Article

Clinical Utility of Next Generation Sequencing in Diagnosis, Prognosis and Treatment of Acute Myeloid Leukemia and Myelodysplastic Syndrome

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Abstract: Acute myeloid leukemia (AML) and Myelodysplastic Syndrome (MDS) are a heterogeneous group of disorders that share a relatively common biology with higher incidence of morbidity and mortality. In clinical diagnostic laboratories, next-generation sequencing (NGS) has provided opportunities for hematologic patients with initial diagnosis, monitoring the disease progression, and recognition of the relapse. NGS has been used to identify a high degree of recurrent mutations and fusions in AML and MDS. Approximately half of AML and MDS patients had normal cytogenetic results. The recurrent mutations include but not limited to NPM1, FLT3, CEBPA, IDH1/2, TP53, RUNX1, DNMT3A, and ASXL1 in AML, and ASXL1, ETV6, EZH2, RUNX1, and TP53 in MDS. In this article, we have analyzed NGS data from 3867 patients. The results demonstrated the clinical utility of NGS in diagnosis, prognosis, and treatment of AML and MDS.

Keywords: Acute myeloid leukemia (AML), Myelodysplastic syndrome (MDS), Next-generation sequencing (NGS), mutation, fusion

Introduction

AML and MDS are myeloid malignancies that have a continuous disease spectrum with several unique features. The diseases occur predominantly in the elderly, usually start with early-stage MDS, which may progress to advanced MDS, AML, cured AML or resistant AML. These disorders are genetically heterogeneous, characterized by abnormal accumulation of blast cells in the bone marrows and peripheral blood in AML, or functional impairment of hematopoiesis and abnormal bone marrow morphology in MDS [1, 2].

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Traditionally, we use cytogenetic markers for stratifying patients into three risk categories: favorable, intermediate, and unfavorable. Approximately 50% of the patients with AML and MDS have normal karyotype, which makes it difficult for risk stratification and treatment decision for these patients due to the high clinical heterogeneity. Therefore, the identification of genetic mutations in addition to the classical cytogenetic markers has been useful in identifying new entities [1, 3]. The updated World Health Organization (WHO) 2016 classification, European Leukemia Net (ELN) and National Comprehensive Cancer Network (NCCN) have provided the guidance on the genetic abbreviations in AML and MDS. NGS analysis has made it possible

for genetic profiling and stratification of patients into molecularly defined subgroups that can lead to diagnosis, risk stratification, a more effective treatment option for MDS and AML, and response assessment in minimal residue disease for AML [4–7]. For relapsed AML patients, several molecularly directed treatment options have recently emerged including FLT3 inhibitors, IDH1/2 inhibitors ivosidenib and enasidenib, respectively [8]. In this article, we have sequenced over 3,000 patients with hematologic disorders including AML and MDS by NGS and demonstrated the clinical utility of NGS in diagnosis and prognosis of MDS and AML. The results were used to guide individualized treatment and improvement of the patient care.

Materials and Methods

All patients diagnosed with hematologic disorders including AML and MDS from 2018 to 2021 were included in this study. This study was approved by internal research board at Banner Health System. Bone marrow or leukemic blood samples from 3,867 patients were used for NGS analysis. The library preparation, templating and sequencing followed manufacturer's procedures for Ion Torrent™ Oncomine™ Myeloid Assay (Thermo Fisher Scientific, Waltham, MA). The assay includes 74 genes that are associated with a wide range of hematologic malignancies and allows concurrent analysis of DNA and RNA to detect multiple types of variants, including single nucleotide variant (SNV), insertion and deletion (INDEL), and gene fusion, in a single workflow. This test has an analytical sensitivity for detecting 2.5% SNVs and 5% INDEL in a background of non-mutated DNA sequence; fusion detection is limited to a set of specified driver and acceptor genes at 2.5% analytical sensitivity. The NGS run was planned and monitored through Ion Torrent Server 5.16 (OS: Ubuntu 18.04). The results were analyzed by Ion Reporter Software (version 5.16). Oncomine™ Reporter (OR 5.2) was used for NGS reporting, which follows the standards and guidelines recommended by the American College of Medical Genetics and the College of American Pathologists and the joint consensus recommendation of AMP, ASCO and CAP [9–12].

Results

Mutations detected by NGS

We have performed NGS testing on 3,867 patients in this study. These patients had clinical indications of AML, MDS, myeloproliferative neoplasm (MPN) and other hematologic disorders. Among these patients, 2,107 (54.5%) revealed abnormal results with variant(s) or fusion(s) detected; 1,682 (43.5%) showed normal results; 78 (2%) were quantity not sufficient (QNS) with DNA and/or RNA.

Of the mutations detected by NGS, TET2, DNMT3A, TP53, JAK2, ASXL1, SF3B1, CSDE1 and FLT3 ITD/TKD were the most common variants with >1.84% of the cases examined. Table 1 shows mutations with the number of cases and the percentage of cases identified.

Fusions detected by NGS

Of the 3,867 samples studied, NGS identified 32 different fusions in 317 cases. The most common fusions include BCR-ABL1, CBFB-MYH11, PML-RARA, RUNX1-RUNX1T1, KMT2A and RUNX1 rearrangements with various partners (Table 2). These fusions were present in various hematologic diseases with different diagnostic and prognostic information [13, 14]. BCR-ABL1 encodes a chimeric protein, which is present in more than 95% of chronic myeloid leukemia (CML), 20% of acute lymphoblastic leukemia (ALL) and 3% of AML patients. CBFB-MYH11 and RUNX1-RUNX1T1 are the core-binding factor (CBF) that are seen in 20% of adult AML, treated with standard therapy with higher complete remission (CR) rate. However, along with KIT and FLT3 mutations, CBF AML confers poor progno-

Table 1: Mutations Detected by NGS

Mutations	Number of Cases	Percentage (%)
TET2	292	7.55
DNMT3A	195	5.04
TP53	169	4.37
JAK2	151	3.90
ASXL1	117	3.03
SF3B1	110	2.84
CSDE1	99	2.56
FLT3 ITD/TKD	71	1.84
NRAS	67	1.73
IDH1	62	1.60
NPM1	59	1.53
WT1	55	1.42
IDH2	54	1.40
U2AF1	49	1.27
RUNX1	45	1.16
CALR	42	1.09
PTPN11	30	0.78
KRAS	28	0.72
KIT	27	0.70
MFSD11	27	0.70
SRSF2	21	0.54
NF1	19	0.49
SETBP1	18	0.47
MPL	18	0.47
BCOR	16	0.41
STAG2	16	0.41
CBL	15	0.39
ETV6	14	0.36
CEBPA	10	0.26
MIR636	8	0.21
ABL1	8	0.21
CSF3R	7	0.18
PHF6	7	0.18
SH2B3	6	0.16
MYD88	5	0.13
GATA2	4	0.10
ZRSR2	4	0.10
IKZF1	3	0.08
BRAF	3	0.08
PRPF8	3	0.08
EZH2	1	0.03
RB1	1	0.03

sis [15–17]. PML-RARA is characteristic in acute promyelocytic leukemia (APL) [18]. KMT2A rearrangements were seen in many different hematologic disorders including AML, MDS, B-ALL, T-ALL, bi-phenotypic leukemias and therapy-related myeloid neoplasms, almost all with poor prognosis [19, 20]. RUNX1 rearrangements were associated with AML, MDS, MPN, therapy-related myeloid neoplasms. ETV6-RUNX1 is seen in B-ALL [21].

Mutations detected in AML patients

Among 3,867 patients tested, 1,270 (32.8%) had clinical indication of AML. Abnormal results with mutation or fusion detected were seen in 831 (65.4%) patients. Mutations from DNMT3A (n=84), TET2 (n=78), TP53 (n=72), CSDE1 (n=51), NPM1 (n=37), WT1 (n=37), FLT3 ITD/TKD (n=36), NRAS (n=35), IDH1/2 (26/35), and ASXL1 (n=29) were the most common variants identified (Figure 1). These mutations were also reported in many other studies [22, 23].

Fusions detected in AML patients

Among the AML patients, the most common fusions identified include CBFB-MYH11 (n=38), PML-RARA (n=35), KMT2A rearrangements (n=30), RUNX1-RUNX1T1 (n=25), BCR-ABL1 (n=19), DEK-NUP214 (n=7), and RUNX1-CBFA2T3 (n=5), etc. (Figure 2). In total, NGS identified fusions in 176 cases. CBFB-MYH11, PML-RARA, and RUNX1-RUNX1T1 are associated with favorable prognosis, while most of the KMT2A rearrangements are associated with poor prognosis (http://atlasgeneticsoncology.org/).

Mutations detected in MDS Patients

In the last decade, NGS has identified over 50 recurrent mutations in MDS though only some of these mutations are pathogenic and contribute to the clinical heterogeneity of the disease course, therefore, influence the prognosis of patients [24–26]. Among the 3,867 samples analyzed, 1,266 had clinical indication

Table 2: Fusions Detected by NGS

Fusions	Number of Cases	Cytogenetic Abnormalities
BCR-ABL	102	t(9;22)(q34;q11)
CBFB-MYH11	57	inv(16)(p13q22)/t(16;16)(p13;q22)
PML-RARA	35	t(15;17)(q24;q21)
RUNX1-RUNX1T1	31	t(8;21)(q22;q22)
KMT2A-ELL	19	t(11;19)(q23;p13.1)
RUNX1-MECOM	9	t(3;21)(q26;q22)
DEK-NUP214	8	t(6;9)(p22;q34)
KMT2A-MLLT3	8	t(9;11)(p22;q23)
KMT2A-MLLT10	8	t(10;11)(p12.31;q23)
RUNX1-CBFA2T3	5	t(16;21)(q24;q22)
ZMYM2-FGFR1	5	t(8;13)(p11;q12)
KMT2A-MLLT4	3	t(6;11)(q27;q23)
PICALM-MLLT10	3	t(10;11)(p12.31;q14.2)
ETV6-MECOM	3	t(3;12)(q26;p13)
KMT2A-MLLT6	2	t(11;17)(q23;q12)
KMT2A-CASC5 (KNL1)	2	t(11;15)(q23;q15.1)
NUP98-KMT2A	2	inv(11)(p15q23)
RUNX1-ZFPM2	2	t(8;21)(q23;q22)
ETV6-ACSL6	1	t(5;12)(q31;p13)
ETV6 - RUNX1	1	t(12;21)(p13;q22)
ETV6-PGDFRB	1	t(5;12)(q33;p13)
KMT2A-AFF1	1	t(4;11)(q21;q23)
KMT2A-CBL	1	del(11)(q23q23)/t(11;11)(q23;q23)
KMT2A-EPS15	1	t(1;11)(p32;q23)
FIP1L1-PDGFRA	1	del(4)(q12q12)
RBM15-MKL1	1	t(1;22)(p13;q13)
RUNX1-MRPS6	1	t(21;21)(q22.11;q22)
TRIM24-BRAF	1	t(7;7)(q33-34;q34)
ZBTB16-RARA	1	t(11;17)(q23;q21)
NUP214-ABL1	1	t(9;9)(q34.13;q34.12)
SET-NUP214	1	t(9;9)(q34.11;q34.13)

of MDS. The mutations with higher rate identified from the current study include TET2 (n=121), SF3B1 (n=67), TP53 (n=59), DNMT3A (n=54), JAK2 (n=39), ASXL1 (n=38), CSDE1 (n=24) and RUNX1 (n=22), etc. (Figure 3). These mutations were described in terms of biological pathways and clinical relevance earlier [27]. Among these mutations, TET2 was found with highest incidence, and was predicative for response to standard hypomethylating agents (HMAs) with conflicting data [28–30]. SF3B1 has been included as a parameter of the revised World Health Organization (WHO) classification of myeloid neoplasms. It is recommended in all low-risk cases given the excellent prognosis [13, 31]. SF3B1 was seen in 5% of ring sideroblasts (RS) instead of 15% need to be detected for the diagnosis of MDS with RS [27]. In general, SF3B1 and deletion of 5q are in low-risk group MDS. TP53 is an independent prognostic indicator, which added more value for the evaluation of allogeneic hematopoietic stem cell transplantation (HSCT) along with deletion of 5q or complex karyotype. DNMT3A was found in all MDS subtypes, co-mutated with SF3B1 in refractory anemia with RS, and associated with unfavorable clinical

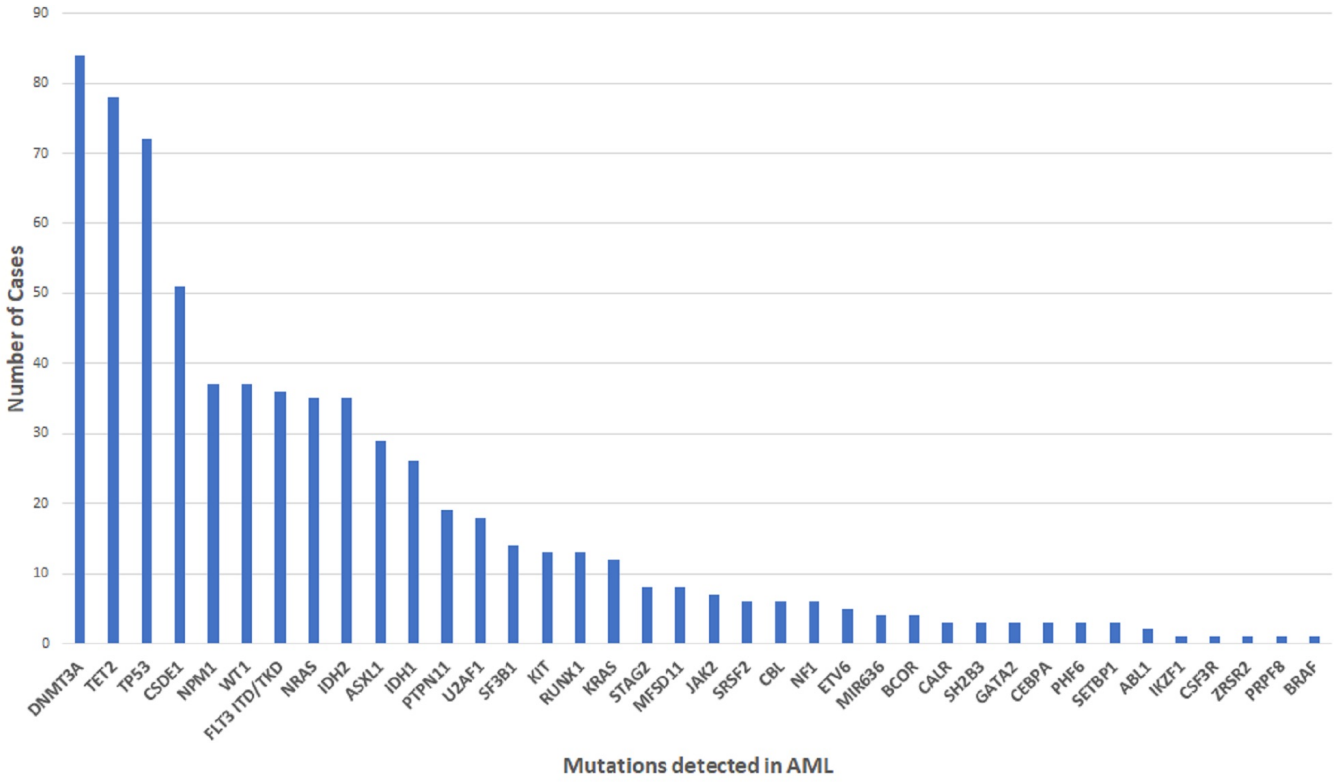

Figure 1: Mutations detected in 1,270 AML patients by NGS.

outcome [27]. While RUNX1 or ASXL1 are among the poor-risk mutations [31].

Fusions detected in MDS patients

Several fusions were detected in 1,266 MDS cases including CBFB-MYH11 (n=5), RUNX1-MECOM (n=3), RUNX1-CBFA2T3 (n=2), KMT2A-ELL (n=1), RUNX1-ZFPM2 (n=1), RUNX1-MRP36 (n=1) and TRIM24-BRAF (n=1) (Figure 4). These were the limited fusions identified in MDS. Most of these cases had pancytopenia, which were associated with high complete remission rate with median survival of 5 years. Some cases were seen in MDS before transformed to AML [32, 33]. RUNX1-MECOM was seen in patients who had poor survival compared with blast crisis of CML, and most of these were co-existing with t(9;22)(BCR-ABL1) [34, 35]. RUNX1-MECOM was also found in therapy-related MDS (t-MDS) or AML (t-AML) [36]. RUNX1-CBFA2T3 has been reported in MDS, pediatric or adult AML or t-AML with similar risk as other core-binding factor leukemias [37]. RUNX1-ZFPM2 was seen in MDS, with uncertain prognosis due to lack of data. KMT2A-ELL has been seen in many hematologic disorders including mixed phenotype acute leukemia with t(v;11q23), AML, and therapy-related myeloid neoplasms with poor prognosis [20].

Discussion

Therapeutic biomarkers in AML and MDS

So far, the therapeutic biomarkers in AML include FLT3 ITD/TKD, IDH1/2, RAS, KIT, BCR-ABL1, PML-RARA, and TP53 while the treatment options

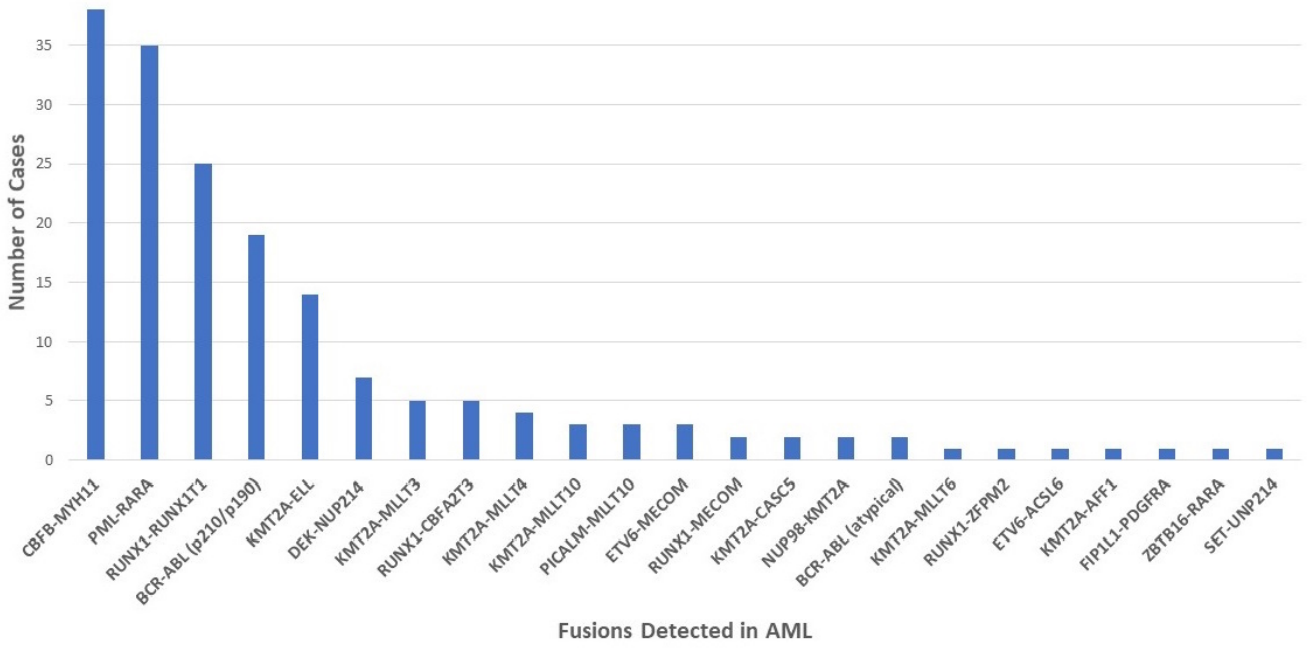

Figure 2: Fusions detected in 1,270 AML patients by NGS.

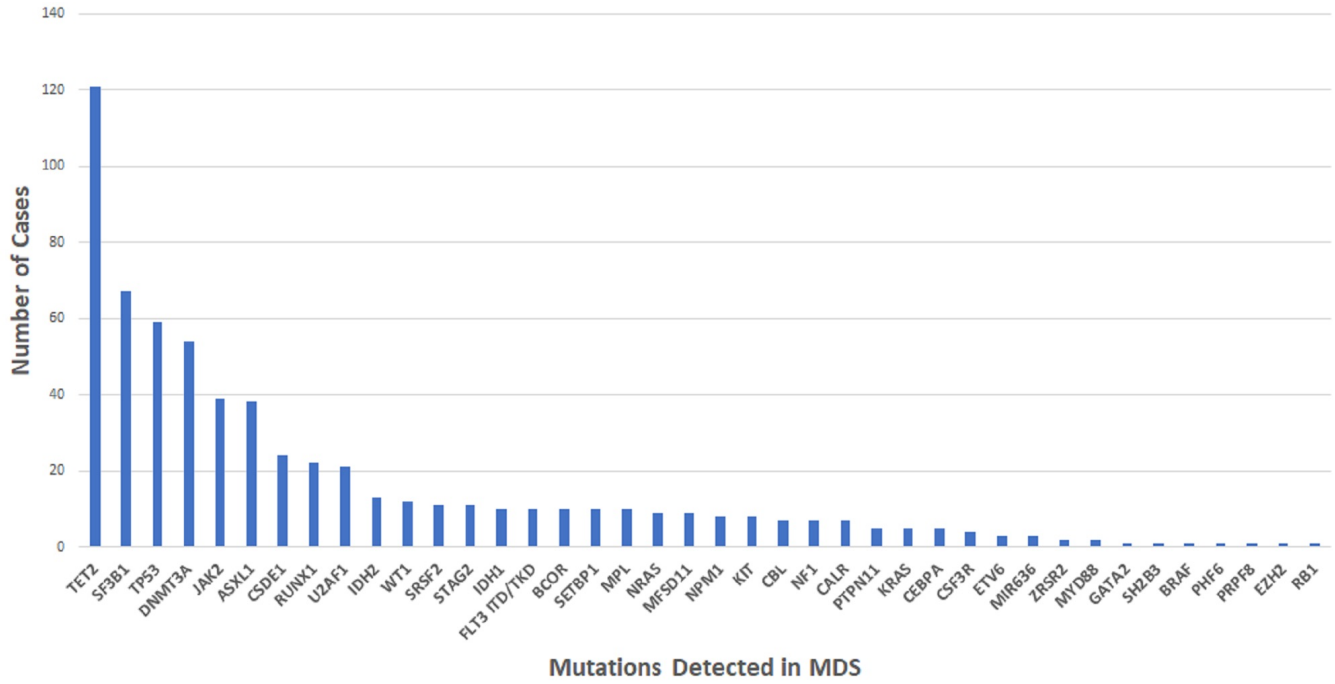

Figure 3: Mutations detected in 1,266 MDS cases.

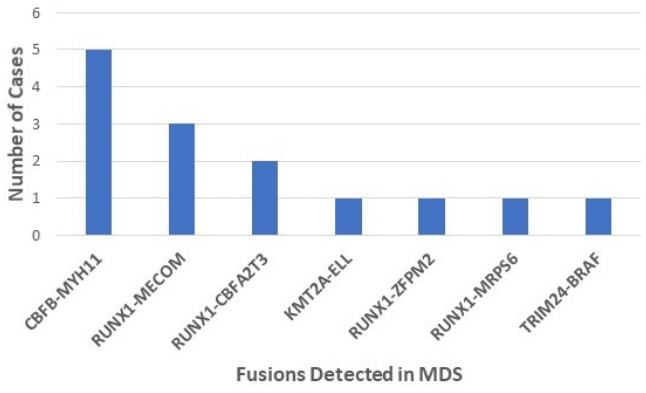

Figure 4: Fusions detected in 1,266 MDS cases.

in MDS are limited [38]. Thanks to the high throughput sequencing technologies, molecular profiling has provided the information on recurrent mutations in AML and MDS patients [39]. These results have shed light on treatment options. According to European Leukemia Net (ELN) classification, NPM1, biallelic CEBPA mutations and inv(16), t(16;16) and t(8;21) have favorable prognosis. However, if a patient has more than one mutation such as FLT3, KIT, JAK2 and RAS, it may confer a different prognosis.

On January 16, 2018, arsenic trioxide (Trisenox) was approved by the FDA in combination with the all-trans retinoic acid (ATRA) agent tretinoin to treat adults with newly-diagnosed low-risk acute promyelocytic leukemia (APL) with the t(15;17) translocation or PML-RARA gene expression (https://ascopost.com/issues/january-25-2018/fda-approves-arsenic-trioxide-with-tretinoin-for-first-line-treatment-of-acute-promyelocytic-leukemia/). One study showed that in relapsed ATRA treated APL patients, FLT3-ITD was in 46% and 37% cases and FLT3-D835+ in 22% and 12% of cases, respectively, at diagnosis and relapse (n=41). These patients also had additional chromosome abnormalities. The study suggested that more genetic aberration collected from relapsed APL patients will benefit some patients that do not respond well to standard therapy [40].

Retrospective studies in high-risk MDS and AML patients showed that patients with unfavorable TP53 mutations were more likely to respond to dose-intense decitabine (20 mg/m^2 over 10 days), an intensive epigenetic treatment than those with other mutations [41]. On April 3, 2020, FDA approved luspatercept-aamt for the treatment of anemia and MDS/MPN with RS and thrombocytosis (MDS/MPN-RS-T) (https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-luspatercept-aamt-anemia-adults-mds).

BCR-ABL

The BCR-ABL1 fusion gene, observed in most CML cases, encodes an active protein tyrosine kinase (PTK) that affects various cellular activities including enhanced proliferation and decreased apoptosis [42]. It is also seen in AML that was transformed from CML. Imatinib, also known as Gleevec, was the first tyrosine kinase inhibitor (TKI) used in treatment of CML [43]. About 40% of patients experiencing resistance to the Gleevec treatment, they were identified with ABL1 T315I mutations. In patients with ABL1 T315I mutation, the second-generation TKIs were developed to target majority of imatinib resistant mutations, such as dasatinib [44] or nilotinib [45]. The presence of T315I also confers resistance to the secondary TKIs, the third generation TKI ponatinib was also available [46, 47]. New drug development is still needed for treatment while more resistance mutations are being identified.

FLT3 Mutations

The FLT3 mutations have been reported in about 30% of AML patients [48]. There were two types of mutations identified: In-frame duplications within the juxtamembrane region (FLT3-ITD) and point mutations in the tyrosine kinase domain (FLT3-TKD). FLT3-ITD was seen in 25% of AML and FLT3-TKD

was observed in 7% of the cases. FLT3-ITD mutations with high allelic burden are associated with an adverse outcome while FLT3-TKD mutations are being reported with better survival with some controversial reports [49–51]. The first generation of FLT3 inhibitors such as midostaurin, sorafenib, lestaurtinib, are shown to be effective [52–54]. The second-generation FLT3 inhibitors include quizartinib, crenolanib, and gilteritinib, and demonstrate a more selective inhibitory activity, as well as a higher potency, when compared to first-generation compounds [55–57].

IDH1/2

IDH1 and IDH2 mutations have been reported in about 20% of all AML cases and 5-12% of MDS. IDH1 occurs in 7-14% of patients, IDH2 occurs in 8-19% of patients [58]. The IDH1 inhibitor, Ivosidenib, was tested in AML patients [59]. IDH2 inhibitor, enasidenib (AG-221), is an oral, selective inhibitor of mutant IDH2. It was first studies in human phase I/II clinical trial, exploring the safety and tolerability of the drug in AML patients [60]; Based on these studies, enasidenib was approved by FDA in advanced mutant IDH2 AML (https://www.fda.gov/drugs/resources-information-approved-drugs/fda-granted-regular-approval-enasidenib-treatment-relapsed-or-refractory-aml).

Core-binding factor AML

Core binding factor acute myeloid leukemia (CBF-AML) codes for two types of recurrent chromosome rearrangements referred to as t(8;21)(q22;q22) and inv(16)(p13q22) or t(16:16)(p13;q22), commonly as t(8;21) and inv(16) [17, 33]. CBF-AML) represents 4-12% of all AML, 15% of adults and 25-30% of pediatrics 16. Patients with CBF-AML have high complete remission (CR) rates (86-88%), however, 30-50% of patients relapse, and the 5-year survival is only 50% [16].

Among 3,867 patients, we identified CBFB-MYH11 translocation in 57 patients, and RUNX1-RUNX1T1 in 31 patients. Eight patients had KIT mutations (three located in exon 8 and 5 located in exon 17) along with CBFB-MYH11 rearrangement. Seven patients had KIT exon 17 mutations along with RUNX1-RUNX1T1 rearrangement. According Park et al, c-KIT exon 17 mutation was associated with poor prognosis in patients with t(8;21) [61]. They are related to lower CR, shorter overall survival (OS) and event-free survival (EFS) in t(8;21) AML adult patients, but it had no effect on inv(16) AML [15, 61].

FLT3-ITD mutations can be found in 20-30% of AML patients, and are more common in normal karyotype (NK)-AML, but less common in CBF-associated AML, while 5-10% of patients with CBF AML have FLT3-ITD mutations [62–65]. Among 3,867 patients, we identified FLT3 mutations in 2 cases with CBFB-MYH11 translocation, and 3 cases along with RUNX1-RUNX1T1 translocation. Studies showed that FLT3 mutation resulted in reduced EFS and OS in patients with CBF-AML. In both subtypes, FLT3 mutation is predictive of short progression-free survival (PFS) in patients with inv(16), whereas not in t(8;21) [66, 67].

In conclusion, the current guidelines for the diagnosis and clinical management of MDS and AML include cytogenetic results, as well as mutations in FLT3, NPM1, CEBPA, IDH1/2, KIT and TP53, etc. and various fusion genes. The advancement of NGS technologies has becoming the tier 1 molecular testing for MDS and AML patients with abundant information regarding genomic profiling [68]. The information obtained from the NGS results has guided clinicians for risk stratification, treatment options and predicting patients' response to the drugs. In addition, NGS opens the opportunity for targeted therapies in clinical trials. The clinical utility of NGS will increase in the next few years with the application of NGS in minimal residual disease (MRD) monitoring. Due to the heterogeneity of AML and MDS, identifying a pattern of multiple biomarkers

will provide a solution for achieving personalized medicine.

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