

Current Progress in The Research of NPM1-Mutated AML

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Abstract. Acute myeloid leukemia (AML) with a mutation in nucleophosmin 1 (NPM1) is the most prevalent form, making up around 30% of cases. Because of the several roles that NPM1 plays in preserving normal cell activity, mutations in this protein can have a serious negative impact on the human body by causing anti-apoptosis and cell proliferation, which can ultimately lead to cancer. Numerous therapies have demonstrated efficacy; while they focus on distinct areas, it is envisaged that they may be combined to provide greater results down the road. AML with solely NPM1 mutations has been shown to have better results than AML with co-mutations, while the prognosis of NPM1-mutated AML varies on several parameters. Monitoring for minimal residual disease (MRD) is used to track and forecast the course of AML with a mutation in NPM1. It is anticipated that more studies will help identify new therapy targets while reducing adverse effects. Choosing the proper guidelines and techniques for keeping an eye on this illness. This article focused on the structure and normal function of NPM1 as well as the mechanisms causing AML. In addition, the treatment, prognosis, and monitoring methods of AML based on NPM1 are proposed in this paper, which has a reference role for the treatment of AML in the future.

Keywords: NPM1-mutated; AML; prognosis; MRD monitoring.

1. Introduction

AML is a hematological malignancy, accounting for about 1.1% of all cancer diagnoses. It is the second most common form of leukemia, and its mortality is quite high. AML originates from bone marrow and is characterized by infiltration of the peripheral blood system and other organs. Most patients with AML will experience symptoms related to bone marrow failure, including infection, fever, bleeding, and anemia. In rare cases, AML may also show myeloid sarcoma [1,2].

A remarkable feature of AML is that it is always represented by the clonal proliferation of progenitor cells and hematopoietic stem cells (HSCs) [3]. This clonal proliferation leads to abnormal cell differentiation and selective growth advantage, which severely impairs normal hematopoiesis. The normal hematopoietic process is disturbed by abnormal cells, which makes the body unable to produce the required blood cells normally, resulting in anemia, easy infection, easy bleeding, and other symptoms.

AML is mainly seen in the elderly, and more than two-thirds of patients are older than 50 years. In China, the median age of AML diagnosis is 68 years, which means that most patients face the challenge of this disease in their later years. With the growth of age, the functions of the human immune system and the hematopoietic system will decline, which may be one of the reasons why older people are more prone to AML [2].

Regardless of age, the overall 5-year survival rate of AML was low around 21% according to the statistical data in 2019 which is similar to that of severe solid tumors such as lung cancer, liver cancer, and brain cancer. Also, the prognosis is related to the genetic characteristics and the patient. Without proper treatment, it is a fatal disease, asymptomatic patients can also have life-threatening complications [2], for example, serious infection and anemia-related symptoms.

Molecular biology has been developing at a rapid pace in recent years, the AML-related research has gone into the level of protein or gene levels. It has been discovered that Dehydrogenase 1 and 2 (IDH1 and IDH2, respectively) TP53 are all important markers for AML but the NPM1 is the most essential one. NPM1 plays a key role in many biological activities therefore NPM1 mutation could have severe effects on human bodies. NPM1 mutation is the most common one in AML [1]. Approximately, 30% of AML patients have the mutation of NPM1, the rate is lower among children

patients, with about 8% of them having the mutated NPM1 [1]. Due to the high fatality of AML, researchers and medical practitioners need to know the NPM1 mutation types, the mechanism of NPM1-mutated caused AML, the appropriate treatment method for it, and also the future directions of research.

2. Structure and functions of NPM1

NPM1 is a 12-exon protein that is found on chromosome 5q35. It is composed of three distinct domains: histone-binding sections, oligomerization domains, and putative metal-binding domains [1].

NPM1wt and other nucleolar components are separated from the surrounding nucleoplasm by a process known as "liquid-liquid" phase separation (LLPS), which forms the nucleolus, a membrane-free organelle [4]. The directionality of ribosomal biogenesis across several subcellular compartments and nucleolar phase separation depends on these heterotypic interactions [4]. The N-terminus of NPM1 is rich in nonpolar amino acids and contains two nuclear export signal sequences (NES). These NES are essential for the transport function of NPM1 from the nucleus to the cytoplasm. The central part of NPM1 is the ribonuclease active region, which contains a nuclear localization signal sequence. The C-terminus contains a 76 amino acid residue segment, which is a nucleic acid binding domain and contains a nuclear localization signal. In addition, NPM1 also has a specific domain that enables it to shuttle between the nucleus and cytoplasm. Its conserved NES enables migration from the nucleoplasm to the cytoplasm, which is essential for binding a range of proteins. The C-terminal domain contains two nucleolar localization signals (NOL), which are critical for the localization of NPM1 back to the nucleolus. The central domain contains two nuclear localization signals (NLS), which guide the movement of NPM1 from the cytoplasm to the nucleoplasm [1]. Figure 1 depicts NPM1's fundamental structure.

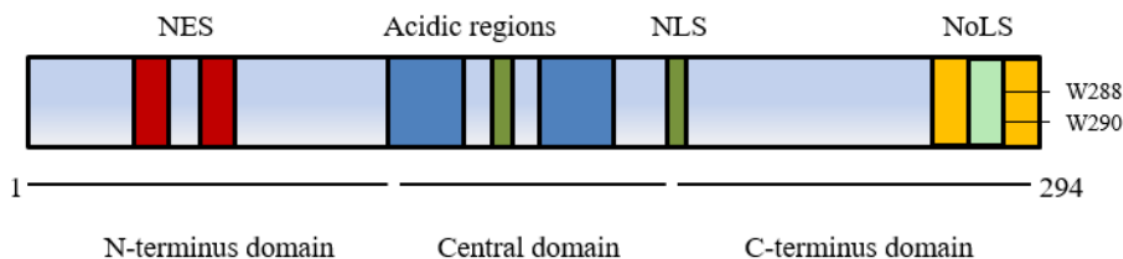


Fig 1. Structural diagram of NPM1 [1]

The capacity of NPM1 to move intracellularly between the nucleolus, nucleoplasm, and cytoplasm has been linked to a variety of activities [1]. NPM1 has been demonstrated to have a significant role in preserving genomic integrity and controlling apoptosis in addition to being involved in ribosome biogenesis, messenger RNA (mRNA) processing, chromatin remodeling, and embryogenesis [5]. Apart from that, NPM1 can support cell division and is strongly linked to centrosome activity [1]. Lastly, one of its most remarkable abilities is that it can both repair DNA and inhibit tumors by upregulating the activity of the tumor suppressor protein TP53, which in turn triggers cell death [1]. Maintaining proper cell biology depends on this. Furthermore, studies conducted by Nachmani et al. showed that NPM1 controls the binding of short nucleolar RNA (snoRNA), which is essential for the correct maintenance of hematopoietic stem cells, by controlling the 2'-O-methylation of ribosomal RNA (rRNA) [6]. All of these suggest that NPM1 is an essential gene in cancer, if NPM1 undergoes mutation, there is a bigger chance of cancer and it has been discovered that NPM1 mutation acts as a powerful biomarker of cancer and disease relapse. Figure 2 illustrates the basic functions of NPM1.

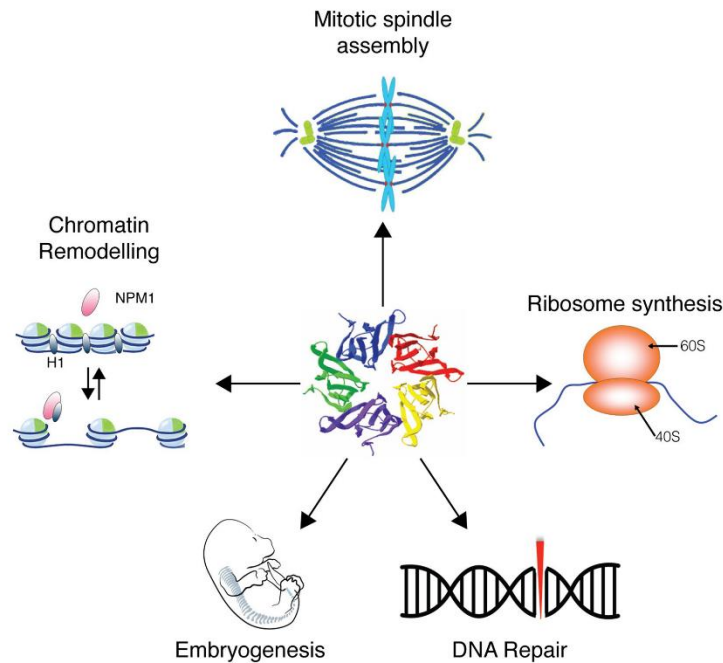


Fig 2. Overview of NPM1 functions [5]

3. The mechanism of mutated NPM1 causing AML

About 50 different types of NPM1 mutations have been identified; type A, which includes TCTG tetranucleotide replication and accounts for 75–80% of all NPM1 mutations, is the most prevalent form. The majority of NPM1 mutations are found in exon 12. The second most prevalent NPM1 mutation, type B, accounts for 10% of cases. At the same nucleotide location as type A, a 4 base pair (bp) insertion (CATG) is made. Anomalies related to abnormal cytoplasmic NPM1 localization have also been reported in exons 5, 9, and 11 due to rare mutations in NPM1. Recent research suggests that terminal deoxynucleotidyl transferase (TdT) is involved in the duplication seen in NPM1 mutations. To be more precise, the primary cause of NPM1 mutations in AML is replication slippages. The development of leukemia appears to be fueled by persistent cytoplasmic localization, a property that all mutation types produce while having differing properties [1]. The NPM1 gene's structure and frequent mutations in AML are seen in Figure 3.

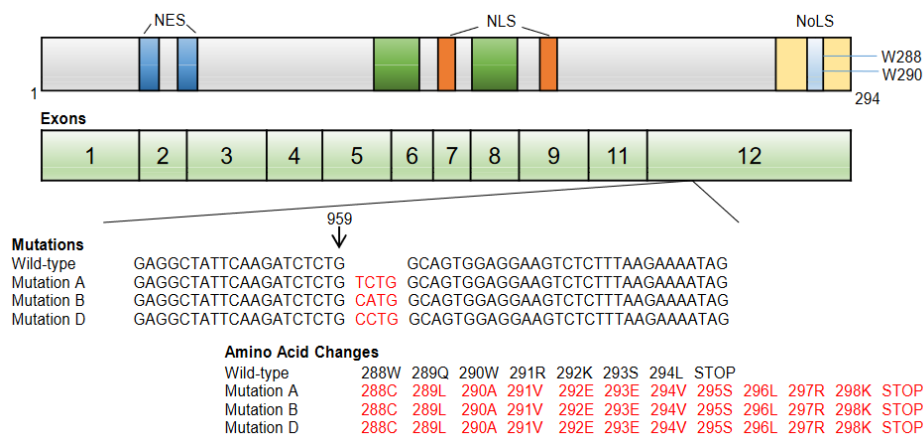


Fig 3. The structure of the NPM1 gene and prevalent mutations in AML [3]

NPM1 mutation can disrupt multiple mechanisms and cause severe results. Mutations in NPM1 result in the production of additional NES and loss of NoLS [7], which leads to a greater capacity for NPM1 to export nuclear and ultimately results in cytoplasmic localization and this can cause loss function of some chromatin binding factors which directly induces malignant transformation of

hematopoietic cells [7]. NPM1 has been identified as having a major part in preserving gene stability. Thus, NPM1 loss of function or altered NPM1 function results in gene instability, and genomic instability is one of the reasons for cell transformation and the emergence of cancer [5]. In addition to flaws in the apoptotic pathway that enable cancer cells to cope with and survive the levels of genetic instability that frequently result in cell death, this instability allows cancer to develop and adapt to the environment in which it functions [5]. In addition, the mutation of NPM1 affects the level of TP53, with the low level of tumor suppressor protein, tumors can grow without limitations. Moreover, it has been approved that NPM1c⁺ can cause microRNA dysregulation and also enhance the adhesion, migration, and invasion potential of leukemia cells.

However, NPM1 mutation may not be enough to cause AML, it always requires the co-mutations of other genes [3], for instance, methyltransferase 3 alpha (DNMT3A) and fms-like tyrosine kinase 3 (FLT3). Figure 4 illustrates the mechanism of DNMT3A and NPM1 coexistence in AML. Moreover, abnormal cytoplasmic localization of associated proteins such as mouse double minute 2 (MDM2), apurinic/apyrimidinic endonuclease 1 (APE1), and alternative reading frame (ARF) may contribute to NPM1mut leukemia [1]. These proteins are involved in differentiation, apoptosis, and DNA repair processes. As per the studies, the transcriptional factor PU.1/spi-1, responsible for the differentiation of monocytic-granulocytic cells, translocates to the cytoplasm around NPM1mut. This might potentially facilitate the arrest of differentiation and result in the monocytic features that are distinctive of NPM1mut AML [1].

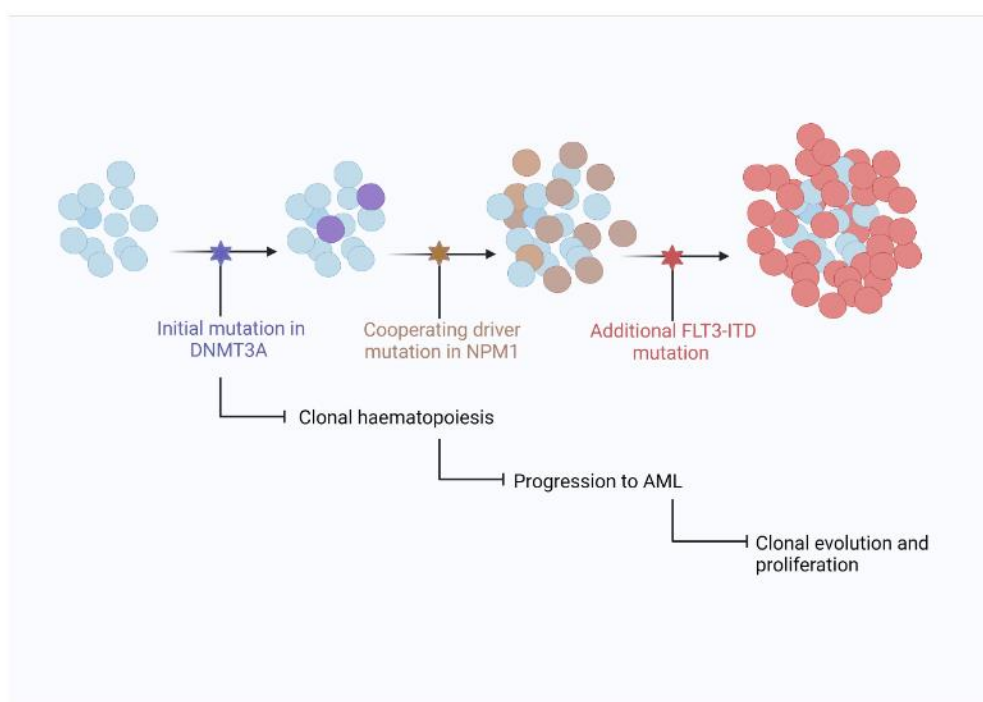


Fig 4. Mechanism of DNMT3A and NPM1 co-existed AML [1]

4. The treatment methods of NPM1-mutated AML

There are various methods of treating NPM1-mutated AML, they may target different molecules or proteins but they all have been promising ways to better the outcomes, as is shown in Figure 5.

First of all, since NPM1 mutation is the most common mutation in AML and also the role it plays in maintaining the stability of the genome, it may be the new target for cancer therapeutic strategies. Meanwhile, a longer mutant protein that acts as a neoantigen is produced by a 4 bp frameshift insertion at the C-terminal of NPM1mut [1], a driver mutation. Because this protein is expressed in a malignant phenotype and is unlikely to be eliminated by immunoediting, it is a promising target for

immunotherapy [8]. It has been demonstrated, for instance, that some compounds can cause cell death by interfering with NPM1's stability and capacity to interact with other DNA repair proteins.

In addition, NPM1 localization is the goal. Among the therapeutic targets are inhibitors of exportin 1, or XPO1, a nuclear exporter that is implicated in the cytoplasmic localization and nuclear export of mutant NPM1, which disrupts cellular homeostasis and promotes the growth and survival of leukemia cells [9]. For example, the Selinexor seeks to rectify NPM1mut's aberrant cytoplasmic localization [1]. Due to the multidomain conformation of NPM1, it is a potential target for small molecule therapy. Small molecules can be designed to bind to any part of a given protein to modulate its function and to achieve certain therapeutic goals [9].

Another treatment method is to target the nucleoli of NPM1 mutant cells. NPM1 acts as a nucleolar stress sensor, AML patients with NPM1 mutation can be vulnerable to stress [9], and the nucleolar stress can activate the p53 protein so that it is possible to suppress the tumor growth. Thus, induction of nucleolar stress is also an option for treating NPM1-mutated AML. There is another way to activate p53 and then increase the apoptosis of cancer cells. This way can be seen as targeting NPM1's protein level [9]. NPM1 mutation causes cytoplasmic localization of mutant proteins due to the replacement appearing on C-terminus, NPM1-mutated patients are highly sensitive to oxidative stress and apoptosis. It has been discovered that leukemia cell lines showed enhanced apoptosis when treated in conjunction with Arsenic trioxide(ATO) and all-trans retinoic acid(ATRA) due to p53 activation and proteasome-mediated degradation [10]. ATRA induces the degradation of mutated-NPM1 so that it can facilitate the cell response to chemotherapy or ATO. According to some reports, ATRA managed to increase the survival rate of senior patients with mutated NPM1 but without FLT3-internal tandem duplication(FLT3-ITD) mutation [8]. A proteolytic-targeted chimera (PROTAC) can also target the mutant NPM1, enabling ubiquitination and proteasome-mediated destruction of the target protein [9]. PROTACs have the potential to be used for AML with a mutation in NPM1 since they facilitate the breakdown of fusion oncoproteins in the MLL leukemia subtype. The selective degradation of mutant NPM1 by degron tagging, which inevitably causes differentiation and growth arrest in NPM1 mutant cell lines, serves as evidence for the efficacy of this strategy in this respect [9].

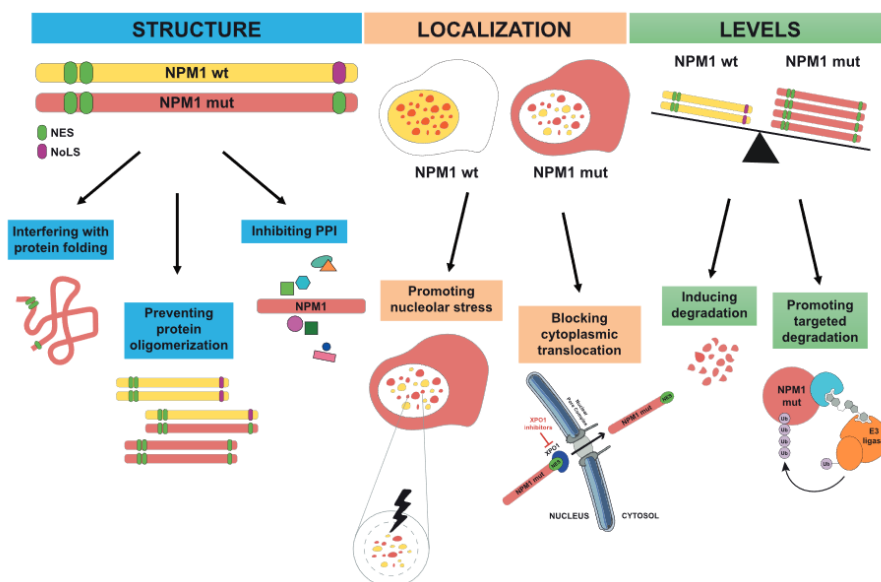


Fig 5. Various targeting methods of treating NPM1-mutated AML [9]

Furthermore, one of the most effective ways to combat AML is to target the apoptotic pathway. AML survival depends on B-cell lymphoma-2 (BCL-2) family proteins, which are also critical for the mitochondrial apoptotic response [9]. The anti-apoptotic proteins BCL-2 and myeloid cell leukemia 1 (MCL1) inhibit apoptosis by isolating the pro-apoptotic protein BIM[9]. Using Venetoclax as an example, this selective BCL-2 inhibitor demonstrated anti-leukemic effectiveness

in 60–70% of AML patients when combined with hypomethylating drugs or low-dose cytarabine (LDAC) [9]. It's among the most promising approaches to treating AML in senior citizens [1].

There are more target locations than NPM1. The homeotic genes come first (HOX). AML cells with NPM1 mutations exhibit high expression of the HOXA and HOXB clusters, which are critical for the preservation of the leukemic state [9]. The histone modifiers MLL1 and DOT1L control the expression and differentiation of HOX and FLT3 in NPM1-mutant AML [8]. As a result, inhibitors of menin-MLL1 and DOT1L together have demonstrated synergistic efficacy against primary AML cells [8]. The two most highly expressed genes in NPM1-mutant AML are CD33 and CD123; in fact, CD123 even exhibits robust expression during the disease's relapse [9]. This implies that they are significant immunotherapy targets. Furthermore, FLT3 mutations can increase CD123 expression, meaning that AML patients with both NPM1 and FLT3 mutations may benefit most from anti-CD123 targeted therapies [9]. Furthermore, the best targets are PD-1 and PD-L1. Anti-PD-1 antibody nivolumab can boost leukemia-associated antigen-stimulated cytotoxic T cells [9], resulting in better patient outcomes throughout treatment. Apart from that, it has been discovered that NPM1mut and nuclear factor- κ B (NF- κ B) interact. The protein NF- κ B is essential for immunological response and inflammation. It also promotes cell division, growth, and resistance to apoptosis [1]. It is activated in AML cells which results in cancer progression [1]. Therefore, it is also a potential target for future therapy.

A gene target may not be necessary for treating NPM1-mutated AML using other techniques. Consider dactinomycin. It is a well-known antibiotic that inhibits RNA polymerases and topoisomerases to exhibit potent antibacterial and anticancer activities [8].

Nowadays, a "7+3" regimen—cytarabine for seven days and anthracyclines for three days—is typically used to treat AML patients with NPM1 mutations [11]. Because of its satisfying therapeutic results, it is regarded as a regular procedure. For younger patients, the total remission rate was 60–80%, but for older patients, it was 40–60% [11]. But because of their aromatic rings and attached amino sugars, anthracyclines are a class of potent chemotherapy drugs that lack selectivity. This can lead to serious side effects, such as cardiac abnormalities in older AML populations [11], so more research is needed to find alternatives or solutions.

Treatment strategies for the prevention of cancer recurrence, stage of relapse, and prognosis recovery are critical to achieve better treatment outcomes. Hematopoietic stem cell transplantation (HSCT) is the greatest way to reduce the likelihood of cancer recurrence, but there are a few other considerations that clinical practitioners should make before using HSCT [2]. These include donor availability, patient health status and comorbidities, conditioning regimens, and patient preferences [2]. Moreover, venetoclax-based regimens, immunotherapy, and 5-azacitidine may be advantageous for patients experiencing molecular recurrence [4]; although, the precise mode of action remains unclear. Elderly individuals may require supportive care, such as pain management, antibiotics, blood transfusion assistance, and referrals to palliative care. It has been shown that adding palliative care to a patient's regimen can improve outcomes, manage symptoms, and promote discussions about care goals and end-of-life planning [2]. Medical professionals must also keep a careful eye on them.

5. The prognosis of NPM1-mutated AML

The European Leukemia Network (ELN) genetic risk categorization is the most widely accepted risk stratification approach for AML [11]. It has been noted that the prognosis is excellent for cytogenetically normal NPM1-mutant AML without FLT3-ITD since they are in a favorable risk category [11]. It has been found that mutant NPM1 is immunogenic to T cells derived from peripheral blood and that the patient benefits when T cells transduced with the mutant NPM1 TCR can destroy NPM1-mutant AML cells both in vitro and in vivo. Both the survival rate and total remission exhibit an increasing trend. About 50% of patients experience relapses, while the overall survival rate is about 40% and the total remission rate is about 80% [8]. Several factors can affect the outcomes. For instance, the mutation stage of NPM1 and also the age. It has been reported that the disease-free

survival and overall survival rate are still disappointing among the old patients group and far worse than the younger patients [8]. This is probably because of the limited treatment options for older people and also drug tolerance.

However, if NPM1 mutations co-exist with other mutations, the prognosis can be worsened. For example, FLT3-ITD-positive, cytogenetically normal, NPM1-mutant AML falls into the intermediate-risk category of the categorization system. FLT3 is involved in JAK-STAT, RAS, and P13K signaling [1]. About 25% of AML patients have been documented to have mutations that result in constitutively active FLT3 kinase, which causes abnormal cell proliferation [1]. It exhibits poor outcomes. In recent years, FLT3 inhibitors have been developed, which to some extent, improve the patient's survival time but finding ways to prevent co-mutations and, at the same time searching for potential targets against the co-mutated AML are still essential parts in the future. Another type of mutation is DNA methyltransferase 3 alpha(DNMT3A) mutation coexists with NPM1 mutation. DNA methylation is regulated by the protein DNMT3A, which is mutated in around 20% of AML cases and co-mutated with NPM1 in 50% of cases [1]. However, the DNMT3A mutation is characterized as a preleukaemic mutation that frequently manifests in remission, suggesting that it is not the primary cause of AML formation [1]. Despite that, there is research indicating that the median survival time of patients with DNMT3A mutation AML is much shorter(only about 12.3 months)than that of patients(around 41.1months)free of DNMT3A mutation so more research is needed in this area as well.

6. MRD used in monitoring AML with NPM1 mutations

Since NPM1 mutations are stable, highly expressed, and do not occur in people with clonal hematopoiesis, they are a perfect target for MRD surveillance [4]. Molecular MRD evaluation using qPCR and ddPCR, as well as flow cytometry, are traditional methods for MRD identification [11]. When hematopoietic stem cell transplantation (HSCT) is performed on AML patients, a positive MRD by flow cytometry before HSCT is significantly linked to a decline in both overall survival and leukemia-free survival (LFS) as compared to MRD-negative individuals. In high-risk AML patients with a normal karyotype, there is a significant correlation between a positive flow cytometry mean ratio and recurrence-free survival. A decreased risk of recurrence is also associated with a negative MRD before HSCT [11]. These all suggest that MRD monitoring is a helpful tool in predicting the survival rate and also the recurrence after HSCT.

For greater accuracy, it is important to master the proper monitoring time. It has been discovered that MRD assessment of peripheral blood after 2 cycles of chemotherapy is more accurate compared to genetic-based prognosis in predicting recurrence risk. In addition, according to the recommendations, RQ-PCR testing should be performed on patients undergoing standard therapy at least once during the diagnosis process, twice throughout treatment cycles, and once the treatment is coming to an end. Every three months throughout the first years of follow-up, MRD monitors should be taken. According to the risk of relapse, MRD monitoring time should then be customized. These recommendations may also be used in the follow-up of patients after allogeneic stem cell transplantation [4].

7. Conclusion

AML is a rarely seen hematological disease with a high death rate which is worthy of more research. NPM1, as the most common mutation in AML has attained much attention. NPM1 has a complex structure and it is an essential gene in the human body with various functions such as remodeling chromatin, involving in ribosome biogenesis, and also affecting the level of tumor suppressor gene p53. Due to its multiple functions, the mechanisms that lead to AML are diverse. Nowadays, researchers have found various treatment methods against NPM1-mutated AML. NPM1 itself appears to be an ideal target, either targeting its structure or targeting the localization, and any

other ways have shown promising results. Still, there are other potential targets, for example, the HOX, CD33, CD123, PD-1, and others. Based on these promising targets, specific drugs and other new therapeutic methods such as CAR-T cell therapy can be involved. As for older patients, special care and monitoring may be needed. The prognosis of the single NPM1-mutated AML is not that disappointing however it also depends on the stage of mutations and patients' age. The outcomes of co-mutations are still barely satisfactory. MRD monitoring is a useful method for predicting the outcomes of NPM1-mutated AML. To obtain more reliable data, the timing of the test is essential.

Although big progress has been made in recent years, there are still some aspects in which further research is needed. First of all, is the combination of different therapies. There are always severe side effects, for example, the hematologic-related adverse by-effects when applying a single therapeutic method, the combination of various methods may play an important role in alleviating or reducing the side effects. Secondly, target therapy is a hot topic nowadays. Researchers need to elucidate the underlying pathways driving the pathogenesis of NPM1 mutant AML to find new targets. Last but not least, the golden criteria for monitoring the disease and also the best method for measurement and prediction need to be discovered in the future. With all the future efforts, it can be expected that the clinical outcome of NPM1-mutated AML will be better.

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