016; 374(23): 2209–2221, doi: 10.1056/NEJMoa1516192, indexed in Pubmed: 27276561.
36. Eisfeld AK, Kohlschmidt J, Mims A, et al. Additional gene mutations may refine the 2017 European LeukemiaNet classification in adult patients with de novo acute myeloid leukemia aged <60 years. Leukemia. 2020; 34(12): 3215–3227, doi: 10.1038/s41375-020-0872-3, indexed in Pubmed: 32461631.
37. Appendix 2. In: Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. ed. WHO Classification of Tumours of Haemopoietic and Lymphoid Tissues. Revised 4th edition. IARC, Lyon 2017: 1023–1024.
38. Xu Z. AML with myelodysplasia-related changes masquerades as acute panmyelosis with myelofibrosis. Blood. 2017; 130(15): 1775, doi: 10.1182/blood-2017-06-793554, indexed in Pubmed: 29025719.
39. Thiele J, Kvasnicka HM, Schmitt-Graeff A. Acute panmyelosis with myelofibrosis. Leuk Lymphoma. 2004; 45(4): 681–687, doi: 10.1080/10428190310001625692, indexed in Pubmed: 15160939.
40. Thiele J, Kvasnicka HM, Zerhusen G, et al. Acute panmyelosis with myelofibrosis: a clinicopathological study on 46 patients including histochemistry of bone marrow biopsies and follow-up. Ann Hematol. 2004; 83(8): 513–521, doi: 10.1007/s00277-004-0881-8, indexed in Pubmed: 15173958.
41. Orazi A, O'Malley DP, Jiang J, et al. Acute panmyelosis with myelofibrosis: an entity distinct from acute megakaryoblastic leukemia. Mod Pathol. 2005; 18(5): 603–614, doi: 10.1038/modpathol.3800348, indexed in Pubmed: 15578075.
42. Koenig KL, Sahasrabudhe KD, Sigmund AM, et al. AML with myelodysplasia-related changes: development, challenges, and treatment advances. Genes (Basel). 2020; 11(8), doi: 10.3390/genes11080845, indexed in Pubmed: 32722092.
43. Østgård LSG, Nørgaard JM, Sengeløv H, et al. Comorbidity and performance status in acute myeloid leukemia patients: a nationwide population-based cohort study. Leukemia. 2015; 29(3): 548–555, doi: 10.1038/leu.2014.234, indexed in Pubmed: 25092141.
44. Montalban-Bravo G, Kanagal-Shamanna R, Class CA, et al. Outcomes of acute myeloid leukemia with myelodysplasia related changes depend on diagnostic criteria and therapy. Am J Hematol. 2020; 95(6): 612–622, doi: 10.1002/ajh.25769, indexed in Pubmed: 32112433.
45. Falini B, Macijewski K, Weiss T, et al. Multilineage dysplasia has no impact on biologic, clinicopathologic, and prognostic features of AML with mutated nucleophosmin (NPM1). Blood. 2010; 115(18): 3776–3786, doi: 10.1182/blood-2009-08-240457, indexed in Pubmed: 20203266.
46. Naous R, Gentile T, Vajpayee N. 201 Evaluation of bone marrow fibrosis in NPM-positive AML: a retrospective study. Am J Clin Pathol. 2018; 149(Suppl_1): S85–S86, doi: 10.1093/ajcp/aqx121.200.
47. Falini B, Nicoletti I, Bolli N, et al. Translocations and mutations involving the nucleophosmin (NPM1) gene in lymphomas and leukemias. Haematologica. 2007; 92(4): 519–532, doi: 10.3324/haematol.11007, indexed in Pubmed: 17488663.
48. Bacher U, Haferlach C, Kern W, et al. Prognostic relevance of FLT3-TKD mutations in AML: the combination matters — an analysis of 3082 patients. Blood. 2008; 111(5): 2527–2537, doi: 10.1182/blood-2007-05-091215, indexed in Pubmed: 17965322.
49. Boddu P, Kantarjian H, Borthakur G, et al. Co-occurrence of -TKD and mutations defines a highly favorable prognostic AML group. Blood Adv. 2017; 1(19): 1546–1550, doi: 10.1182/bloodadvances.2017009019, indexed in Pubmed: 29296796.
50. Lachowiez CA, Loghavi S, Kadia TM, et al. Outcomes of older patients with NPM1-mutated AML: current treatments and the promise of venetoclax-based regimens. Blood Adv. 2020; 4(7): 1311–1320, doi: 10.1182/bloodadvances.2019001267, indexed in Pubmed: 32251497.
51. Kasper S, Breitenbuecher F, Heidel F, et al. Targeting MCL-1 sensitizes FLT3-ITD-positive leukemias to cytotoxic therapies. Blood Cancer J. 2012; 2(3): e60, doi: 10.1038/bcj.2012.5, indexed in Pubmed: 22829255.
52. Uckelmann HJ, Kim S, Wong E, et al. Therapeutic targeting of preleukemia cells in a mouse model of NPM1 mutant acute myeloid leukemia. Science. 2020; 367(6477): 586–590, doi: 10.1126/science.aax5863, indexed in Pubmed: 32001657.
53. Klossowski S, Miao H, Kempinska K, et al. Menin inhibitor MI-3454 induces remission in MLL1-rearranged and NPM1-mutated models of leukemia. J Clin Invest. 2020; 130(2): 981–997, doi: 10.1172/jci129126, indexed in Pubmed: 31855575.
54. Fischer MA, Friedlander SY, Arrate MP, et al. Venetoclax response is enhanced by selective inhibitor of nuclear export compounds in hematologic malignancies. Blood Adv. 2020; 4(3): 586–598, doi: 10.1182/bloodadvances.2019000359, indexed in Pubmed: 32045477.
55. Cela I, Di Matteo A, Federici L. Nucleophosmin in its interaction with ligands. Int J Mol Sci. 2020; 21(14), doi: 10.3390/ijms21144885, indexed in Pubmed: 32664415.
56. Voso MT, Larson RA, Jones D, et al. Midostaurin in patients with acute myeloid leukemia and FLT3-TKD mutations: a subanalysis from the RATIFY trial. Blood Adv. 2020; 4(19): 4945–4954, doi: 10.1182/bloodadvances.2020002904, indexed in Pubmed: 33049054.
57. Carreras E, Dufour C, Mohty M, Kröger N. ed. The EBMT handbook. Hematopoietic stem cell transplantation and cellular therapies. Springer Open, Cham 2019: 507–521.
58. Perry M, Bertoli S, Rocher C, et al. FLT3-TKD mutations associated with NPM1 mutations define a favorable-risk group in Patients With Acute Myeloid Leukemia. Clin Lymphoma Myeloma Leuk. 2018; 18(12): e545–e550, doi: 10.1016/j.clml.2018.06.006, indexed in Pubmed: 30082225.