REVIEW ARTICLE

Next-generation Sequencing in Acute Myeloid Leukemia

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ABSTRACT

Acute myeloid leukemia (AML) is a disease that affects any age group with a poor outcome using the traditional chemotherapy and the bone marrow transplantation as well. The World Health Organization published several reports on AML classification depending on morphology, immunophenotyping, and cytogenetic; however, the impact on treatment approaches was not encouraging unless in AML-M3 where the fusion gene acute promyelocytic leukemia-retinoic acid receptor alpha is a major target for all-trans-retinoic acid with better survival rates compared with other subtypes. During the past two decades, efforts were made to introduce new technologies to reveal the most important gene alterations responsible for AML pathogenesis. The first technique was the polymerase chain reaction which is able to detect a definitive gene status and quantitative copies of such genes. Recently, the next-generation sequencing (NGS) has been introduced as a model through which a complete gene profile can be mapped for any kind of diseases including hematologic malignancies. NGS in AML can help to detect the most important genes driving myeloid blasts into malignancy and it can help design new treatment approaches to improve both response and survival rates.

Key words: Acute leukemia, myeloid, next generation sequencing

INTRODUCTION

Acute myeloid leukemia (AML) is one of the most lethal leukemias which can affect any age group. Prognosis is still bad even with the introduction of new therapies. The mainstay of treatment is chemotherapy, targeted agents and allogeneic stem cell transplantation. The FDA began to approve new therapies depending on phenotype such as the anti CD33 Gemtuzumab ozogamicin in both newly diagnosed and relapsed CD33 positive AML. Also, midostaurin was approved in newly diagnosed FLT-3 mutant AML as well as the anti-BCL-2 venetoclax which was approved in older patients with AML where chemotherapy is not tolerable. Those few examples reflect the new reality where genetic testing will lead the treatment approach in both newly diagnosed and refractory diseases.

IMPACT OF NEXT-GENERATION SEQUENCING (NGS) ON DIAGNOSIS OF MYELOID MALIGNANCIES

Gene profile by NGS is introduced in specialized hematologic laboratories using targeted sequences of DNA and analyzes more than 50 genes according to their function as follows: Splicing function (i.e. U2AF1, SF3B1, SRSF1, and ZRSR2), epigenetic function (TET2, DNMT3A, BCOR, ASXL1, IDH1, and IDH2), cohesion (STAG2, RAD21, and SMC3), transcription factors (RUNX1, WT1, and ETV6), signaling proteins (NF1, NRAS, CBL, PTPN11, JAK2, and FLT3), and modifiers of chromatin (EZH2 and ASXL1).[1-4] NS is able to detect more than 90% gene mutations in patients with myelodysplastic syndrome (MDS).[5] Some new entities are

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Table 1: The frequency of gene mutations in AML patients detected by NGS and the current treatment approaches along with the ongoing clinical trials basing on NGS results

Mutated gene	Frequency	Current trial and treatment approach
ABL1	0.43%	Fludarabine, cyclophosphamide, and total body irradiation
ABL2	14.29%	ECT-001 expanded cord blood
AFF1	NA	Fludarabine, cyclophosphamide, and mycophenolate mofetil
ASXL1	18.71%	Allogeneic hematopoietic stem cell transplantation, azacitidine, and antineoplastic immune cells
ATM	2.72%	Min9709, allogeneic hematopoietic stem cell transplantation, and cyclophosphamide
BCL-2	NA	Bet inhibitors gsk525762
BCL-6	0.37%	Bet inhibitors gsk525762
BCOR	9.4%	Busulfan, fludarabine, and venetoclax
BCR	NA	Fludarabine, cyclophosphamide, and total body irradiation
BIRC3	NA	Min9708, allogeneic stem cell transplantation, and cyclophosphamide
CBFA2T3	1.32%	Allogeneic-specific allogeneic Type 1 regulatory T cells t-allo 19, non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation, and rabbit antithymocyte globulin
CBFB	2.38%	Cytarabine, fludarabine, and total body irradiation
CBL	2.75%	Anti-EphA3 monoclonal antibody KB004, busulfan, and fludarabine
CEBPA	4.19%	Idarubicin, cytarabine, and etoposide
CREBBP	3.3%	Alloantigen-specific allogeneic Type 1 regulatory T cells t-allo 10, non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation
CRLF2	NA	ECT-001 expanded cord blood
CSF1R	NA	ECT-001 expanded cord blood
DEK	NA	Fludarabine, cyclophosphamide, and allogeneic stem cell transplantation
DNMT3A	21.37%	Allogeneic CD56-positive CD3-negative natural killer cells, interleukin-2, and superagonist interleukin-15: interleukin-15 receptor alpha su/fc fusion complex alt-803
ELL	NA	Fludarabine, cyclophosphamide, and mycophenolate mofetil
EPOR	NA	ECT-001 expanded cord blood
ERBB2	1.09%	Autologous NKG2D chimeric antigen receptor-expressing T lymphocyte CM-CS1, granulocyte-macrophage colony-stimulating factor, and galinpepimut-S
ERG	NA	Allogeneic-specific allogeneic Type 1 regulatory T cells t-allo 10
ETV6	17.38%	Non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation
EZH2	4.69%	Azacitidine, busulfan, and fludarabine
FGF2	NA	Azacitidine and nintedanib
FGFR3	1027%	CDK/JAK2/FLT-3 inhibitors TG02 citrate, MLN9708, and allogeneic stem cell transplantation
FLT3	11.3%	Gilteritinib, midostaurin, and sorafenib are already approved in FLT-3 + AML
FUS	0.37%	Allogeneic-specific allogeneic Type 1 regulatory T cells t-allo 10
GLIS2	NA	Non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation, rabbit antithymocyte globulin, and cyclophosphamide
HOXA9	NA	Non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation, rabbit antithymocyte globulin, and cyclophosphamide
HRAS	2.27%	Allogeneic stem cell transplantation, allogeneic CD-56 positive CD-3 negative natural killer cells
IDH1	8.17%	Ivosidenib is currently approved for IDH1 mutated AML patients

(*Contd...*)

Table 1: (*Continued*)

Mutated gene	Frequency	Current trial and treatment approach
IDH2	10.59%	Enasidenib is currently approved in IDH2 mutated AML patients, IDH2 inhibitor AG-221, azacitidine, and enasidenib are in trials as well
IGH	NA	Cyclophosphamide, fludarabine, and CDK/JAK2/FLT-3 inhibitors T02 citrate
IKZF1	1.92%	Cyclophosphamide, fludarabine, and mycophenolate mofetil
IL7R	NA	ECT-001 expanded cord blood
JAK1	1.19%	ECT-001 expanded cord blood
JAK2	3.13%	Antineoplastic immune cells, ECT-01 expanded cord blood, and allogeneic stem cell transplantation
JAK3	0.45%	ECT-001 expanded cord blood
KAT6A	NA	Allogeneic-specific allogeneic Type 1 regulatory T cells t-allo 10, non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation
KDM5A	NA	Non-myeloablative TCR alpha/beta-depleted haploidentical stem cell transplantation, rabbit antithymocyte globulin, and cyclophosphamide
KIT	2.2%	Fludarabine, cytarabine, and cyclophosphamide
KMT2A	1.44%	Fludarabine, cyclophosphamide, and total body irradiation
KRAS	3.77%	Binimetinib, busulfan, and fludarabine
MDM2	NA	MDM2/MDMX inhibitor ALRN-6924 and cytarabine
MDM4	0.73%	MDM/MDMX inhibitor ALRN-6924 and cytarabine
MECOM	1.51%	Fludarabine, cyclophosphamide, and allogeneic stem cell transplantation
MLF1	NA	Azacitidine, fludarabine, and cyclophosphamide
MLLT1	NA	Fludarabine, cyclophosphamide, and mycophenolate mofetil
MLLT10	NA	Fludarabine, cyclophosphamide, and mycophenolate mofetil
MLLT3	NA	Fludarabine, cyclophosphamide, and mycophenolate mofetil
MLLT4	NA	Fludarabine, cyclophosphamide, and mycophenolate mofetil
MNX1	NA	Non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation rabbit antithymocyte globulin and cyclophosphamide
MYC	2.2%	BET inhibitor gsk525762, allogeneic bone marrow transplantation, and busulfan
MYH11	NA	Cytarabine, fludarabine, and total body irradiation
NF1	7.33%	Busulfan, fludarabine, and venetoclax
NPM1	16.7%	Azacitidine, fludarabine, and cytarabine
NRAS	9.81%	Binimetinib, busulfan, and allogeneic bone marrow transplantation
NSD1	NA	Non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation, rabbit antithymocyte globulin, and cyclophosphamide
NUMA1	NA	Beta all trans retinoic acid and idarubicin
NUP214	NA	Fludarabine, cyclophosphamide, and allogeneic bone marrow transplantation
NUP98	NA	Non-myeloablative TCR alpha/beta-depleted haploidentical hematopoietic stem cell transplantation, rabbit antithymocyte globulin, and cyclophosphamide
PBX1	NA	Fludarabine, cyclophosphamide, and mycophenolate mofetil
PDGFRB	2.2%	ECT-001 expanded cord blood
PML	3.95%	Cytarabine, idarubicin, and fludarabine
PTPN11	4.9%	Busulfan, fludarabine, and venetoclax
RARA	0.37%	Cytarabine, idarubicin, and fludarabine
RIT1	NA	Busulfan, fludarabine, and venetoclax

(*Contd...*)

Table 1: (*Continued*)

Mutated gene	Frequency	Current trial and treatment approach
RPN1	NA	Fludarabine, cyclophosphamide, and allogeneic bone marrow transplantation
RUNX1	15.6%	Cytarabine, fludarabine, and cyclophosphamide
RUNX1T1	1.2%	Cytarabine, fludarabine, and total body irradiation
SF3B1	3.46%	Fludarabine, MLN9708, and splicing inhibitor H3B-8800
SH2B3	1.1%	ECT-001 expanded cord blood
SRSF2	10.73%	Splicing inhibitor H3B-8800, busulfan, and fludarabine
STAG2	6.95%	Busulfan, fludarabine, and venetoclax
TCF3	0.75%	Fludarabine, cyclophosphamide, and mycophenolate mofetil
TET2	17.55%	Cytarabine, azacitidine, and daunorubicin
TP53	13.22%	Fludarabine, cyclophosphamide, and allogeneic stem cell transplantation
TRA	NA	IL-15 activated cytokine-induced killer cells
TRB	NA	IL-15 activated cytokine-induced killer cells
TRD	NA	IL-15 activated cytokine-induced killer cells
TRG	NA	IL-15 activated cytokine-induced killer cells
U2AF1	4.97%	Splicing inhibitor H3B-8800, busulfan, and fludarabine
WHSC1	NA	MLN9708, allogeneic hematopoietic stem cell transplantation,and cyclophosphamide
WT1	4.61	Anti-CD33 monoclonal antibody bi 836858. IDH2 inhibitor AG-221 and pevonedistat
ZRSR2	1.86%	Splicing inhibitor H3B-8800, busulfan, and fludarabine

AML: Acute myeloid leukemia, NGS: Next-generation sequencing, TCR: T-cell receptor, RARA: Retinoic acid receptor-alpha, IL: Interleukin

newly defined in acute myeloid leukemia (AML) including chromatin-spliceosome, TP53 aneuploidy, and IDH2R172.[2]

In myeloproliferative neoplasms (MPN), the three main driver mutations are JAK2, MPL, and CALR which are well studied; however, there are several non-driver mutations which are detected by NGS but the impact of these genes on practice is to be defined.[6,7] Compared with the traditional cytogenetic and molecular studies, the role of NGS will be in risk stratification in difficult and challenging cases and consequently treatment choices.[8] There are certain co-occurring genes that drive a secondary AML from MPN cases. The high molecular risk genes in primary myelofibrosis include mutations in ASXL1, SRSF2, EZH2, IDH1, and IDH2.[9] The former co-occurring mutations may predict poor prognosis and short survival in acute leukemia patients.[10] Thus, NGS is able to identify patients at high risk of transformation and those who are potential candidates for allogeneic bone marrow transplant.[11] Furthermore, NS can offer a great information in MPN patients who lack the driver mutations (JAK2V617F, CALR, and MPL), in this case, NGS can detect the clone implicated in pathogenesis.[12] In AML, NGS can detect genes that are still found in bone marrow after remission without affecting relapse rate including DNMT3A, TET2, ASXL1, RUNX1, IDH1, and IDH2.[13] NGS is of great value in discriminating between somatic leukemia-associated mutation and clonal hematopoiesis of indeterminate potential (CHIP). For example, JAK2V617 mutation can be detected in 0.1% of the population without any signs of myeloproliferative disease; therefore, we should wait before reaching the diagnosis of MPN in case of JAK2 positivity.[14] Another example is a patient with thrombocytosis with mutations in DNMT3A and TET2 which can be detected in normal clonal hematopoiesis; therefore, the diagnosis of MPN should be carefully excluded. Of note, donation of hematopoietic stem cells from persons with CHIP like DNMT3A may be related to bad engraftment after allogeneic bone marrow transplant.[15]

Several types of gene mutation such as TP53, RUNX1, DATA2, CEBPA, or ASXL1 may arise as a CHIP which is challenging in diagnosis of MPN. The abnormal clonal hematopoiesis has much larger clones and more driver mutation compared with that in benign hematopoiesis. Genes with somatic mutations in hematologic neoplasms were identified in 71% of MDS patients and 62% of idiopathic cytopenia of unclear significance patients.[16]

In the pre-NGS era, detection of the most frequent reciprocal rearrangement was feasible with the polymerase chain reaction and the most important rearrangements detected in AML were promyelocytic leukemia (PML)-retinoic acid receptor alpha (RARA), RUNX1-RUNX1T1, and CBFB-MYH11; however, current NGS technologies are able to detect even very rare aberrations.[17] Compared with data on the genome atlas, more than 9000 fusion genes were discovered during the last several

years using NGS technology which reflects the high sensitivity of the procedure compared with fluorescence *in situ* hybridization (FISH) which is able to detect the exchange in big chromosomal segments; however, more than 75% of fusions detected by NGS are related to intrachromosomal rearrangements.[18]

It is also possible to investigate extended RNA sequences to reach the second partner gene in case of detection of one gene only in presumed AML patients with fusion genes. NGS was able to detect the partner genes in 58% of AML patients in which one partner gene was detected (RUNX1, ETV6, PDGFRB, KMT2A, RARA, NPM1, MECOM, PDGFRA, BCOR, TET2, and NUP98) by means of FISH and chromosome painting.[19] Another variant of PML with the unusual X-RARA was detected by NGS instead of the usual fusion PML-RARA where PR fails to detect it.[20]

GENES PLAYING AN IMPORTANT ROLE IN DIAGNOSIS AND TREATMENT APPROACH OF AML

AML is a clonal expansion of myeloid blasts in bone marrow, peripheral blood, and extramedullary tissues. AML classification according to the World Health Organization (WHO) criteria is divided into four major categories: (1) AML with recurrent genetic abnormalities, (2) AML with multilineage dysplasia, (3) therapy-related AML, and (4) AML not otherwise specified. About 20% of myeloid blasts in bone marrow or peripheral blood are required to put the diagnosis of aml according to the WHO.[21] The most important genetic alterations in AML are DNMT3A, NPM1, ASXL1, TET2, and ETV6.[22] NPM1 mutation, NPM1 W288Cfs*12, DNMT3A mutation, ASXL1 mutation, and TET2 mutation represent the most frequent mutations in patients with AML.

Table 1 demonstrates all isolated genes known to be mutated in patients with AML, in the left column genes are mentioned, in the second one gene frequency is illustrated, and the 3rd column mention the current approved drug or the current clinical trials targeting the gene of interest.

In case of relapse, AML patients need to be studied again using NGS technology to sort out candidate genes for possible treatment approaches such as IDH1, IDH2, and FLT3-ITD/TKD that may result from clonal evolution.

CONCLUSION

AML is a very aggressive disease and patients should be allocated to targeted therapy the earlier we can, therefore, efforts should be made to study the most important top player genes such as IDH1, IDH2 and FLT3 in order to start FLT3 inhibitor before the complete gene profile is revealed by NGS. This very complicated gene profile mentioned in Table 1 needs a multidisciplinary team to put a suitable profile for every patient to decrease cost and improve decision making. Concentration should be directed toward genes that they already have its own approved therapy. Several parameters are important when we decide which genes to be studied and which are more important in treatment decision making. The most important parameter is gene frequency in AML patients, what the extent the gene plays in pathogenesis and if there is an actual approved treatment in case of mutation. How to differentiate between the real somatic mutations from CHIP and germline ones? The landscape seems to be easier than we expect and we should wait several years where experience should be accumulated before NGS becomes a routine practice in AML treatment decision making.

NGS results should be interpreted with caution in MPN since the presence of high-risk molecular markers such as mutations in ASXL1, EZH2, and IDH1/IDH2 is an indicator of poor prognosis and leukemic transformation.[10] The eradication of most important mutation at the end of treatment and the persistence of other genes such as ASXL1, DNMT3A, or TET2 indicates a clonal hematopoiesis or germline background.[13] Therefore, a close relationship should be constituted between NGS laboratory, hematologist, and pathologist to better understand and interpret results.

REFERENCES

1. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. Blood 2013;122:4021-34.
2. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, *et al*. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 2016;374:2209-21.
3. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, *et al*. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 2013;122:3616-27.
4. Duncavage EJ, Tandon B. The utility of next-generation sequencing in diagnosis and monitoring of acute myeloid leukemia and myelodysplastic syndromes. Int J Lab Hematol 2015;37 Suppl 1:115-21.
5. Bacher U, Kohlmann A, Haferlach T. Mutational profiling in patients with MDS: Ready for every-day use in the clinic? Best Pract Res Clin Haematol 2015;28:32-42.
6. Tefferi A, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L, *et al*. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood 2014;124:2507-13.
7. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2017 Update on diagnosis, risk-stratification, and management. Am J Hematol 2017;92:94-108.
8. Tefferi A. Primary myelofibrosis: 2017 Update on diagnosis, risk-stratification, and management. Am J Hematol 2016;91:1262-71.
9. Vannucchi AM, Lasho TL, Guglielmelli P, Biamonte F,

Pardanani A, Pereira A, *et al*. Mutations and prognosis in primary myelofibrosis. Leukemia 2013;27:1861-9.
10. Guglielmelli P, Lasho TL, Rotunno G, Score J, Mannarelli C, Pancrazzi A, *et al*. The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: An international study of 797 patients. Leukemia 2014;28:1804-10.
11. Alduaij W, McNamara CJ, Schuh A, Arruda A, Sukhai M, Kanwar N, *et al*. Clinical utility of next-generation sequencing in the management of myeloproliferative neoplasms: A single-center experience. Hemasphere 2018;2:e44.
12. Rumi E, Cazzola M. Advances in understanding the pathogenesis of familial myeloproliferative neoplasms. Br J Haematol 2017;178:689-98.
13. Jongen-Lavrencic M, Grob T, Hanekamp D, Kavelaars FG, Al Hinai A, Zeilemaker A, *et al*. Molecular minimal residual disease in acute myeloid leukemia. N Engl J Med 2018;378:1189-99.
14. Nielsen C, Birgens HS, Nordestgaard BG, Bojesen SE. Diagnostic value of JAK2 V617F somatic mutation for myeloproliferative cancer in 49 488 individuals from the general population. Br J Haematol 2013;160:70-9.
15. Gibson CJ, Kennedy JA, Nikiforow S, Kuo FC, Alyea EP, Ho V, *et al*. Donor-engrafted CHIP is common among stem cell transplant recipients with unexplained cytopenias. Blood 2017;130:91-4.
16. Kwok B, Hall JM, Witte JS, Xu Y, Reddy P, Lin K, *et al*. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood 2015;126:2355-61.
17. Wang E, Zaman N, Mcgee S, Milanese JS, Masoudi-Nejad A, O'Connor-McCourt M. Predictive genomics: A cancer hallmark network framework for predicting tumor clinical phenotypes using genome sequencing data. Semin Cancer Biol 2015;30:4-12.
18. Mertens F, Johansson B, Fioretos T, Mitelman F. The emerging complexity of gene fusions in cancer. Nat Rev Cancer 2015;15:371-81.
19. Stengel A, Nadarajah N, Haferlach T, Dicker F, Kern W, Meggendorfer M, *et al*. Detection of recurrent and of novel fusion transcripts in myeloid malignancies by targeted RNA sequencing. Leukemia 2018;32:1229-38.
20. Welch JS, Westervelt P, Ding L, Larson DE, Klco JM, Kulkarni S, *et al*. Use of whole-genome sequencing to diagnose a cryptic fusion oncogene. JAMA 2011;305:1577-84.
21. National Cancer Institute. NCI the Saurus Version 18.11d. Bethesda, Maryland: National Cancer Institute; 2018. Available from: http://www.ncit.nci.nih.gov/ncitbrowser. [Last accessed on 2018 Aug 28; retrieved on 2018 Sep 21].
22. AACR Project GENIE Consortium. AACR project GENIE: Powering precision medicine through an international consortium. Cancer Discov 2017;7:818-31.

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